Impact of Tillage on Soil Biota

An investigation into the effect of crop establishment on soil health

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Abstract

Soil health is a key component of the UK government's "25 Year Plan for the Environment" and is an important factor in defining the sustainability of agricultural systems. Understanding the effect agriculture has on long-term soil health is therefore vital for sustainability and ensuring profitable farming practices.

However, how we define soil health still requires evaluation particularly for long term implications on agricultural management practices. Soil biology plays a pivotal role as a soil health indicator however it is less regularly measured in comparison to soil chemistry and physical properties.

Soil ecosystems take time to stabilise after perturbations, and this is likely to vary under crop establishment techniques which involve different levels of tillage intensification. To investigate tillage intensity on soil health, a long-term trial was selected. The long-term experiment was established in August 2012 at Quarry Field, Harnhill Manor Farm, Cirencester. This comprised three different cultivation treatments (plough and power harrow drill; minimum tillage and direct drill) replicated six times in a randomised block design.

To assess soil health, complete analysis of soil biological, physical and chemical parameters were undertaken as well as consideration given to environmental factors. The parameters investigated included atmospheric and soil temperatures, rainfall, soil compaction, soil profile composition, organic-, carbon- and moisture content, nitrate, ammonium, and phosphorus content as well as diversity and abundance of microfauna (nematodes), mesofauna (collembola and acari) and macrofauna (earthworms and arthropods). Samples were taken at four intervals over a twelve-month period (summer, autumn, winter and spring).

Significant variance was consistently found between inversion tillage (conventional) and non-inversion (minimal till and direct drill) types, particularly in soil organic matter which consistently showed higher percentage means in both direct drill and minimal tillage plots when compared to conventional tillage plots. Collembola showed higher abundance counts in all sampling intervals, but in different taxonomic orders for non-inversion types and anecic earthworms showed significant differences in spring samples for minimal tillage plots alone when compared to conventional tillage plots. Significant differences in soil physical properties were limited to either one or two sampling intervals. Soil organic

matter standardised sampling methods came under scrutiny as significance varied when sampling method was altered to compensate for clay composition of soil. Non-inversion methods were interchangeable in terms of their performance when compared to inversion tillage which consistently performed worse in all significant results indicating that traditional cultivation practices are fundamentally impacting soil health and therefore potentially impacting crop yields.

Author's Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author and in no way represent those of the University.

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Abbreviations

BD – Bulk Density

CT – Conventional Tilling

DD – Direct Drilling

GM – Gravitational Moisture Content/ Soil Water Content by Mass

LOI – Loss on Ignition

MT – Minimal Tilling

NT – No Tilling

RT – Reduced Tilling

SOM / OMC – Soil Organic Matter Content

SOC /OC – Organic Carbon Content

VESS – Visual Evaluation of Soil Structure

VM – Volumetric Moisture Content / Soil Water Content by Volume

Introduction

Agricultural Land Management Practices

Agriculture – the science of land cultivation - has been shaping the landscape since the last ice age, and as the demand for food security has increased, so has land-use intensity (Bélanger & Pilling, 2019). This focus on maximising food provision has come at the expense of a sustaining soil ecosystem's ability to provide its other vital services, such as regulating climate, sequestering carbon, limiting erosion, retaining and purifying water, effectively recycling nutrients and providing niches for specialised soil organisms (Bardgett, 2005) collectively known as ecosystem multifunctionality (Byrnes et al., 2014). This subsequent loss of biodiversity, land degradation and soil ecosystem destabilisation has led to an increased attention in quantifying soil health to sustain future food security and improve resilience against threats such as climate change (Ferrier, Harwood, Ware, & Hoskins, 2019).

Modern intensive agriculture demands continuous and constant trade-off between provisioning (food) and regulating/supporting (soil formation, climate regulation, nutrient cycling, flood regulation, carbon sequestration and habitat provision) (Balvanera et al., 2014; DEFRA, 2011; Lal, 2013) services. Productivity aims to increase the rate of provisioning services to the detriment of regulating services, however when regulating and supporting ecosystem services are disrupted, food production is affected, creating a feedback loop of degradation (FAO, 2011).

Currently, the level of empirical evidence on the impacts intensive agriculture has on soil biodiversity are highly variable depending on region, crop and physical characteristics of soil and study target (macro-, meso- or microfauna) (FAO, ITPS, GSBI, SCBD, & and EC, 2020). Furthermore, there is a lack of defining the tilling intensity outside of the basic categorisation of soil inversion methods such as ploughing known as conventional tillage and non-inversion methods such as reduced tillage or no tillage.

Variability in tillage practices

Tillage is defined as the mechanical manipulation of the soil to optimise productivity and alleviate constraints of soil (Townsend, Ramsden, & Wilson, 2016). Current tillage systems can be divided into two broad categories; soil inversion – known as conventional tillage whereby a complete soil inversion takes place to bury or incorporate crop residue accompanied by a secondary cultivation to prepare the seedbed. Non-inversion tillage can be subset into two groups, firstly one that involves fewer passes than CT, incorporating crop residue from the upper soil profile (usually around 10cm) whilst still leaving at least 30% of crop residue on the soil surface known as reduced tillage or minimal tillage. Secondly, a no tillage or direct drilling approach would be to leave the soil completely undisturbed until crop sowing, whereby all crop residues remain on the surface, only creating a narrow incision where the seed is delivered.

The implementation of tillage systems however varies amongst farmers. Table 1 provides an overview of variation in tillage systems described in Townsend et al. (2016) which explored cultivation practices in the UK. In this experiment conventional tillage refers to soil inversion techniques, minimal tillage (MT) is high intensity non-inversion techniques and direct drill (DD) is used to refer to low intensity non-inversion techniques. Table 3 describes specific machinery, depths and techniques implemented at the site in the last 7 years (T. Norris, personal communication, October 2021), however tillage intensity has remained the same since inception.

Table 1: Description of tillage practices

Tillage Type	Description
Conventional Tillage	Entails inversion of the top 20-50 cm of soil as a primary cultivation, followed by a secondary cultivation that breaks up the larger particles to produce a smoother surface enabling seed insertion
Non-Inversion Tillage, Reduced Tillage, Minimal Tillage, High Intensity Non-Inversion Tillage, Low Intensity Non-Inversion Tillage	No soil inversion entailing a combing of the top 10-25cm of soil and or specification of crop residue left on soil surface usually 30% or more
No Tillage, Direct Drilling	Minor soil disturbance where the seed is drilled directly into the stubble of the previous crop
Mixed Tillage	The use of both conventional and reduced tillage with either rotational ploughing (once every few years) or strategic tillage (site specific)
Subsoiling	A practice to loosen top layer of soil usually conducted after harvest to reduce compaction after heavy rain season
Primary Cultivation	Initial land preparation to deepest planned depth
Secondary Cultivation	A shallower and finer scale tillage practice to smooth out surface and prepare for seeding usually proceeding main tillage practice

Weed, pest and disease control have been the primary drivers of land cultivation, however due to an increase in herbicide availability and the economic pressures faced by farmers, the feasibility of reduced tillage has become more prominent (Morris, Miller, Orson, & Froud-Williams, 2010).

Culturally, the appearance of clean, ploughed land is still seen by many farmers as a well-managed land (Jones et al., 2006), and CT is still used to counteract compaction cased from field traffic during previous crop by inducing a reduction in soil aggregates (Alvarez & Steinbach, 2009).

According to an analysis of implemented cultivation techniques in England, 32% of arable land was established under reduced tillage (RT), with 44% of farms using some form of RT and only 4% of this is under no till (NT) with insufficient data available on specific tillage practices at farm level (Townsend et al., 2016). Furthermore, it was found that individual crops impacted choice of land preparation more than any other reason. The average depth of tillage was only slightly shallower for RT compared to ploughing.

Reducing tillage intensity has shown to reduce soil health indicators, environmental impacts and improve agricultural outputs in many studies (Alvarez & Steinbach, 2009; Benito, Sombrero, & Escribano, 1999; Celik et al., 2020; Cerdà et al., 2020). Reduced tillage generally forms part of a wider agronomic practice which include residue management, diverse crop rotations as well as the addition of cover crops (Busari, Kukal, Kaur, Bhatt, & Dulazi, 2015; Zibilske, Bradford, & Smart, 2002). Furthermore, much research shows that intensive ploughing leads to soil-related problems which include soil compaction and soil erosion by leaving soil bare and exposed, resulting in lower productivity and soil quality (El Titi, 2002; Lal, Reicosky, & Hanson, 2007).

It is now empirically evident that conventional agricultural practices reduces biodiversity within the soil (Briones & Schmidt, 2017; House & Parmelee, 1985; Mathew, Feng, Githinji, Ankumah, & Balkcom, 2012; Nunes, van Es, Schindelbeck, Ristow, & Ryan, 2018) and furthermore a reduction in biodiversity leads to a reduction in an ecosystem's resilience to stresses (Balvanera et al., 2014; Byrnes et al., 2014; Loreau, Naeem, & Inchausti, 2002; Soliveres et al., 2016; Wagg, Bender, Widmer, & van der Heijden, 2014).

The Importance of Soil Health

Soil health has become an important factor in defining the sustainability of agricultural systems. However, there is still little evidence of how longer-term agricultural management influences soil health. Our understanding of biological indicators of soil health is less developed than indicators of soil physical and chemical properties, the aim of this study would be to improve that knowledge. Soil health underpins sustainable agriculture and provides ecosystem resilience to climatic stresses (Powlson et al., 2012). The UK's 25-year environmental plan has set out ambitious goals to manage resources more efficiently from nature, with a specific commitment to managing all UK soils sustainably by 2030. A further goal of the plan is to develop a soil health index to improve and restore soil health (DEFRA, 2018). In November 2020 the Agriculture Act 2020 was introduced in which the Secretary of State committed to providing financial assistance for "managing land or water in a way that protects or improves the environment". This commitment will be implemented over the next seven years starting with 2021 (Coe & Finlay, 2020).

Definition of soil health

Soil health is defined as the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (Doran & Zeiss, 2000). The definition helps emphasise that soil is a dynamic, life-sustaining ecosystem that supports multi-trophic food webs by providing food, fibre and shelter, as well as recycling nutrients, water and air for all life on earth. Retaining the health of soil is essential to protect this fragile resource and therefore thoughtful consideration needs to be given to the careful management of it.

Comprehensive soil health assessment tools (Andrews, Karlen, & Cambardella, 2004) have been developed in recent years integrating multiple types of measured data ranging from soil physical, chemical and biological components whereby inherent soil property information is able to provide assessments that are sensitive to land management practices and are strongly correlated with ecosystem services (Karlen, Veum, Sudduth, Obrycki, & Nunes, 2019).

Identifying and quantifying the benefits as well as adverse impacts of agricultural practices on soil resources is essential to develop guidelines and implement practices that will improve soil health, advance food security, and ensure environmental sustainability.

Soil Health Indicators

Soil health indicators are set from measurable soil physical, soil chemical and soil biological properties and attributes which relate to functioning soil processes and ecosystem services which can be used to evaluate the soil's health status, as affected by land management and climate stress drivers.

Role of Soil Physical Properties

The Importance of Soil Aggregate Stability

Soil aggregate stability is an important index of soil quality, and soil physical property affects soil productivity and sustainability. Soil aggregates are groups of soil particles that stick together as result of cohesive forces among particles and interaction of organic matter, cations, and anions with soil particles. Soil fungi produce a protein, Glycoprotein glomalin, sticky in nature and resistant to decomposition, that binds soil aggregates aiding resilience to erosion and desertification (FAO et al., 2020).

Aggregate stability indices define the capability of soil particles to oppose dispersion and degeneration of soil peds/clods. Tilling the soil exerts a series of disruptive forces that disintegrates soil particles, thus reducing stability index of soil (Alakukku et al., 2003; Blanco-Canqui, Wienhold, Jin, Schmer, & Kibet, 2017). Soil with a high stability index has been shown to be a more productive of producing a higher crop yield, while a low stability index, soil erosion losses are high. Soil compaction reduces formation of soil aggregates and becomes worsened when high axle loads are put upon soils with a high moisture content (Alakukku et al., 2003; Botta, Jorajuria, Balbuena, & Rosatto, 2004).

Soil Moisture

A soil's ability to store water plays a key role in managing the risk of flooding and agricultural run-off. It has also been associated with increasing flood risk; with soil erosion and degradation directly linked to an increase in the risk of flooding to downstream communities (CIWEM, 2019; European Commission, 2010; Graves et al., 2015). Soil moisture content also determines aggregate stability and tensile strength of soil aggregates (Six, Bossuyt, Degryze, & Denef, 2004).

Soil water content is the moisture that exists both in between soil aggregates (inter-aggregate pore space) within the pore spaces as well as within soil aggregates (intra-aggregate pore space) which is either completely filled with water (saturation) and or air (dry), but usually a combination of both. An optimum environment for plant growth and aerobic microbial activity would be where pores are predominantly filled with water, and a pocket of air remains in the pore space. In contrast, waterlogged (saturated) soils stimulate anaerobic microbial activity and can kill plants as water content within soil affects the amount of nutrients available to plants by acting as a transportation vehicle as well as influencing the presence of oxygen and the soil's aeration status (Susha, Singh, & Baghini, 2014). "Field capacity" is a term referred to when a soil holds the maximum amount of water in between and within soil aggregates after excess water has been drained away by gravity action (Santos, Borém, & Caldas, 2015). Soil moisture content can either be measured on a volume or mass basis. Gravimetric moisture content is the mass of water measured by subtracting the wet soil mass from the mass of the soil subjected to being oven dried (typically 105°C for 24 hours) and expressed as a percentage of the total weight (Susha et al., 2014). Volumetric moisture content is the percentage of water content based on a volume of soil and measured by subtracting the wet soil volume from the dry soil volume after being oven dried, expressed as a percentage of the total weight. Soil water content however is only one element characterising the status of water in soil, water potential must also be known (Cambell & Mulla, 1990). Water content refers to how much water is in the soil, whereas water potential refers to the amount of water available for plant uptake and microbial activity. Water is held in the soil by its adhesion to the surfaces of organic and mineral particles and by cohesion to itself which is responsible for surface tension. These forces combined with gravity forces affect the energy status within the soil. The energy status of soil affects how tightly water is held as well as which direction it moves (Voroney & Sharpe, 2019). When the soil pore space becomes filled with water it is referred to as being saturated. Soil water content however drops rapidly after rainfall due to gravity depending on soil texture, structure, and treatment, therefore its water holding capacity becomes an important factor for plants and soil biota which depend greatly on its presence. Water potential is the equivalent of the total soil pore space at saturation point which is available to plants and soil biota.

Water tends to move from areas of high potential to areas of lower potential and the gradient between those two areas becomes the driving force that governs water flow (Cambell & Mulla, 1990). Plants and microbes are only able to obtain water from the soil if an internal water potential can be maintained which is below that of the soil and since there is a limit to the lowest potentials attainable by living organisms, water intake may be restricted.

Soil moisture content has a dominant influence on root growth and a general response to sub-optimal availability is increased biomass allocation to roots at the expense of above ground growth. This in itself increases water capture and decreases water use – a negative feedback loop equating to a “functional equilibrium” (Lynch, Marschner, & Rengel, 2012). In dry conditions, the rainfall may not be able to saturate the soil profile and water tends to remain only available in the top-soil layers, however as soils dry, water usually remains in the deeper soil layers which prompt the plant to either invest in shallow and fine “rain roots”, or deeper roots all at the expense of shoot growth.

Role of Soil Chemical Properties

The soil profile is a natural organic pool of nutrients required for plants; however, they are in stabilised forms which are unavailable for plant uptake. The process requires the aid of the microbial community such as soil bacteria, fungi and actinomycetes to convert organic compounds to inorganic compounds that are water soluble and thus available for plant uptake. The plant's requirements vary throughout the growth cycle and therefore prompts the microbes to convert the required nutrients through the release of root exudates which feed and proliferate microbial populations, stimulating nutrient conversion (Richard D Bardgett & Wardle, 2010).

The nitrogen cycle is of particular ecological interest as it drives key ecosystem processes such as primary production and decomposition (Bernhard, 2010). Nitrogen is present in many forms namely inorganic (plant available) such as ammonium NH_4 (positively charged) nitrite NO_2 (negatively charged) nitrate NO_3, gaseous forms or organic forms such as nitrogen in living organisms, in humus, or in organic matter. There is a constant transformation of forms from one to the other. This is carried out by the microbial community either by storing stable forms of nitrogen within their bodies, or by directly harvesting its energy.

Bacteria or fungi convert organic nitrogen into ammonium in a process called mineralisation. The conversion of ammonium to nitrate is primarily performed by soil living bacteria and other nitrifying bacteria. This is referred to as nitrification which is a multiple step process involving microbial conversion from ammonium to ammonia, then nitrites to nitrates, and this is carried out by different groups of microbial organisms.

Phosphorus (P) together with nitrogen has been described as the main plant macronutrient that limits plant growth. Although P is abundant in many soils, its available form is low (Ohyama, 2017).

A plant requires a multitude of nutrients in varying ratios, however the macro nutrients required are nitrogen, phosphorus, potassium, and sulphate. Fertiliser application is a common way for nutrients to be made available to plants and is applied rapidly on a large scale to increase crop yields. However, if the plant does not utilise the compounds, they leach into the water course which is of great environmental concern. Assessing nitrate and ammonia levels in soils is a direct way to assess and consider the nitrification and denitrification process (Motavalli, Goyne, & Udawatta, 2008).

One of the most overlooked roles of soil is the storage and cycling of carbon, despite it being the largest carbon store (1600 billion tonnes) in directly accessible natural systems (Lal, 2016; Smith, 2005). This presents an opportunity to further store carbon within soil organic matter, thus displacing the burden of carbon storage from the limited capacity present in the atmosphere and hydrosphere (Environment Agency, 2019; European Commission, 2012).

Role of Soil Organic Matter

Soil Organic Matter (SOM), and more specifically the carbon content within SOM (SOC) is the basis of physical, chemical, biological, and ecological transformation in the soil, and is recognized as the most important soil health indicator (Doran & Parkin, 1997; Environment Agency, 2019; Lorenz, Lal, & Ehlers, 2019; Nunes, Karlen, Veum, Moorman, & Cambardella, 2020; Sustainable Soils Alliance, 2018). SOC along with the other soil biological indicators are essential for 1) maintaining soil structure and aggregation, which govern soil tilth and aeration; 2) ensuring water retention and water use efficiency, which control tolerance to drought, heat waves, and abrupt climate change; 3) nutrient recycling and retention, which moderate nonpoint source pollution and water quality; 4) regulating rhizospheric processes, which influence elemental transformations and creation of disease-suppressive soils; 5) control of gaseous emissions, which moderate atmospheric chemistry and regulate climate change; and 6) agronomic productivity (Lal, 2016).

Organic matter forms the fundaments of the soil food chain instigated by microbiological activity and interactions between plant and microbe (Lorenz et al., 2019; Nunes et al., 2020; T. Shah, Lateef, & Noor, 2020). The loss of organic matter not only affects the nutrient cycle in soil ecosystems, but has a cascading effect on the carbon released into the wider biosphere (FAO et al., 2020). Furthermore, high organic matter results in a higher stability index, higher soil quality and productivity, while lower organic matter contents in soil make soil more susceptible to soil compaction, erosion, biodiversity loss and climate regulation (Wortmann & Jasa, 2003).

Role of Soil Biota

Soil ecosystems are dynamic environments in which organisms are constantly interacting forming positive and negative feedback loops resulting in complex food webs regulating nutrient cycling (Hunt & Wall, 2002). The energy and nutrient flow within an ecosystem occurs across a wide range of scales and trophic levels, from above ground to below ground, that define the hierarchy of food webs (Steffan et al., 2015). The activity at one trophic level might influence the process happening at other trophic levels (Wollrab, Diehl, & De Roos, 2012). The correct functioning of the food webs is highly dependent on nutrient cycling, and bacteria are major engines of such substance turnover and biogeochemical cycles on the earth (Graham et al., 2016).

Species richness is the number of species found in a community or ecosystem. Species diversity is a measurement of species richness combined with evenness, meaning it takes into account not only how many species are present but also how evenly distributed the numbers of each species are (Colwell, 2009).

The diversity and abundance of species is directly and primarily dependent on the availability of SOC which instigates nutrient cycling by way of organic matter composition which are all influenced by and influential over soil structure, productivity integrity and composition (Plante & Parton, 2007).

Primary producers such as plants initiate carbon and nutrient cycles by way of releasing carbon through plant exudates. An exchange of nutrients takes place between primary producers and plant symbionts (bacteria & fungi). This in turn leads to food availability for microbial feeders (nematodes, arthropods), which in turn leads to food availability for predacious species (nematodes, arthropods & mammalian species). Alongside this, keystone species such as earthworms provide services such as soil structure enhancement, organic matter decomposition and nutrient cycling (FAO et al., 2020).

Thus, biodiversity at multiple trophic levels is imperative for ecosystem multi-functionality and subsequent loss leads to a reduction in ecosystem services provided (Byrnes et al., 2014; Soliveres et al., 2016; Wagg et al., 2014).

Furthermore, opportunist species (plant parasitic nematodes) constantly threaten symbiotic relationships and therefore an understanding of the drivers and curtailment criteria is necessary in building an understanding of the biological component when determining soil health.

It is important also to note that above-ground biodiversity does not follow below-ground biodiversity (FAO et al., 2020) and subsequently the aim of this research is to consider the below ground communities from a multi-trophic scale, and how the impact of agricultural disturbances such as tillage influence their composition.

Any bioindicator that can easily and rapidly measure the effects of land management impacts is able to contribute to ecosystem management and improve its sustainability. Many studies stress that the main traits required in an indicator taxon are environment sensitivity, impact responsiveness, ubiquity, functionally important in an ecosystem and representative i.e. able to act as surrogate for other taxa, as well as easy to sample, identify and assess (Greenslade, 2007; Mekonen, Petros, Hailemariam, & Conservation, 2017; Menta & Remelli, 2020).

Macrofauna

Since being described as “nature’s plough” in 1881 by Charles Darwin, earthworms (Lumbricina) have been referred to as a keystone species within soil ecosystems and are suggested by many (Le Bayon et al., 2017; Liu et al., 2019; Van Groenigen et al., 2014) as a key bioindicator of soil health. They have been popularised as a prominent study organism when determining soil ecosystem stability (Blouin et al., 2013; Bostroem, 1988; Fox, Miller, Joschko, Drury, & Reynolds, 2017; Le Bayon et al., 2017; Peigné et al., 2009), and although this ecosystem engineer plays a vital and varied role within the soil, it certainly does not work in isolation, nor is it self-reliant. Earthworms are decomposers, and are not just reliant on organic matter as a food source (Brown & Doube, 2004) they also rely on the nutrients as well as the organisms within the soil for their own survival (Kawaguchi, Kyoshima, & Kaneko, 2011). They rely heavily on a ”hands off” approach, showing a reduction in population and diversity presence under high intensity farming practices such as conventional tillage (Kemper, Schneider, & Sinclair, 2011) and agrochemical inputs (Chan, 2001). A cascading effect has to occur within the soil food web whereby lower trophic levels such as nematodes, protozoa and micro-arthropods are in supply for their food source to exist (Satchell, 1980). Their burrows not only provide aeration and drainage within the soil, but also provide biological hotspots for lower trophic levels known as the “driloshpere” (Andriuzzi, 2015).

Diversity, not just abundance of species is important as earthworms also fulfil different ecological functions within the soil. Each ecological group (endogeic, epigeic and anecic) have distinct burrowing, feeding and casting (excretion) habits, therefore they have distinct functions they perform within soil ecosystems (Satchell, 1980). As not all earthworms are uniform in their contribution to the ecosystem functionality, it is important that an ecosystem consists of all functional group types. Over and above nutrient cycling, decomposition and microbial dispersal, endogeic species are least sensitive to disturbances and most prevalent across agricultural landscapes (Briones & Schmidt, 2017). They have short life cycles and high fecundity rates and recover soil quickly after disturbance (Van Groenigen et al., 2014). Epigeic species are surface dwelling earthworms with a litter only diet and incorporate organic matter into the topsoil through their casts (Kawaguchi et al., 2011). Anecic species are k-strategist with long life cycles (up to 10 years), slower maturing rates and most sensitive to soil disturbances due to their size. They ensure soil structure maintenance, aeration, water filtration as well as being found to ameliorate the disturbance of intense rain on plants thus providing evidence that soil biodiversity is likely to contribute to overall ecosystem stability (Andriuzzi, 2015; Andriuzzi, Pulleman, Schmidt, Faber, & Brussaard, 2015).

Meta-analysis studies show that abundance has a high negative correlation with disturbance (Bostroem, 1988; Briones & Schmidt, 2017; Chan, 2001; Fox et al., 2017; Van Groenigen et al., 2014) showing clear evidence that CT negatively affects earthworm abundance, in comparison to NT. However, conflicting results are reported such as in a global meta-analysis (Briones & Schmidt, 2017), which included extensive observations over 65 years across 40 countries. These were attributed to site-specific variability in soil properties, climatic conditions, and agrochemical inputs. Although overall it shows that disturbing the soil less (NT) significantly increases earthworm abundance compared to CT, population responses were more pronounced under minimised tillage (MT) for periods more than 10 years, in warm temperate zones, and in clay soils with pH below 5.5. A retention of crop residue amplified this response to MT. The use of herbicides did not have a significant affect. Epigeic and Anecic groups displayed highest sensitivity to CT, particularly the species *Lumbricus terrestris* which responded most positively to MT.

Mesofauna

Small invertebrates such as collembola (springtails) and acari (mites) – known as micro-arthropods - mainly inhabit topsoil and act as biological regulators by feeding on decaying vegetation, bacteria, fungi as well as other soil organisms. They form a crucial link in the soil food web by activating microflora by fragmenting organic matter and boosting available nutrients (FAO et al., 2020).

Acari are an integral part of soil food webs, and span several trophic levels including fungivores, bacterivores, detritivores and (top) predators. One of the better-studied groups of soil acari are Oribatida which are often most abundant in soils rich in organic matter such as forest soils. Oribatida feed on soil fungi, bacteria, yeasts, algae, lichens and decomposing plant detritus; some facultative predation is also seen among some species in this group (Schneider et al., 2004).

Studies using stable isotopes (13^{C} and 15^{N}) show that bodies of soil mites are enriched in root-derived C and N, suggesting that much of the soil food web is fuelled by nutrients entering the soil from below ground as roots/root exudates as well as above-ground leaf litter (Scheunemann, Digel, Scheu, & Butenschoen, 2015; Zieger, Ammerschubert, Polle, & Scheu, 2017). These nutrients are taken up through the consumption of soil microflora which is passed to microbivores and then to predators feeding on microbivores, or on root-feeding nematodes.

Predatory mites specifically play a role in carbon transformations through their top-down effects on the herbivorous and decomposer fauna on which they feed, as well as through respiration (Ruf, 1998).

Collembolans are likely to be good indicators for soil conditions because they are widely distributed (Hopkin, 1997). Due to short life cycles of the species; composition and abundance of collembolan communities are expected to rapidly adapt to and reflect environmental changes. This response might be further enhanced through their function as secondary decomposers, feeding on fungi and microorganisms, which links them closer to the environment than predatory or herbivorous animals (Greenslade, 2007).

Collembola are among the most diverse, abundant and functionally important of soil organisms (Potapov et al., 2017) and an equally integral part of the soil food web as they directly and indirectly regulate the soil microbial activity and nutrient cycling. The majority of collembola feed on decaying plant material or fungal hyphae and have been shown to influence mycorrhizae growth and reduce fungal diseases in some instances (Hopkin, 1997). Through their population density, their feeding activity, grazing intensity and their role in dead organic matter decomposition; collembola can directly alter nutrient cycling and participate actively in humus formation. Most soils contain high volumes of collembolan faecal pellets which slowly release essential nutrients to plants when broken down by microbes (Hopkin, 1997). Due to their intimate connection with the decomposition process, it has been argued that they are more sensitive to environmental stresses applied to ecosystem function compared to predatory or herbivorous animals (Greenslade, 2007). Collembola influence microbial species composition and biomass and indirectly impact mineralization rates that can lead to greater leaching and export of nutrients (FAO et al., 2020). They are also generally considered to be beneficial as few collembolan species feed off actively growing plants (Richard D. Bardgett, Hopkins, Usher, & British Ecological, 2005). Collembola also have short life cycles and therefore are likely to respond rapidly to environmental changes. Collembola are not social animals, so quantitative sampling would not be biased based on strong aggregation factors. Moisture content in soil is predominant factor when determining distribution; high moisture levels are favourable to collembola and a lack of it is often serious resulting in desiccation (Christiansen, Bellinger, & Janssens, 2009). Collembola resist desiccation by moving into microenvironments of high humidity. Competition amongst species in laboratory cultures, even under long-term and competitive conditions, has at least in a few instances shown no evidence of competitive exclusion. Furthermore, it has been shown that such interactions can either render a positive or a negative result depending on the interaction (Christiansen et al., 2009). Collembola are separated taxonomically by their morphological differences which have clear functions in relation to their feeding and habitat requirements including position in soil profile (Petersen, 1994).

Microfauna

Nematodes are viewed as a useful group of microfauna for community indicator analysis due to more information existing on their taxonomy and feeding roles than exists for other microfauna (Gupta & Yeates, 1997). Furthermore, they form a central part of the food web as they represent multiple trophic levels including primary (plant parasitic herbivores), secondary (bacterivores and fungivores) and tertiary (predators and omnivores) feeders (Yeates, Bongers, De Goede, Freckman, & Georgieva, 1993).

Bacterivores, fungivores, and herbivores are considered as nematode trophic groups in the lower hierarchy of the soil food web; and predators and omnivores are considered as nematode trophic groups in the higher hierarchy of the soil food web (Levin, 2014).

Bacterivores and fungivores promote N and C mineralization by feeding on decomposing bacterial and fungal biomass. In controlled experiments conducted by Coleman et al. (1984) more nitrogen was found to be available in the ammonium form when bacterivorous and fungivorous nematodes were present than when they were absent.

Predators and omnivores maintain ecological balance between decomposition and mineralization by regulating bacterivores and fungivores (Ingham, Trofymow, Ingham, & Coleman, 1985). In addition, predators act as biocontrol agents by feeding on herbivorous nematodes which are more commonly known as plant parasites (Bilgrami & Brey, 2005).

The composition such communities provide valuable information regarding the soil health since nematodes are predominantly specific in their food preferences and abundant in decomposition habitats (Bongers & Bongers, 1998). Many useful indices for nematode faunal analysis have been developed based on trophic groups and colonizer-persister (c-p) scale (Bongers, 1990) which ranks nematodes based on their life history characteristics and survival strategies (life span, fecundity strategies and size). Consequently, nematodes can be used as indicators of structure and function of soil food webs and overall ecosystem conditions (Pothula, Grewal, Auge, Saxton, & Bernard, 2019). Nematode diversity can reflect soil biodiversity, soil resource diversity, and resource utilization diversity, and can serve as a useful indicator of the nutrient status of the ecosystem and the structure of the soil food web (Li et al., 2019).

A plethora of published literature exists on how different ecosystems affect the abundance and species richness of nematodes (Bilgrami & Brey, 2005; Bongers, 1990; Bongers & Bongers, 1998; Ferris, Bongers, & De Goede, 2001; Li, Peng, & Zhao, 2019). However, there is no single consensus in the published literature about the pattern of nematode abundance and richness in different ecosystems of varied disturbance. Some authors have reported that diversity is high in undisturbed ecosystems such as forests, while abundance is high in disturbed ecosystems such as agriculture (Cardoso et al., 2015; Ferris et al., 2001; Yeates, 2007; Yeates & Bongers, 1999); but others have stated the converse (Briar et al., 2007; Darby, Neher, & Belnap, 2007; Kimenju, Karanja, Mutua, Rimberia, & Wachira, 2009; Neher, Wu, Barbercheck, & Anas, 2005). A meta-analysis by Pothula et al. (2019) revealed that overall richness of nematodes were highest in undisturbed ecosystems when compared to disturbed ecosystems. Overall nematode abundance was similar in all ecosystems except highly disturbed ecosystems. Although it is argued that high nematode abundance represents high productivity of the ecosystem (Ritz & Trudgill, 1999), the high abundance in disturbed ecosystems was mostly attributed to high abundance of lower trophic groups known as colonisers and visa-versa. High abundance of upper trophic groups (persisters) was found in less disturbed ecosystems. The abundance of bacterivores, fungivores and herbivores was highest in disturbed ecosystems, whereas the abundance of predators and omnivores was highest in undisturbed ecosystems. Perturbations in an ecosystem may increase the abundance of trophic groups in the lower hierarchy of soil food web (bacterivores, fungivores, and herbivores) but decrease the abundance of nematode trophic groups in the higher hierarchy of the soil food web (predators and omnivores), which play a crucial role in regulating the lower groups including herbivores. Therefore, losing these regulators may be detrimental to nutrient cycling dynamics and agricultural management (Pothula et al., 2019). The results in a recent study showed that significant differences in nematode diversity among land use types were detected by assessment at the order level but not at the family or genus level. The results also showed that significant differences in nematode diversity were better revealed by assessment of trophic groups rather than cp (coloniser-persister) groups. (Li, Peng, & Zhao, 2019)

Threats to Soil Health

Quantifiable soil degradation costs ranged between £0.9 bn and £1.4 bn per year, with a central estimate of £1.2 bn, mainly linked to loss of organic content of soils (47% of total cost), compaction (39%) and erosion (12%) in England and Wales (Graves et al., 2015). Soil health is most threatened by degradation such as erosion, compaction and decline in organic matter and most threats to soil health are directly related to anthropogenic induced changes which include amongst others, agricultural intensification. Intensification of land management practices lead to loss of soil organic matter/carbon, nutrient imbalances, soil compaction and erosion, all of which lead to further soil degradation (FAO et al., 2020). In a global synthesis carried out by the Food and

Agriculture Organisation, the most widespread threat to soil biodiversity is the loss of SOM and SOC which are in return directly linked to land use change as well as with climate change. This clearly highlights the importance of considering the impacts land management practices have on the sustainability of the world's soil resources.

Impact of Land Management Practices

Biological indicators of soil health can change rapidly depending on the agricultural practices and land use decisions adopted (van Es & Karlen, 2019). Traditional land cultivation techniques such as ploughing potentially destabilises soil aggregates, displaces/destroys soil biota, oxidises soil carbon and exposes topsoil to wind and water erosion through continual disturbance by of primary and secondary mechanical tillage. Conventional tillage (CT) for example has been shown to negatively impact microbial communities by disrupting hyphal networks, thus reducing substrate availability, stabilisation of surface area and a reduction of enzyme activity (Holland & Coleman, 1987; Lützow et al., 2006). CT has also been shown to reduce aggregate stability, promote surface crusting and increase soil erosion (Baumhardt, Stewart, & Sainju, 2015). In contrast, reduced tillage practices such as minimal tillage (MT) and no tillage (NT) such as direct drilling (DD) practices have been shown to increase SOM and improve soil health with measurable benefits to biological, physical, and chemical properties (Nunes et al., 2018; Sharma, Singh, & Singh, 2013).

Complementary conservation practices such as incorporating cover crops or perennial cropping systems have been shown to enhance reduced tillage effects on soil health indicators by way of increasing soil's exposure to living plants and root systems as well as reducing wind- or runoff-induced erosion, and thus increasing both the quantity and quality of SOM (Nunes et al., 2018; Veum et al., 2015) which in turn stimulates micro- and macro-organism activity, leading to increased soil carbon (C) and nitrogen (N) inputs. Increased SOM under diversified cropping systems also stimulates soil micro- and macro-organism activity due to an increased supply of root exudates, nutrients, and oxygen (Kumar, Dorodnikov, Splettstößer, Kuzyakov, & Pausch, 2017), thus enhancing biological soil health more than reduced tillage alone (Nunes et al., 2018). Returning crop residues in conjunction with reduced tillage can also enhance soil health due to increased soil C and N inputs (Schomberg, Ford, & Hargrove, 1994).

However, reducing tillage intensity has not been uniformly positive as shown in studies such as Blanco-Canqui et al. (2017) and Nunes et al. (2017) where direct drill - even in the long term - had limited effects on soil hydraulic properties. Blanco-Canqui et al. found CT increased ponded infiltration rates (positive soil water pressure and tension) significantly when compared to no till and Nunes et al. found percolation rates were slower in no till when compared to CT irrespective of the soil layer. Other studies reported NT increased soil compaction in clay soils, with the 7- to 20-cm layer being characterized by high bulk density, high root penetration resistance, reduced air and water permeability, and limited crop root growth (Nunes, Denardin, Pauletto, Faganello, & Pinto, 2015; Reichert, Suzuki, Reinert, Horn, & Håkansson, 2009) all factors known to affect biological soil health.

Although there is widespread belief that reduced tillage favours C sequestration, others have concluded it may be an artefact of sampling. According to Baker et al. (2007), sampling the entire soil profile generally shows no cumulative C sequestration advantage for reduced tillage practices, and in fact, often finds greater C stocks in conventionally tilled systems. Similarly Lou, Wang & Sun (2010) found that changing from CT to NT did not increase SOC. Both studies highlight inconsistencies and question whether NT really enhances C sequestration in agricultural soils. Previous meta-analyses (Luo et al., 2010) have been conducted to quantify tillage intensity effects on SOC, but most studies have primarily focused on two tillage intensities (NT *vs.* CT) and have generally examined only a few soil depth increments.

In a review conducted by D'Hose et al. (2018) on impact of non-inversion tillage on soil biota across Europe, they found a large variety of non-inversion tillage techniques reported in the published literature. They distinguished three tillage classes based on tillage depth: no tillage (NT), shallow non-inversion tillage (SNIT) comprising all tillage practices with a maximum working depth of 15 cm and deep non-inversion tillage (DNIT) with a working depth greater than 15 cm.

In the latest Farming Practices Survey 2021, cultivation practices were not part of the questions asked, nor in the greenhouse gas emissions survey, it was asked in 2010, but that appears to be the last time information about cultivation practices and specifically RT preferences were recorded.

In 2010 the status of RT in UK indicated that 4% of land was cultivated using direct drilling, 40% of reduced tilling and 56% on inversion tillage (CT).

Soil type can have a substantial influence on the impact of cultivation method for example self-structuring clay soils are better suited to non-inversion tillage as it reduces the risk of creating very dry cloddy seedbeds in wet conditions which can prohibit crop establishment. In contrast sandy soils tend to form a tightly compacted angular structure, restricting the movement of air and water which are key factors affecting soil health - through the soil profile. Therefore good drainage and a naturally stable structure is required to avoid soil compaction making calcareous soils clay soil more suited to RT as they "self mulch" (Powlson et al., 2012) compared to sandy soils (Morris et al., 2010). RT has also been shown to require greater herbicide use to control weeds (Melander et al., 2013).

Erosion

Soil erosion is one of the major threats to soil health and global water quality and is accelerated by unsustainable land management practices, particularly intensive agriculture (Lee, Chu, Guzman, & Botero-Acosta, 2021). Erosion of soil is a natural occurrence that impacts all landscapes as soil particles are detached and transported by erosive forces such as wind and water. In agricultural settings, this process is manifested as an accelerated displacement of topsoil that can negatively impact soil's fertility. In addition, it facilitates the transport of nutrients as they carry organic and inorganic material through watercourses which impair water bodies by nutrient, microbial and toxic pollution (Montanarella et al., 2016). Specific intensive practices such as conventional tillage have been identified as a major sources of soil erosion and important consideration is given to more conservative practices which include reducing the intensity of tillage (Montgomery, 2007). Erosion rates specifically from reduced tillage and direct drill systems have been shown to mimic natural soil erosion rates due to the absence of heavy and repeated mechanical operations that further disturb and disrupt the soil ecosystems (Cerdà et al., 2020; Montgomery, 2007). Direct drill and reduced tillage practices leave the soil virtually undisturbed between harvests which have been shown to improve water holding capacity, improve long term crop production, increase organic matter content in addition to reducing fuel, labour, and machinery costs (Claassen, Bowman, McFadden, Smith, & Wallander, 2018); potentially reducing nutrient loss through leaching and runoff (Soane et al., 2012). In this context, reduced till farming has the potential to ensure long-term productivity of agricultural farming whilst promoting environmentally friendly and sustainable practices (Visser, Keesstra, Maas, & De Cleen, 2019).

Soil Compaction

Soil compaction reduces agricultural productivity and intensity of agriculture appears to be correlated with compaction (Botta et al., 2004; Nawaz, Bourrie, & Trolard, 2013). Reasons appear to be high mechanical surface load, mono-crop plantations, intensive grazing, irrigation, low organic matter, animal trampling engine vibrations and high moisture content (Alakukku et al., 2003; Håkansson & Reeder, 1994; Nawaz et al., 2013). Soil compaction increases bulk density, decreases porosity, aggregate stability index, conductivity and nutrient availability thus reducing soil health and a reduction in soil health results in lowered crop performance via stunted shoot and reduced root growth (A. N. Shah et al., 2017).

According to a recent review soil moisture content appears to be the most influencing factor that makes soil susceptible to compaction; the reason being, is an increase in moisture content decreased macropore spaces thus leading to load support decline (A. N. Shah et al., 2017).

Soil moisture contribution to soil compaction is dependent on deformability of soil, precompression value, stress dissemination ability, and mechanical contact area depending on optimum soil moisture limitations as the drier the soil, the lower the stress transformation and subsequently the lower the deformation in soil structure (Batey, 2009). Furthermore, a decrease in macropores resulted in the development of anoxia conditions, thus interferes with crop growth and development. Soil compaction reduces pore spaces and consequently inhibits the transfusion and transportation of air and water within soil profile as well as its water retention abilities (Dexter, 2004). Alteration in pore size distribution due to compaction resulted in increased runoff, decreased infiltration, and high erosion losses. The destruction effect of compact zone has also been reported (Koch, Heuer, Tomanová, & Märländer, 2008), pointing out that compacted zone negatively affects macropore volume and air permeability of the topsoil (0.05–0.1 and 0.18–0.23 m) and subsoil (0.4–0.45 m) layers.

Loss of Organic Matter

Organic matter/residues on soil surface have been shown to cushion the effects of soil compaction (Hamza and Anderson 2005). A layer of surface crop residues might be compressed under compressing action of heavy machineries, but they can retain their shape and structure once the traffic has passed, therefore organic residues may act like a sponge that can be compressed but comes back to its normal shape.

Organic residues in soil profile are more important than on the surface, as this organic matter attached to soil particles especially clay particles bind micro-soil and macro-soil aggregates, thus preventing soil from becoming compacted by the action of heavy machines. Conclusively, soil organic matter is a very important soil property, which can determine the magnitude of soil compaction. More soil organic matter make soils less susceptible to soil compaction (FAO et al., 2020; Six et al., 2004).

Loss of Biodiversity

The continuous modification of topsoil by way of altering and destroying microhabitats leads to a one-sided selection of soil biota whereby if thresholds (tipping points) of resilience are exceeded, a regime shift occurs leading to the degradation of the soil's health status (Vogel et al., 2018). Soil organisms across the food web play integral roles in decomposing organic matter, aiding nutrient recycling, creating habitats.

The most recent study conducted by the IES reveals a collective interdisciplinary understanding that soils are interconnected with all ecosystems as soil plays a crucial role in buffering other natural systems, therefore its health has direct and indirect consequences across the globe (Lewis, 2020). Soil biodiversity supports several ecosystem functions simultaneously, underpinning its crucial role in ecosystems worldwide (Bender, Wagg, & van der Heijden, 2016). Optimal function of such ecosystems require maintenance and indeed enhancement of soil biodiversity.

Traditional soil testing primarily focussed on chemical composition (i.e. pH and nutrients) to determine soil fertility and a transition has been made in recent years to consider soil functionality and the ecosystem as a whole – in other words its multi-functionality (Doran, 2002; Kibblewhite, Ritz, & Swift, 2008; Soliveres et al., 2016). This transition is important since the traditional narrow focus on soil chemistry and plant nutrition may have unintentionally contributed to soil physical and biological degradation (Andrews & Carroll, 2001).

Climatic Stresses

In the UK, extreme weather poses a great risk to future food production and many farmers are concerned about the impact higher temperatures, changing patterns of rainfall, increased evaporation and reduced water availability will have on crop production (Knox, Morris, & Hess, 2010). An increase in wet autumns will also constrain agriculture production by adversely affecting the timing of farming operations and could also directly exacerbate soil compaction and erosion. Furthermore there is considerable uncertainty as to the severity of these extreme weather events as well as the rate of field recovery following perturbations (UK Climate Resillience Programme, 2021). The hot dry summer of 2018 that proceeded a cold late spring contributed to 6% lower wheat yields and 10% lower spring barley yields (5 year comparative average) but slightly above average yields for oilseed rape (NFU, 2019). In 2019 a year of extreme weather was experienced; record-breaking heat and rain, with autumn and winter being exceptionally wet resulting in operational delays and real-time harvest losses for wheat, oilseed rape and barley down on average 15% (Centre for Ecology and Hydrology, 2020; DEFRA, 2019). In the DEFRA (2020) report, harvested production of wheat decreased by 40% when compared to the previous year and although a global health pandemic was at play, 2020 was another year of extreme weather by being both warmer and sunnier than usual as well as being one of the wettest years on record resulting in flooding in many areas.

Current Knowledge Base on Soil Biota

Current knowledge accepts that although there is a lack of understanding as to the dynamics of soil community interactions and the weight they bear on individual ecosystem functions i.e. what the baseline of functionality is required in soil ecosystems (Byrnes et al., 2014), what is empirically accepted is that intensity of land management is highly correlated with a reduction in community composition and furthermore that reduction in biodiversity reduces ecosystem's resilience to climatic stresses (FAO et al., 2020).

The aim of this study is to investigate which biological factors are good determinations of soil health as our understanding of biological soil health indicators are less developed than physical and chemical indicators. Furthermore, the study will help improve knowledge on how longer-term agricultural management influence soil biodiversity. The study will look to explore the impact of tillage on soil health, identify gaps in the knowledge. In addition, it will look to the impact long-term tillage treatments have on soil micro- and mesofauna diversity, activity and abundance as well as investigate the impact of tillage intensity on earthworm and macrofauna populations.

Methods

Site Description

This study was conducted in an experimental field comprising 18 plots (100m x 30m) with a total trial area of 5.4ha (figure 1). The field was previously owned by the Royal Agricultural University in Harnhill Manor Farm, Cirencester, UK (51°42′ N, 01°59′ W). The field's altitude is 135m above sea level, and the soil series is Evesham 1 (411a) (National Soil Resources Institute, 2020) with a clay texture (22% sand, 38% silt and 40% clay) (Rial-Lovera, Davies, Cannon, & Conway, 2016). The trial was established in 2010 and managed organically for 2 seasons, after which it was managed non-organically with a seasonal rotational crop system of winter oilseed rape, winter wheat and winter barley. The last agrochemical input (glyphosate) was July 2020, after which the field was converted to organic crops under the instruction of the new owners to a diverse legume and herb rich sward, sown on the 7th of September 2020 as a fertility building ley (table 2).

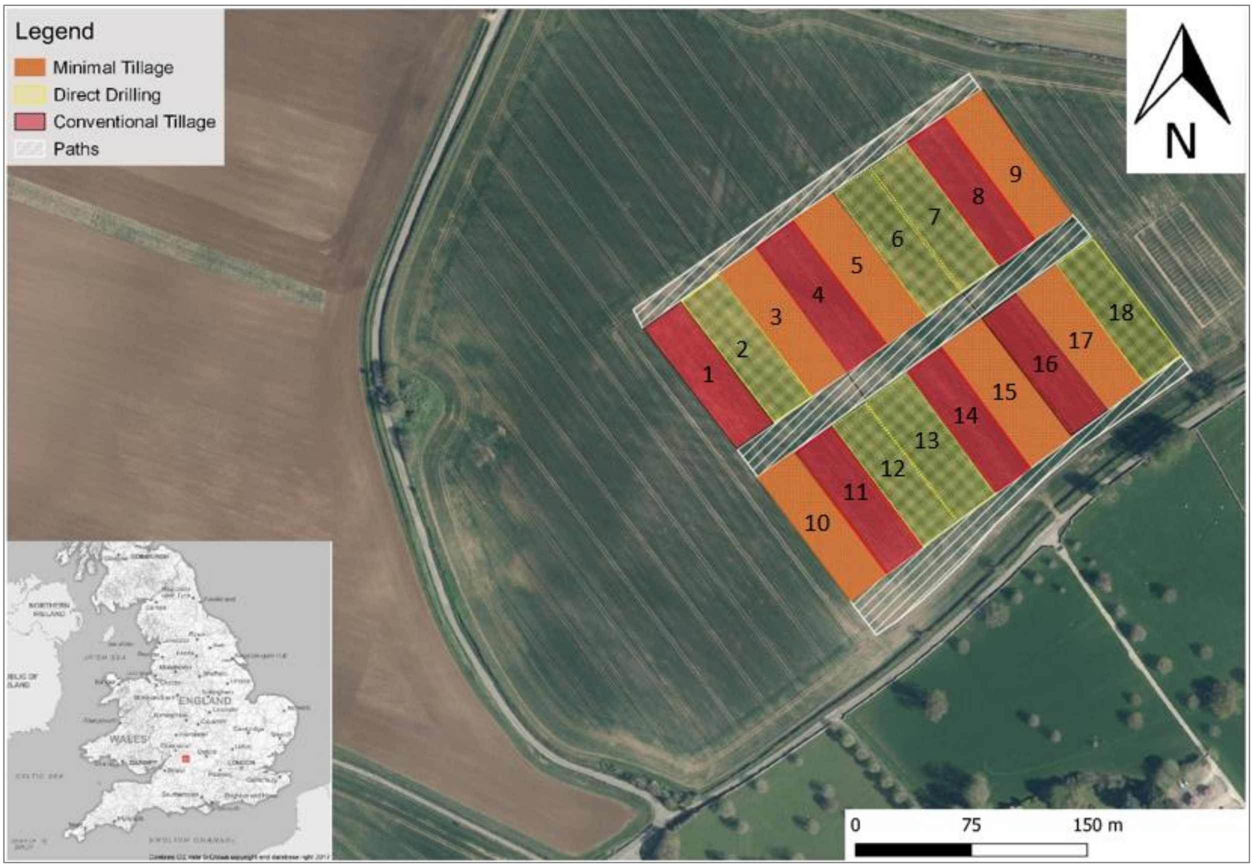


Figure 1: *GIS site map created in QGIS detailing location, sample plots by tillage type; conventional tillage, minimal tillage, direct drilling and tractor pathways. Main map: High Resolution (25cm) Vertical Aerial Imagery [JPG geospatial data], Scale 1:500, Tiles: sp0600, sp0601, sp0700, sp0701, updated: 29 October 2018, Getmapping, Using EDINA*

Aerial Digimap Service, https://digimap.edina.ac.uk, Downloaded: 2020-01-20 07:39:58.242. Insert map Contains OS data © Crown Copyright

.

Table 2: Seed mixture composition - legume and herb-rich sward

Species	Composite
Certified HUSAR ORGANIC cocksfoot	12%
Certified Calibra tetraploid perennial ryegrass	12%
Certified AberGreen perennial ryegrass	5%
Certified Polar King Timothy	10.9%
Certified Pardus meadow fescue	5%
Certified Fawn tall fescue	4%
Certified Dawn alsike clover	1.5%
Certified Milvus red clover	5%
Commercial sweet clover	3.5%
Certified Virgo Pagblerg yellow trefoil	0.8%
Commercial Sainfoin	20%
Certified Luzelle lucern - RHIZOBIUM INNOCULATED	2.5%
Certified Toro birdsfoot trefoil	3%
Commercial Lacerta chicory	4%
Burnet forage herb	7.2%
Sheeps Parsley forage herb	2%
Ribgrass forage herb	1.5%
Lesser Knapweed (Centaurea nigra) wildflower	0.1%

Weather Data

The RAU campus weather station (51^{o}42'N, 01^{o}54'W) records daily local rainfall and atmospheric temperatures. Figure 3 indicates minimum, maximum, and mean temperatures for June 2020 to June 2021 and figure 2 indicates total rainfall. Table 4 lists average monthly rainfall and temperature for each defined stage in data collection intervals (see Sampling Intervals).

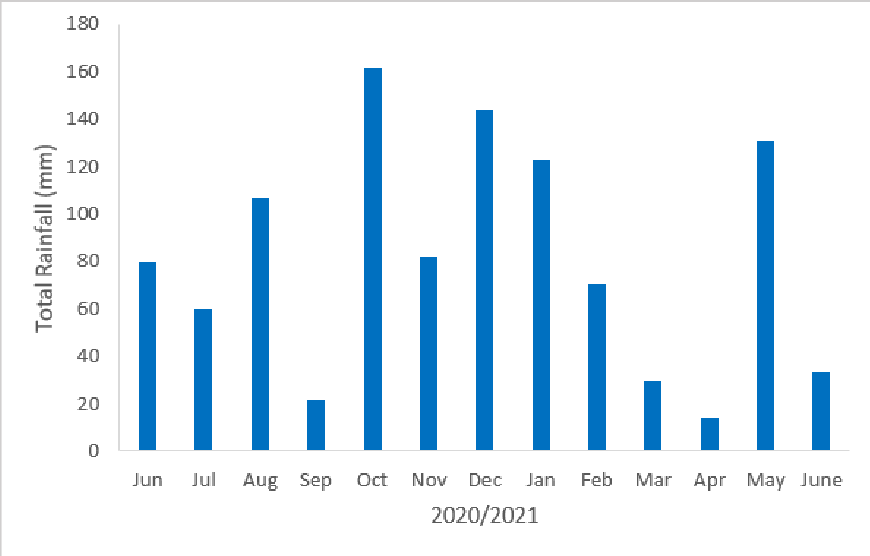


Figure 2: Total rainfall measurements in millimetres taken at Royal Agricultural University campus weather station for June 2020 to June 2021.

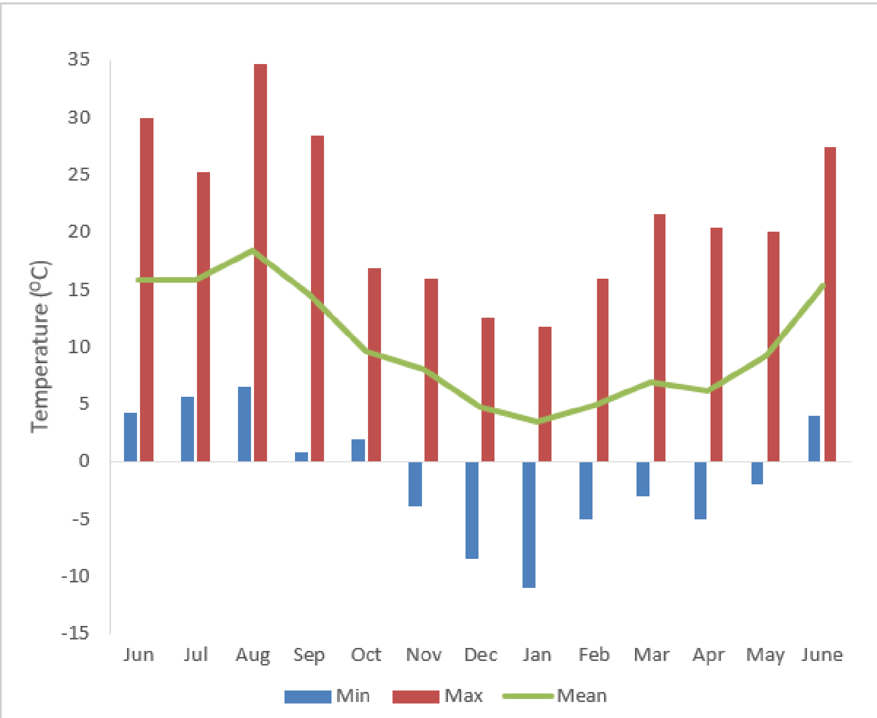


Figure 3: Minimum, maximum and mean temperature measurements in degrees Celsius taken at Royal Agricultural University campus weather station for June 2020 – June 2021.

Plot Treatments

The trial is a randomised complete block design replicated six times (figure 4). Each block (90 m × 100 m) is divided into three tillage treatments of 30 m × 100 m (main plots) – Conventional plough-based Tillage (CT); Minimal Tillage (MT); and Direct Drilling (DD).

Details of the tillage treatments used are specified in table 3. All plots were subsoiled in July 2019 due to concerns over compaction after heavy rainfall in that year.

A			B			C		
1	2	3	4	5	6	7	8	9
CT	DD	MT	CT	MT	DD	DD	CT	MT

D			E			F		
10	11	12	13	14	15	16	17	18
MT	CT	DD	DD	CT	MT	CT	MT	DD

Figure 4: Diagram of plot arrangements, letters A-F indicating blocks, numbers 1-18 indicating individual plots and CT,MT & DD indicating tillage type in randomised design.

Table 3: Tillage implemented at Harnhill Quary Farm

Tillage Type	Pre-Cultivation Treatment	Primary Cultivation		Secondary Cultivation	
Conventional Tillage (CT)	Glyphosate Spray	Five furrow Kverneland reversible plough harrow drill (18cm)	Cambridge Roll	Five furrow Kverneland reversible plough (18cm) + Cambridge roll + Kuhn power harrow combination seed drill (8cm)	Cambridge Roll
Minimal Tillage (MT)	Glyphosate Spray	2 passes of Horsch Jocker (7.5cm) disk cultivator	Cambridge Roll	Horsch sprinter drill (8cm)	Cambridge Roll
Direct Drilling (DD)	Glyphosate Spray	1 pass of Moore Unidrill (7.5cm)		Horsch sprinter drill (8cm)	Cambridge Roll

Sampling Intervals

Sampling intervals were divided into four stages and referenced either by stage (1-4) or by season (summer, autumn, winter, spring) which are interchangeable throughout the thesis. Details of environmental factors such as field activity, average temperature and rainfall are detailed in table 4.

Three samples were randomly taken from each of the 18 plots for each of the methods conducted (see Methods section). During stage 4 all variables were sampled from the same $1m^2$, selected at random from pre-determined segments (top left, middle right, bottom left) from each plot, whilst stages 1-3 variables were sampled from each plot in succession of one another.

Table 4: Sampling strategy design

Stage	Reference	Dates	Field Activity	Average Rainfall (mm)	Average Temperature °C
1	Summer	17/08/20 - 11/09/20	Post field cultivation and herbicide treatment. Pre seed-sowing of plots	2.4	16.05
2	Autumn	14/09/20 - 28/10/20	Post seeding of plots	6.7	14.6
3	Winter	22/11/20 - 11/12/20	Fully developed plants with flowering visible	3.6	3.75
4	Spring	01/04/21 - 01/05/21	Post grazing of sheep	0.5	7.7

Table 5 below provides a summary of the sampling methods conducted by category (physical, chemical, and biological) and stage.

Table 5: Sampling methods conducted summarised by category and stage

Stage	Physical							Chemical				Biological					
	Moisture Content			Soil Structure			Temperature	Carbon Content	Nitrate	Ammonium	Phosphate	Organic Content		Macro-Fauna		Meso-Fauna	Micro-Fauna
	Oven Dry	Visual Assess-ment	Moisture Meter	Visual Assess-ment	Penetro-meter	Bulk Density	Thermometer	Organic Content Equation	Automated Flow Segmented Analyser			Loss on Ignition	Visual Assess-ment	Hand-sorting	Mustard Extraction	Berlese-Tullgren Funnel Extraction	Wet Tray Extraction
1								✓	✓	✓	✓	✓		✓	✓		
2	✓	✓	✓	✓		✓	✓	✓				✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓		✓	✓	✓				✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓		✓	✓				✓	✓	✓	✓	✓	✓

Soil Physical Properties Methods

Moisture Content

Oven Dry Method

Three random cored samples from each plot were taken with an intact soil corer (8.8 x 6.3cm) from the topsoil profile at stage 2, 3 & 4. Samples were weighed ensuring all twigs, stones and visible roots were removed and oven dried at 105°C for 24 hours. Samples were allowed to cool in the oven and then weighed again. Gravitational moisture content - water content by mass - was calculated using standardised methods (Radojevic & Bashkin, 2007) in percentage using the following equation:

Water content (%) = 100 x $(M_1-M_2)/M_1$ where M_1 is the initial weight and M_2 the final weight.

Volumetric moisture content - water content by volume - was calculated using the gravimetric method whereby dry bulk density results from stages 2 & 3 were multiplied by their water content mass ($(M_1-M_2)/M_1$) as described by Cambell and Mulla (1990).

Visual Assessment Method

Three random samples from each plot were taken at Stages 2,3 & 4 using a 20cm^3 mechanical extraction and graded based on the moisture content of the soil profile from 1 (low) to 5 (high). This is an experimental approach to assessing moisture content and method was adopted and adapted based on the Visual Evaluation of Soil Structure method as described by Ball et al. (2007).

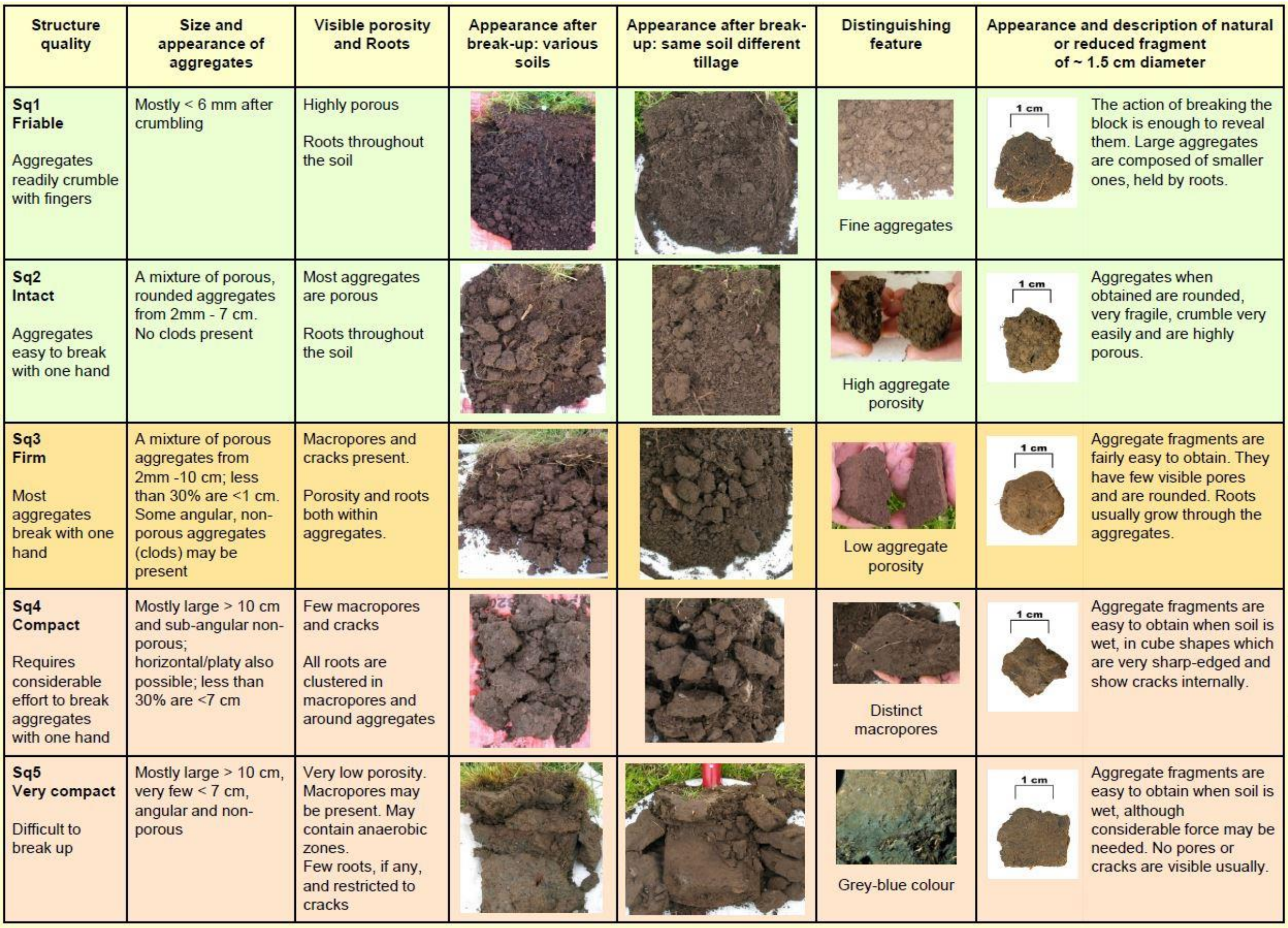
Moisture Meter Method

A soil moisture meter (Lutron PMS-714, Lutron Electronic Enterprise Co Ltd) was used to probe 15cm into topsoil profile to record volumetric moisture content. Three samples from each plot were taken at random following a V-formation on each plot from Stages 2, 3 & 4.

Soil Structure

Visual Assessment Method

Three random samples of undisturbed soil blocks (20 x 20 x 20cm) were extracted by spade at stages 2,3 & 4. The profile was then evaluated using an illustrated chart (figure 5) by scoring each layer with contrasting structures from 1 (loose, crumbly, porous soil) to 5 (compact, dense, solid) and assigned a *Sq* score based on the Visual Evaluation of Soil Structure (VESS) method described by Ball et al (2007).



Structure quality	Size and appearance of aggregates	Visible porosity and Roots	Appearance after break-up: various soils	Appearance after break-up: same soil different tillage	Distinguishing feature	Appearance and description of natural or reduced fragment of ~ 1.5 cm diameter
Sq1 Friable Aggregates readily crumble with fingers	Mostly < 6 mm after crumbling	Highly porous Roots throughout the soil			Fine aggregates	1 cm The action of breaking the block is enough to reveal them. Large aggregates are composed of smaller ones, held by roots.
Sq2 Intact Aggregates easy to break with one hand	A mixture of porous, rounded aggregates from 2mm - 7 cm. No clods present	Most aggregates are porous Roots throughout the soil			High aggregate porosity	1 cm Aggregates when obtained are rounded, very fragile, crumble very easily and are highly porous.
Sq3 Firm Most aggregates break with one hand	A mixture of porous aggregates from 2mm -10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present	Macropores and cracks present. Porosity and roots both within aggregates.			Low aggregate porosity	1 cm Aggregate fragments are fairly easy to obtain. They have few visible pores and are rounded. Roots usually grow through the aggregates.
Sq4 Compact Requires considerable effort to break aggregates with one hand	Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm	Few macropores and cracks All roots are clustered in macropores and around aggregates			Distinct macropores	1 cm Aggregate fragments are easy to obtain when soil is wet, in cube shapes which are very sharp-edged and show cracks internally.
Sq5 Very compact Difficult to break up	Mostly large > 10 cm, very few < 7 cm, angular and non-porous	Very low porosity. Macropores may be present. May contain anaerobic zones. Few roots, if any, and restricted to cracks			Grey-blue colour	1 cm Aggregate fragments are easy to obtain when soil is wet, although considerable force may be needed. No pores or cracks are visible usually.

Figure 5: Visual Evaluation of Soil Structure for Soil Health scoring chart copyright: Scotland's Rural College available at: https://www.sruc.ac.uk/downloads/file/1121/visual_evaluation_of_soil_structure_score_chart.

Digital Penetrometer Method

At stage 4 the Spectrum Field Scout 900 (Spectrum Technologies Inc.) digital compaction meter (penetrometer) was used as an ultrasonic depth sensor capturing resistance readings measured in KPa (kilopascal) in 2.5cm increments up to 45cm depths. Three random samples were taken from each plot based on a V-formation across each plot.

Bulk Density

Stage 2 & 3 soil cores used for moisture content samples were weighed and analysis with a known volume (intact corer) and bulk density was calculated using a dry weight calculation according to method described in Blake (1965).

Soil Temperature

A digital thermometer (Tenma^{TM} TP101) was used to take soil temperatures during macro-fauna extractions by probing 10-12cm of the topsoil profile for stages 2, 3 & 4.

Soil Chemical Properties Methods

Carbon Content

Organic Content Equation

Organic Content results were calculated utilising the Van Bermmelen factor (Allison, 1965; Nelson & Sommers, 1996) equation:

Organic Carbon (%) = Organic Matter (%) x.058.

Automated Flow Segmented Analyser

Phosphate, ammonium and nitrate tests were conducted on milled, oven dried samples subjected to comminution to reduce aggregate grain size to <2mm from Stages 1 & 4 using an Automated Flow Segmented Analyser as per standardised methods established in ISO 13395:1996 (Seal Analytical, 2013) for nitrate and ammonium and Olsen P method for phosphate determination with a spectrophotometer and calibrated absorbance standards (J. T. Sims, 2000).

Soil Biological Properties Methods

Soil Organic Matter Content

Loss on Ignition Method

Three random samples from each plot were taken at each stage and oven dried at 105˚C for 24 hours. Thereafter, 1 gram from each dried sample was placed in pre-weighed crucible and placed in a muffle furnace at a temperature of 550^{O}C for 4 hours. Samples were then allowed to cool at room temperature in a desiccator and weighed to determine carbon loss based on an established ‘Loss on Ignition’ method (Heiri, Lotter, & Lemcke, 2001; Nelson & Sommers, 1996). Organic Content was expressed in percentage and calculated using the following equation: Organic Content (%) = 100 x $(M_1-M_2)/M_1$ where M_1 is original mass and M_2 is weight after burn.

An additional ‘Loss on Ignition’ test was conducted on samples extracted from stage 1 and 4 and placed in a muffle furnace at a temperature of 400^{O}C for 16 hours to use as a comparative method.

Summer samples were all processed in a Carbolite OAF 10/2 furnace (installed: June 1994), whilst autumn, winter and spring samples were processed using a Carbolite AAF 1100 furnace (installed: September 2020).

Visual Assessment Method

Three random samples from each plot were taken at stages 2, 3 & 4. Soil blocks (20 x 20 x 20 cm) were extracted by spade and graded based on the organic composition (root establishment as well as organic debris) of the soil profile from 1 (low) to 5 (high). This method is an experimental approach to assessing organic matter in soil profile and was adopted and adapted based on the Visual Evaluation of Soil Structure method as described by Ball et al. (2007).

Macrofauna

Hand Sorting Method

Three soil block samples (20 x 20 x 20 cm) were excavated randomly by spade from each plot and sifted through systematically on site at stages 1,2 & 4 and off-site at stage 3. All macrofauna were extracted using the standardised hand sorting method (D. Jones & Eggleton, 2014). Extracted juvenile earthworms were counted and recorded, adult earthworms identified using standardised identification categories (R. W. Sims & Gerard, 1985) and recorded and other invertebrates identified according to Wheater and Read (1996).

Mustard Extraction Method

A (20 x 20 x 20 cm) soil block was extracted and a solution of two tablespoons of mustard powder and 1ltr of water was poured evenly across the extraction quadrat and observed for 20 minutes, removing all earthworms that emerged. Extracted juveniles were counted, and adult earthworms identified using standardised identification categories (R. W. Sims & Gerard, 1985) and recorded. Mustard Extraction was completed for stages 1, 2 & 4.

Mesofauna

Berlese-Tullgren Funnel Extraction

Three cored soil samples (intact soil corer) were taken at random from each plot at stages 2, 3 & 4 and placed on a Tullgren funnel system (built based on specifications of Burkard Manufacturing Co. Ltd, UK whereby 20 funnels with 5mm mesh suspend intact core sample 10cm above a 20W halogen bulbs) allowing invertebrates to migrate through mesh into 70% alcohol container over 7 days. Collected species were then grouped according to taxonomic order.

Microfauna

Wet Tray Extraction

A wet tray extraction method was used as described by Whitehead & Hemming (1965), adapted by Bardgett et al. (1997) to isolate nematodes from 100g cored soil samples using an intact soil corer. Samples were roughly broken up and placed on a thin layer of coarse porosity tissue paper (Mills & Adl, 2006) (KIMWIPES LITE 100), supported by two different sized meshes in shallow trays filled with 400ml of Ultra-Pure water ensuring tissue and water made contact without full coverage. Samples were left for 24 hours and then repeatedly settled and reduced by suction over a period of 7 days to a concentrate of 5ml. Samples were kept at 4˚C during each settlement period. Sample was then mixed and divided into a 1ml extraction for abundance count and the remaining 4ml was diluted with a 4ml 10% formaldehyde solution to preserve and record diversity counts at a later point. An inverted microscope was used to identify nematode abundance. Abundance counts were conducted on 3 x 250 µg samples.

Statistical Analysis

Results were recorded in Microsoft Excel and imported into GenSat for statistical analysis (Verzani, 2018). All data were tested for normality and homoscedasticity, and normalised/transformed with either log or sqrt where possible/applicable. Two-way ANOVA tests were conducted to determine significance of variance at the 5% confidence level (treatment structure: cultivation; block structure: block/cultivation) and Pairwise t-tests were conducted in Excel (T.DIST.2T function) to determine differential significance between cultivation means where applicable (P. Brains, personal communication, October 2021). Correlation was tested using Pearson's product moment correlation using a 5% significance level after checking test assumptions were met.

A statistical power analysis test was conducted (pwr test: 80% power and medium effect size) in order to reduce type II errors when determining overall sampling size at each stage resulting in 3 samples taken from each plot (Lan & Lian, 2010).

Boxplots and scatterplots were generated in Excel to present visual variance in data.

Results

Stage 1 - Summer

Soil Chemical Properties

There were no significant differences in ammonium (figure 6a), or phosphate (figure 6b) readings between cultivations (ammonium: $F_{2, 10} = 1.56$, $P = 0.257$; phosphate: $F_{2, 10} = 1.00$, $P = 0.402$), however there was a trending significance in nitrate (figure 6c) readings between cultivations ($F_{2, 10} = 3.94$, $P = 0.055$). DD plots were on average 1.57 mg/l higher in nitrate compared to CT plots ($P = 0.024$) and on average 1.28 mg/l higher compared to CT plots ($P = 0.058$).

(a) (b) (c)

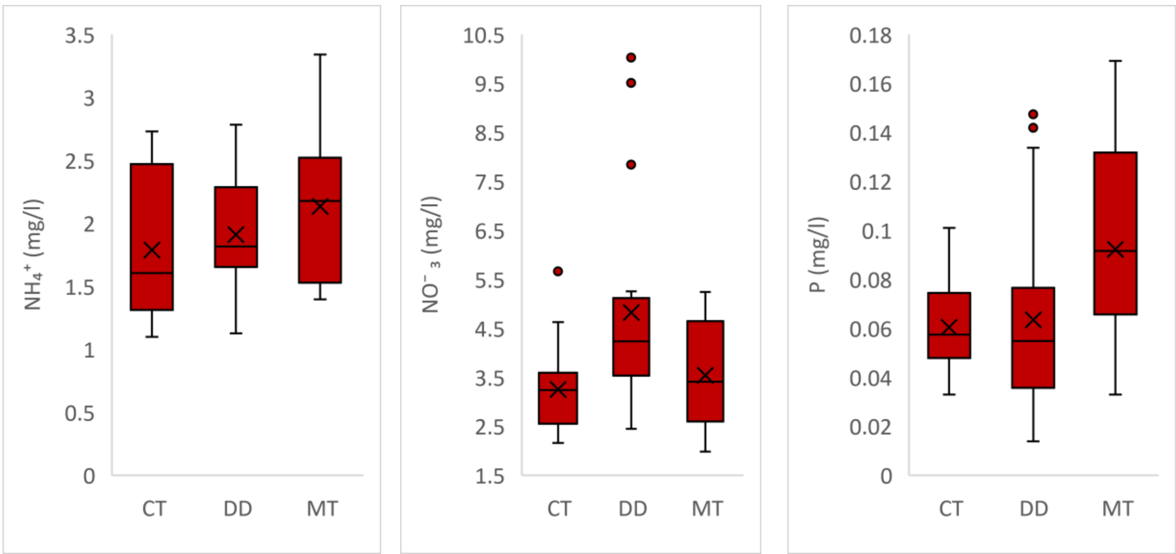


Figure 6: Summer cultivation readings for (a) ammonium (NH_4^+), (b) nitrate (NO_3^-) and (c) phosphate (P) using automated segmented flow analyser and Olsen P method showing means (X), medians (-) and outliers (o) with trending significance for nitrate (P=0.055) in direct drill (DD) compared to conventional tillage (CT) no significance in minimal tillage (MT).

Soil Biological Properties

Soil Organic Matter

No significant difference was found in SOM between cultivations (figure 7) either at a 550°C LOI temperature ($F_{2, 10}$ = 0.80, P = 0.476) or when method was modified to a temperature of 400°C and duration of 16 hours ($F_{2, 10}$ = 0.36, P = 0.707).

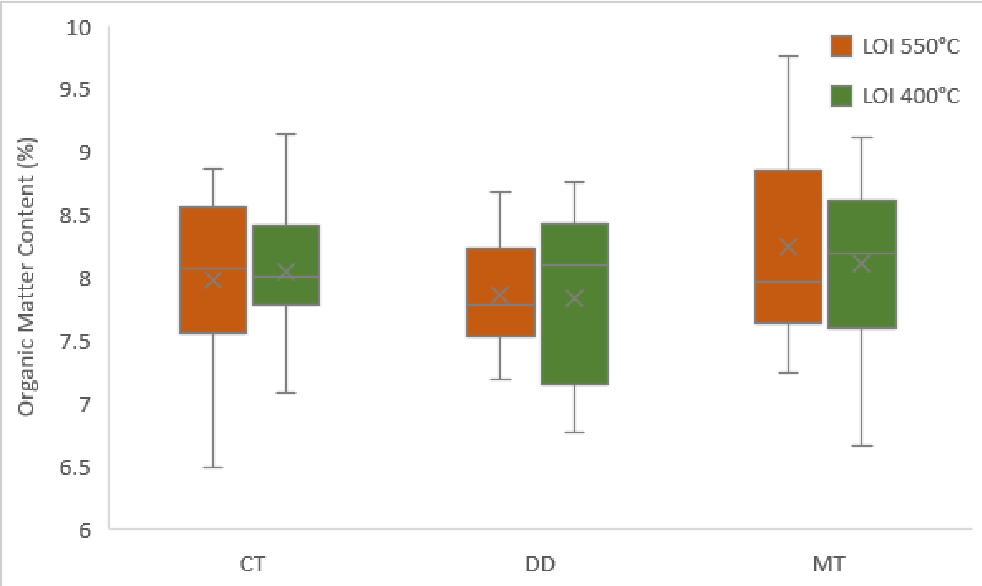


Figure 7: Summer cultivation recordings for soil organic matter content (%) using Loss on ignition rates of 550°C for 4 (LOI 550°C) and modified method of 400°C for 16 hours showing means (X), medians (-) and outliers (o) with no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Macrofauna - Earthworms

There were no significant differences in the total amount of earthworms between cultivations ($F_{2, 10}$ = 0.38, P = 0.696) (figure 9a), nor were there any significant differences in ecological function groups (Endogeic: $F_{2, 10}$ = 0.10, P = 0.902; Epigeic: $F_{2, 10}$ = 2.14, P = 0.168; Anecic: $F_{2, 10}$ = 2.16, P = 0.166) (figure 8) and diversity indices ($F_{2, 10}$ = 3.20, P = 0.084) (figure 9b).

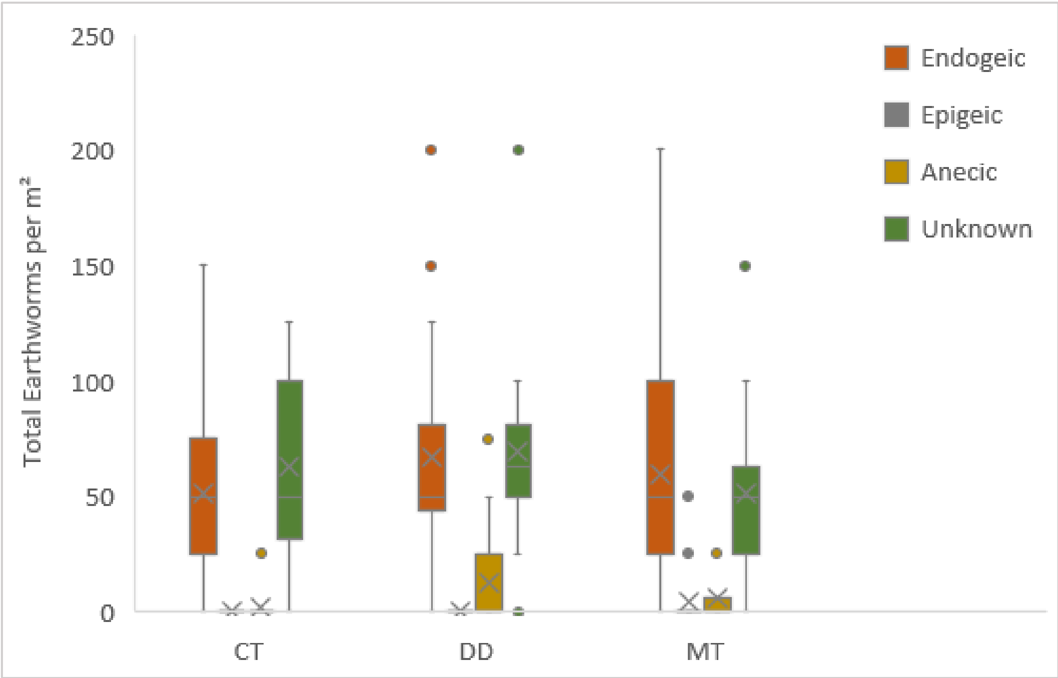


Figure 8: Summer recordings for total earthworms per m² by ecological function groups: endogeic, epigeic, anecic and unknown earthworms using a combination of hand-sorting and mustard extraction method showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

(a) *(b)*

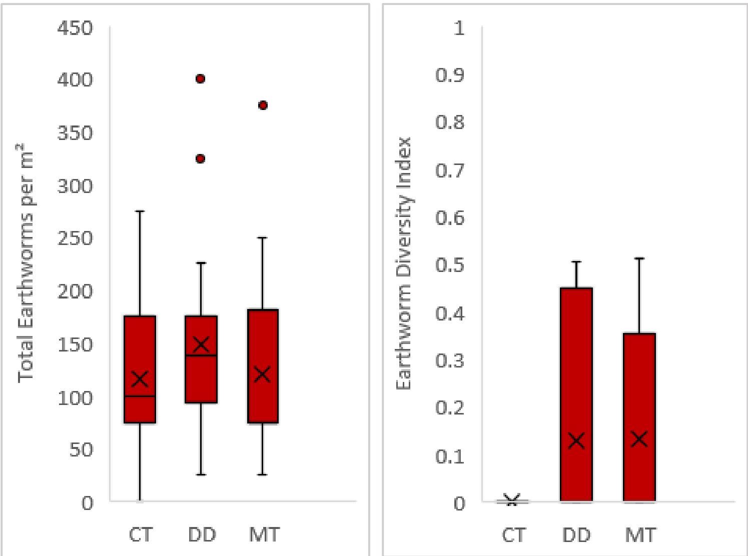


Figure 9: Summer recordings for total earthworms per m² (a) and Simpsons Diversity Index for earthworms (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Macrofauna - Other

There were no significant differences in the total abundance of macrofauna between cultivations ($F_{2, 10} = 0.86$, P = 0.453) (figure 10a) nor were there any significance differences in diversity indices (F2, 10 = 0.52, P = 0.607) (figure 10b).

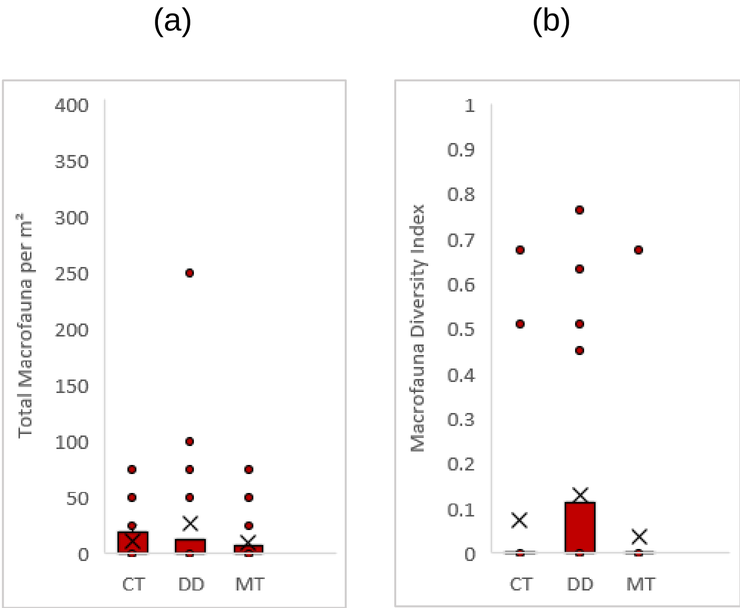


Figure 10: Summer recordings for total macrofauna per m² (a) and Simpsons Diversity Index for macrofauna (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Stage 2 – Autumn

Soil Physical Properties

Moisture Content

There were significant differences in gravitational moisture content between cultivations ($F_{2, 10} = 5.46$, P = 0.025) (figure 12b). MT plots were on average 1.01% higher in gravitational moisture compared to CT plots (P = 0.007). In contrast there were no significant differences in volumetric moisture content using the gravimetric method ($F_{2, 10} = 0.24$, P = 0.794) nor were there any significant differences between cultivations using a moisture meter ($F_{2, 10} = 1.87$, P = 0.205) (figure 11). Additionally, there were no significant differences in the visual evaluation of moisture content ($F_{2, 10} = 0.03$, P = 0.963) (figure 12a).

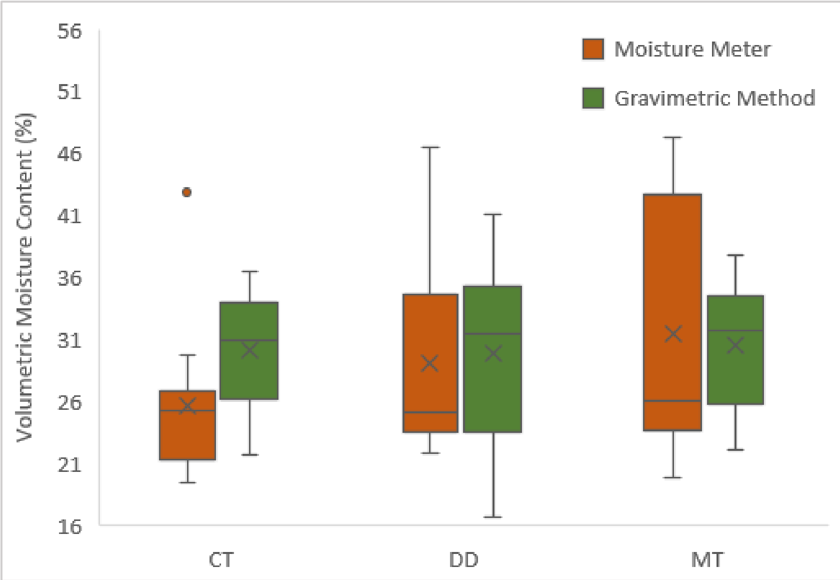


Figure 11: Autumn recordings for volumetric moisture content (%) comparing moisture meter readings to gravimetric method calculations showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

(a) (b)

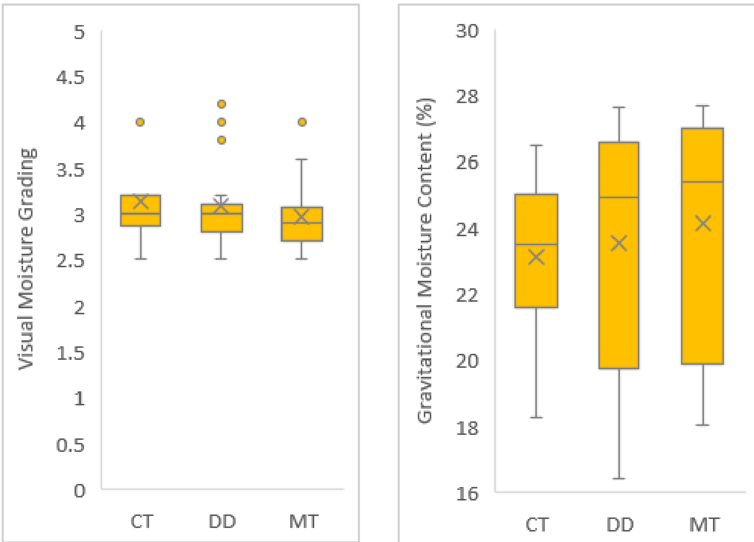


Figure 12: Autumn recordings for moisture content by visual assessment (a) grading soil profile 1 (very dry soil) to 5 (saturated soil) and gravitational moisture content (%) (b) using oven dry method showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD) for visual assessment however significance in gravimetric moisture content of MT when compared to CT (P = 0.025).

Other

There were no significant differences in VESS grading between cultivations ($F_{2, 10}$ = 1.82, P = 0.212) (figure 13a), in soil temperature ($F_{2, 10}$ = 0.72, P = 0.510) (figure 13b), nor were there significant differences in bulk density measurements ($F_{2, 10}$ = 0.193, P = 1.95) (figure 13c).

. (a) (b) (c)

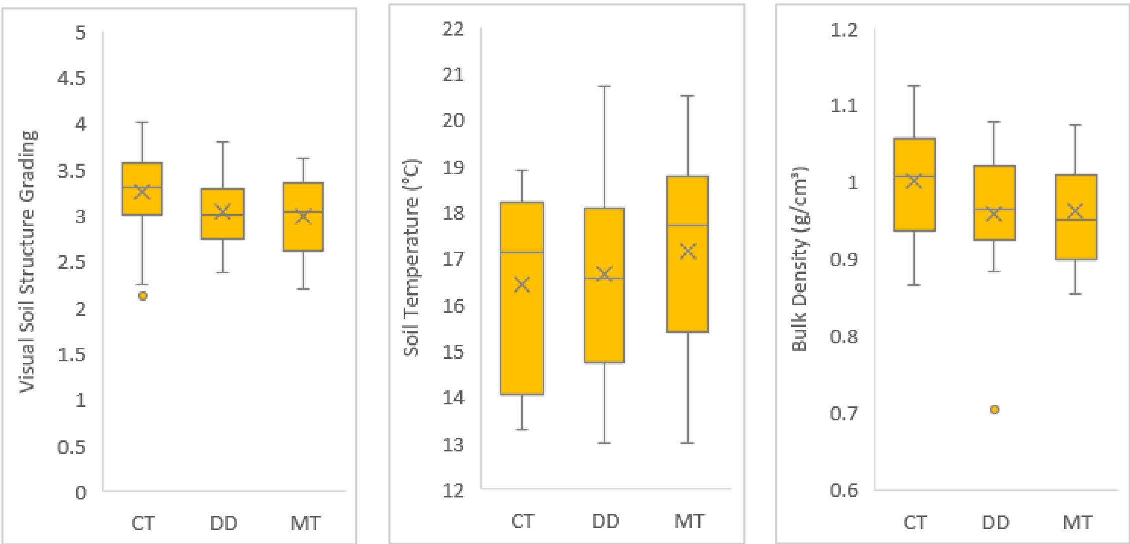


Figure 13: Autumn recordings for Visual Evaluation of Soil Structure (VESS) (a) using a grading system to assess structure of soil profile between 1 (loose, crumbly) to 5 (compact, dense), soil temperature (b) using a thermometer probe and bulk density (c) using oven dry method showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD) for all three measurements.

Soil Biological Properties

Soil Organic Matter

There were highly significant differences in SOM at 550°C between cultivations ($F_{2, 10}$ = 15.70, P < 0.001) (figure 14a). DD plots were on average 0.997% higher in SOM compared to CT (P < 0.001) and MT plots were on average 0.793% higher in SOM compared to CT (P = 0.002). In contrast, there were no significant differences found in the visual evaluation of SOM between cultivations ($F_{2, 10}$ = 3.12, P = 0.100) (figure 14b).

(a) *(b)*

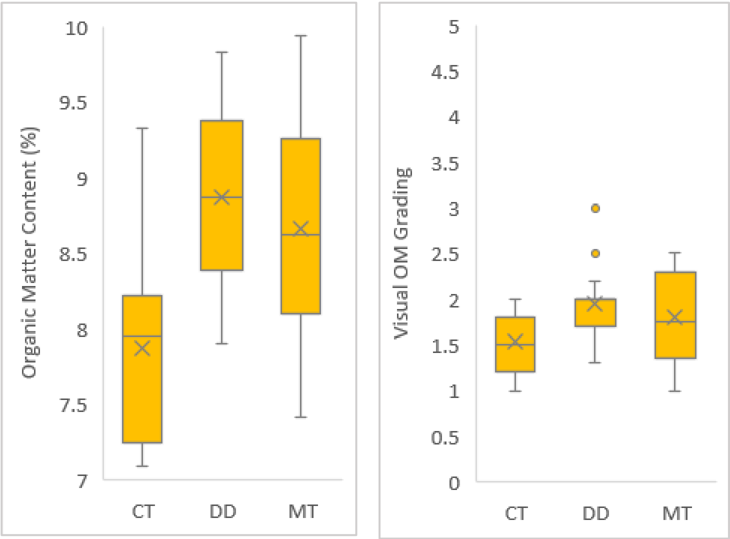


Figure 14: Autumn recordings for soil organic matter (%) (a) using loss on ignition method at 550°C for 4 hours, showing means (X), medians (-) and outliers (o). There were highly significant differences between conventional tillage (CT) when compared to minimal tillage (MT) and direct drill (DD) (P < 0.001). Graph b depicts visual assessment of organic content (b) using an adapted grading method with 1 indicating low organic matter and 5 indicating high organic matter, showing means (X), medians (-) and outliers (o). No significance recorded between cultivations in graph b.

Macrofauna - Earthworms

There were no significant differences in the total amount of earthworms ($F_{2, 10} = 0.10$, P = 0.910) (figure 16a), ecological function groups (Endogeic: $F_{2, 10} = 0.41$, P = 0.675; Epigeic: $F_{2, 10} = 0.25$, P = 0.780; Anecic: $F_{2, 10} = 1.30$, P = 0.316) (figure 15) or diversity indices ($F_{2, 10} = 1.09$, P = 0.374) (figure 16b) between cultivations.

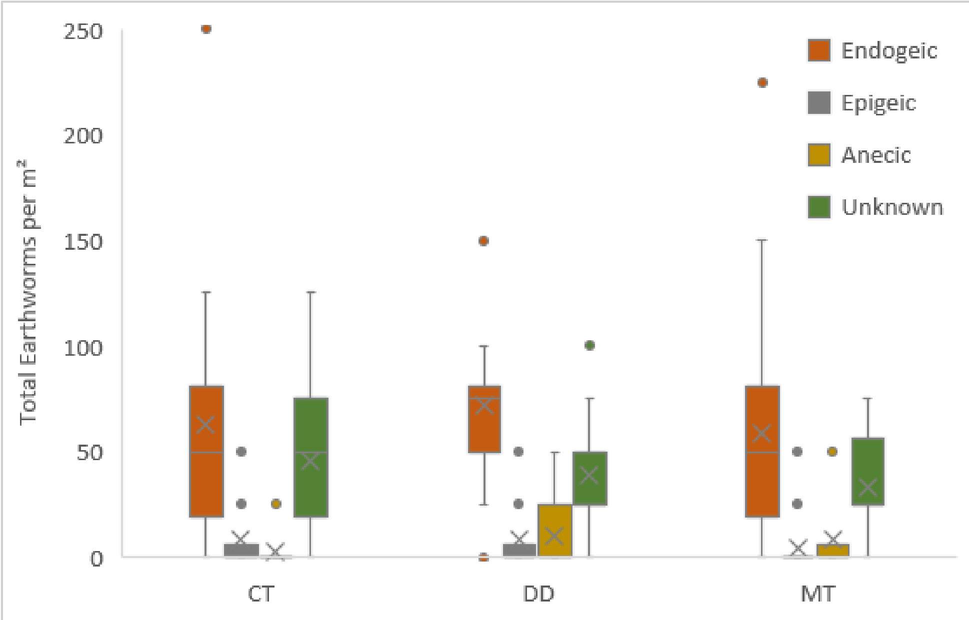
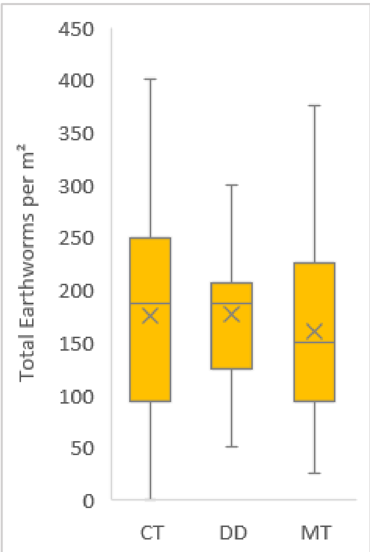


Figure 15: Autumn recordings for total earthworms per m² by ecological function groups: endogeic, epigeic, anecic and unknown earthworms using a combination of hand-sorting and mustard extraction method showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

(a) (b)



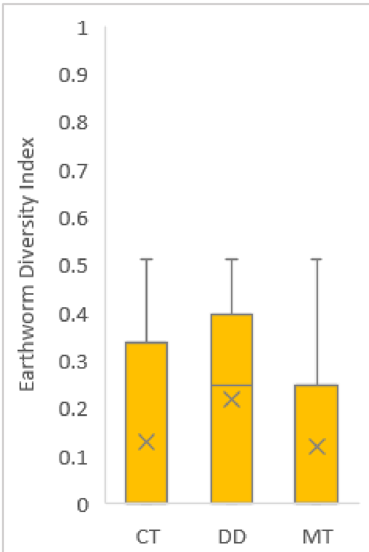


Figure 16: Autumn recordings for total earthworms per m² (a) and Simpsons Diversity Index for earthworms (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Macrofauna - Other

There were no significant differences in the total amount of macrofauna ($F_{2, 10}$ = 1.89, P = 0.202) (figure 17a) or diversity indices ($F_{2, 10}$ = 1.40, P = 0.291) (figure 17b) between cultivations.

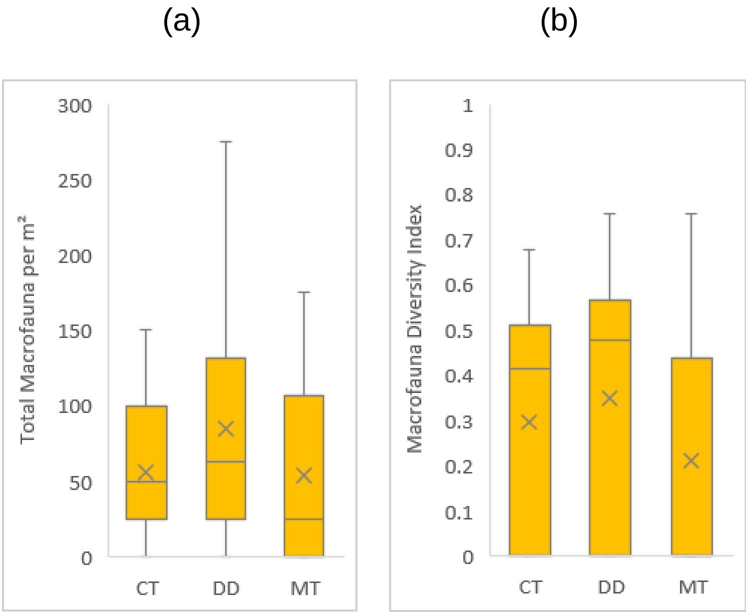


Figure 17: Autumn recordings for total macrofauna per m² (a) and Simpsons Diversity Index for macrofauna (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Mesofauna

There were significant differences in the total amount of collembola between cultivations ($F_{2, 10}$ = 6.36, P = 0.017). MT plots had on average 266 more individuals than CT plots (P = 0.005) and 166 more individuals than DD plots (P = 0.052). There were also significant differences in collembola function group Entomobryomorpha ($F_{2, 10}$ = 6.11, P = 0.018) but no significant difference in Poduromorpha ($F_{2, 10}$ = 0.52, P = 0.610), Symphypleona ($F_{2, 10}$ = 0.59, P = 0.571). MT plots on average had 267 more Entromobryomorpha individuals compared to CT plots (P = 0.008) and 217 more individuals than DD plots (P = 0.023) (figure 18).

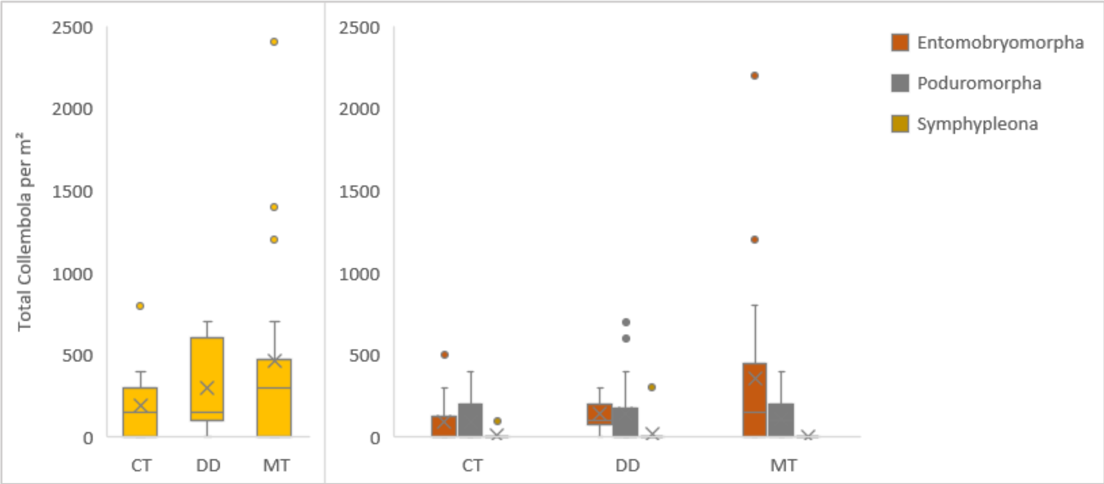


Figure 18: Autumn recordings for total collembola per m² as well as by taxonomic order: Entomobryomorpha, Poduramorpha and Symphypleona using a Tullgren extraction method showing means (X), medians (-) and outliers (o). There were significant differences for total collembola (P = 0.017) and Entomobryomorpha (P = 0.018) in conventional tillage (CT) compared to minimal tillage (MT) and direct drill (DD).

There were no significant differences in the total amount of acari ($F_{2,\,10}$ = 1.51, P = 0.268) (figure 19a), acari to collembola ratio ($F_{2,\,10}$ = 0.30, P = 0.751) (figure 19b) or overall diversity index ($F_{2,\,10}$ = 1.10, P = 0.370) (figure 20a). There were however significant differences in total mesofauna abundance ($F_{2,\,10}$ = 5.23, P = 0.028). CT plots on average had 1124 less individuals compared to MT plots (P = 0.023) and 538 less individuals compared to DD plots (P = 0.01) (figure 20b).

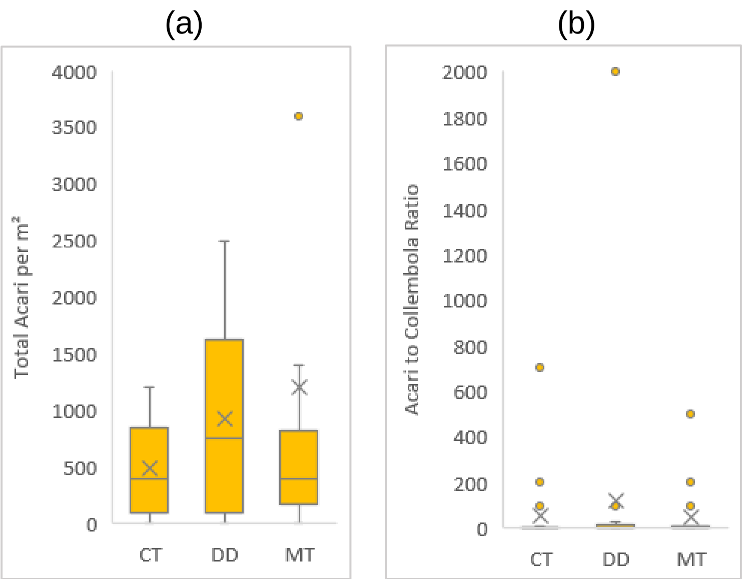


Figure 19: Autumn recordings for total acari per m² (a) and acari to collembola ratio (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

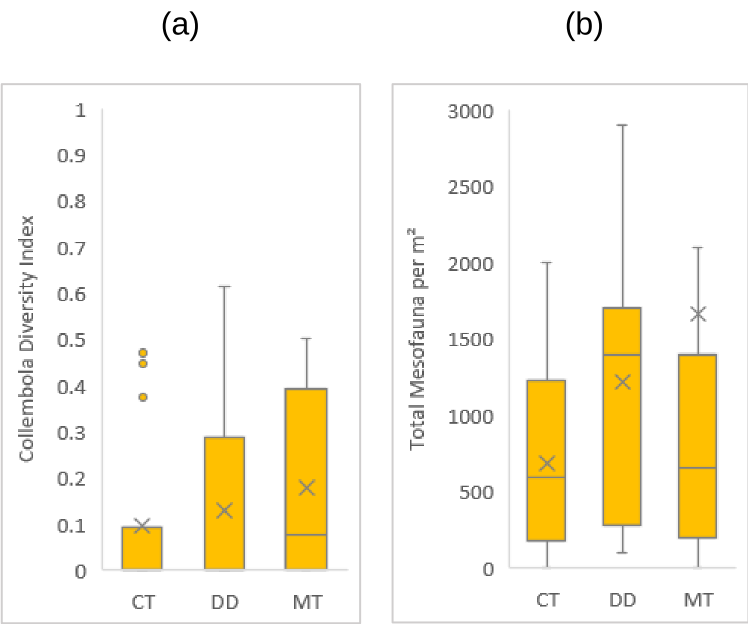


Figure 20: Autumn recordings for Simpson's Diversity Index for collembola (a) and total mesofauna per m² (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD) for diversity index, however there were for total mesofauna. Both MT and DD were significantly higher than CT (P = 0.028).

Microfauna

There were no significant differences in the total amount of nematodes between cultivations ($F_{2, 10}$ = 2.71, P = 0.114) (figure 21).

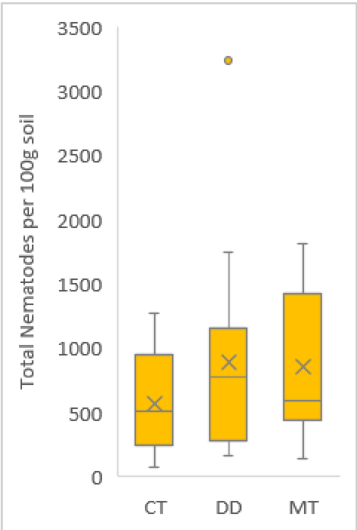


Figure 21: Autumn recordings for total nematodes per 100g soil showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

Stage 3 – Winter

Soil Physical Properties

Moisture Content

There were significant differences in gravitational moisture content between cultivations ($F_{2, 10}$ = 5.33, P = 0.027). CT plots were on average 1.11% lower in GM compared to DD plots (P = 0.034) and on average 1.41% lower in GM compared to MT plots (P = 0.011) (figure 23a). Regarding volumetric moisture content, there were no significant differences in volumetric moisture meter readings ($F_{2, 10}$ = 1.61, P = 0.247) (figure 22), however there were significant differences in volumetric moisture using the gravimetric method between cultivations ($F_{2, 10}$ = 7.97, P = 0.009). CT plots were on average 4.48% lower in VM compared to DD plots (P = 0.005) and on average 4.17% lower compared to MT plots (P = 0.007) (figure 22). Additionally, there were significant differences in moisture content using the visual evaluation method ($F_{2, 10}$ = 7.82, P = 0.009). CT plots were on average graded 0.472 points higher in moisture content compared to DD plots (P = 0.002) and MT plots were graded on average 0.211 points higher in moisture content compared to DD plots (P = 0.054) (figure 23a).

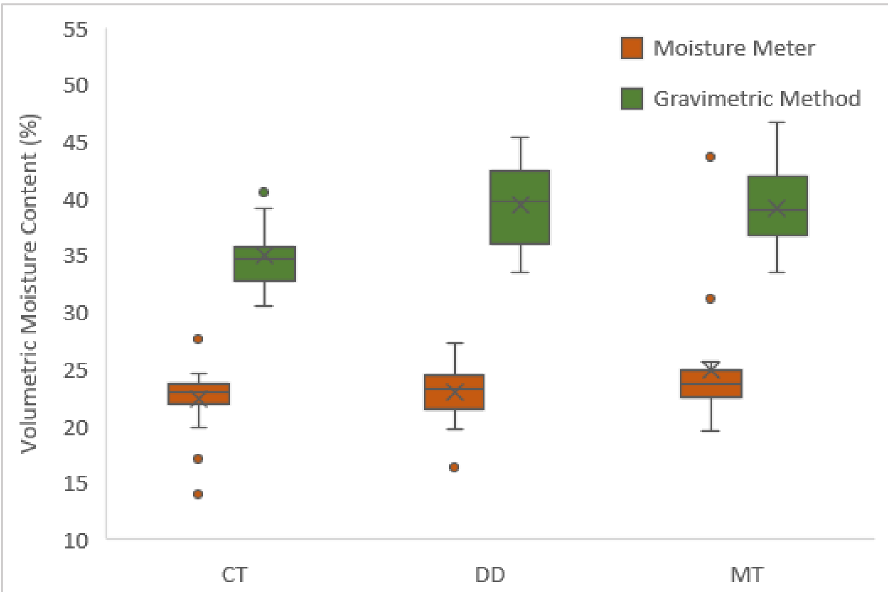


Figure 22: Winter recordings for volumetric moisture content (%) comparing moisture meter readings to gravimetric method calculations showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD) in moisture meter readings, however VM was significantly higher in DD and MT compared to CT (P = 0.009).

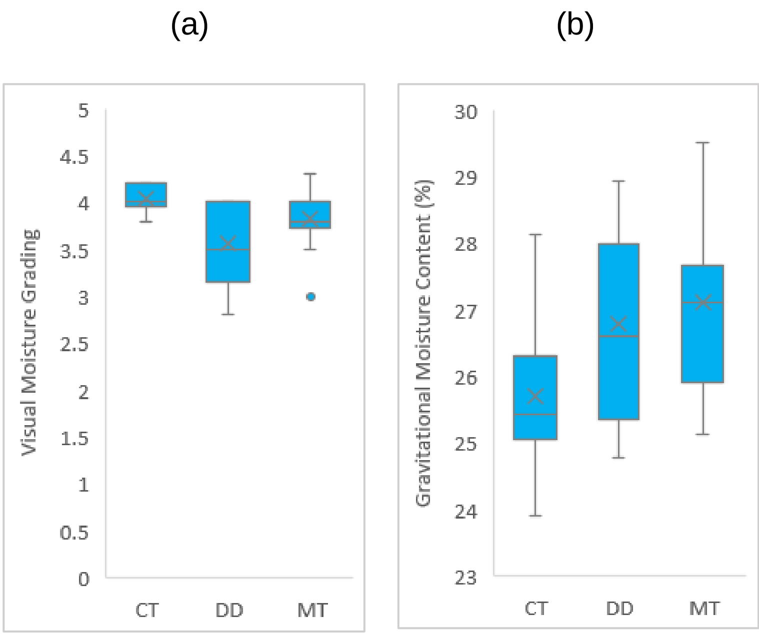


Figure 23: Winter recordings for moisture content by visual assessment (a) grading soil profile 1 (very dry soil) to 5 (saturated soil) and gravitational moisture content (%) (b) using oven dry method showing means (X), medians (-) and outliers (o). For graph (a) there were significant differences between conventional tillage (CT) compared to minimal tillage (MT and direct drill (DD) (P = 0.009) as well as gravitational moisture content (P = 0.009).

Other

There were significant differences in the visual evaluation of the soil structure between cultivations ($F_{2, 10}$ = 7.85, P = 0.009). CT plots on average were graded 0.605 points higher than DD plots (P = 0.004) and 0.549 points higher than MT plots (P = 0.008) (figure 24a). There were no significant differences in soil temperature between cultivations ($F_{2, 10}$ = 2.42, P = 0.139) (figure 24b). There were also significant differences in bulk density between cultivations (F2. 10 = 3.95, P = 0.054). DD plots on average were 0.061 g/m^3 lower in bulk density compared to CT plots (P = 0.019) (figure 24c)

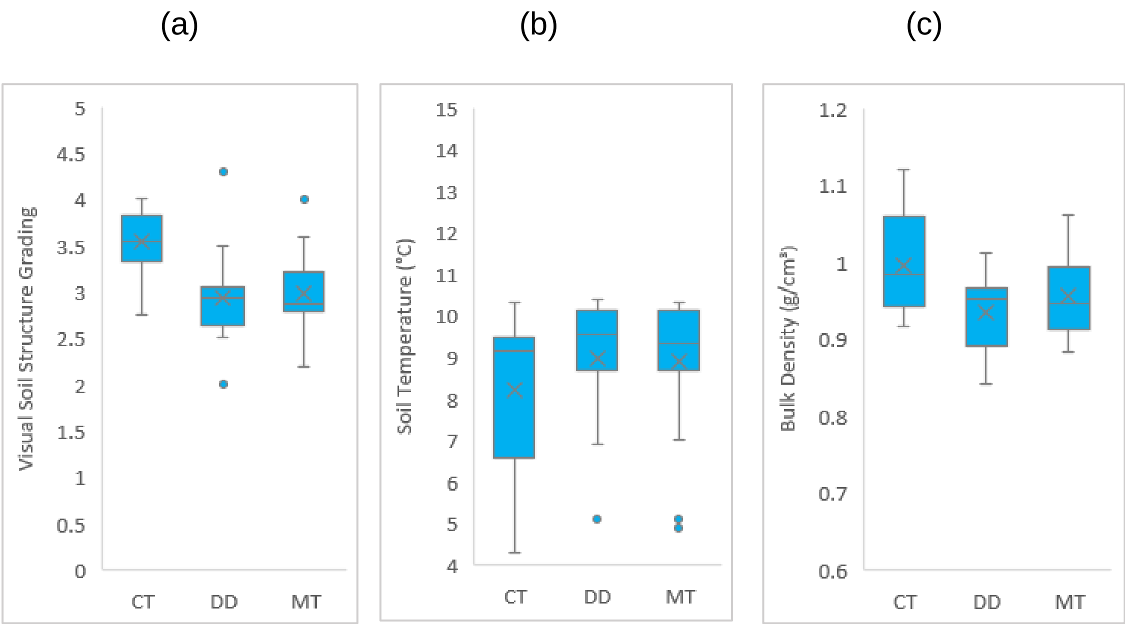


Figure 24: Winter recordings for Visual Evaluation of Soil Structure (VESS) (a) using a grading system to assess structure of soil profile between 1 (loose, crumbly) to 5 (compact, dense), soil temperature (b) using a thermometer probe and bulk density using oven dry method (c) showing means (X), medians (-) and outliers (o). There were significant differences in VESS scoring between conventional tillage (CT) when compared to minimal tillage (MT and direct drill (DD) (P = 0.009) as well as bulk density; DD was significantly lower compared to CT (P = 0.54) but none for soil temperature.

Soil Biological Properties

Soil Organic Matter

There were significant differences in SOM between cultivations at 550°C ($F_{2, 10}$ = 7.75, P = 0.009). CT plots were on average 0.924% lower in SOM compared to DD plots (P = 0.003) and on average 0.621% lower in SOM compared to MT plots (P = 0.026). Additionally, there were significant differences in the visual evaluation of SOM between cultivations ($F_{2, 10}$ = 5.15, P = 0.029). CT plots on average were graded 0.662 points lower in SOM compared to DD plots (P = 0.016) and 0.612 points lower than MT plots (P = 0.023).

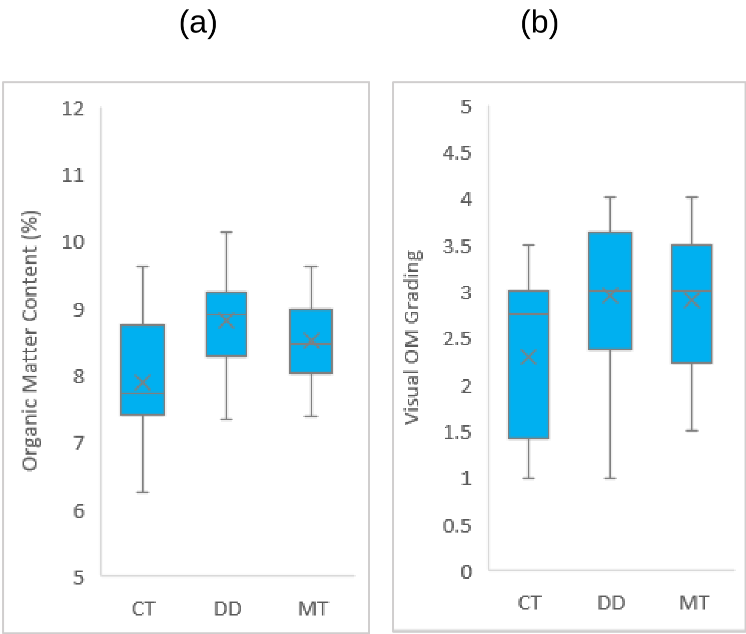


Figure 25: Winter recordings for soil organic matter (%) (a) using loss on ignition method at 550°C for 4 hours, showing means (X), medians (-) and outliers (o). There were highly significant differences between conventional tillage (CT) when compared to minimal tillage (MT) and direct drill (DD) (P < 0.001). Second graph depicts visual assessment of organic content (b) using an adapted grading method with 1 indicating low organic matter and 5 indicating high organic matter, showing means (X), medians (-) and outliers (o). CT was significantly lower when compared to DD and MT in visual evaluation of SOM (P = 0.029).

Macrofauna – Earthworms

There were no significant differences in the total amount of earthworms ($F_{2, 10} = 0.20$, P = 0.825) (figure 27a), or by ecological function group; endogeic worms ($F_{2, 10} = 0.30$, P = 0.749), epigeic worms ($F_{2, 10} = 0.56$, P = 0.590) or anecic worms ($F_{2, 10} = 2.06$, P = 0.178) between cultivations (figure 26). Additionally, there were no significant differences in overall diversity indices ($F_{2, 10} = 0.09$, P = 0.912) (figure 27b).

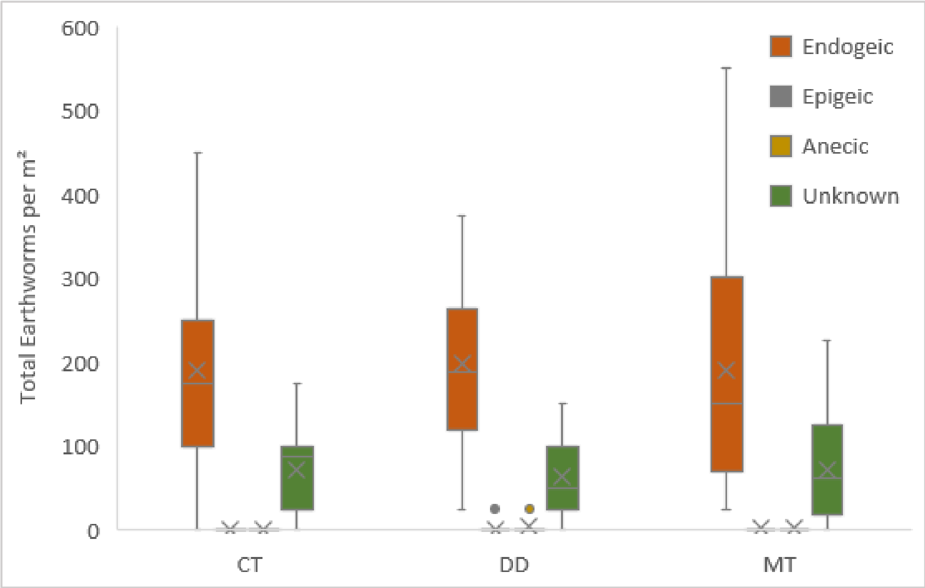


Figure 26: Winter recordings for total earthworms per m² by ecological function groups: endogeic, epigeic, anecic and unknown earthworms using a combination of hand-sorting and mustard extraction method showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

(a) *(b)*

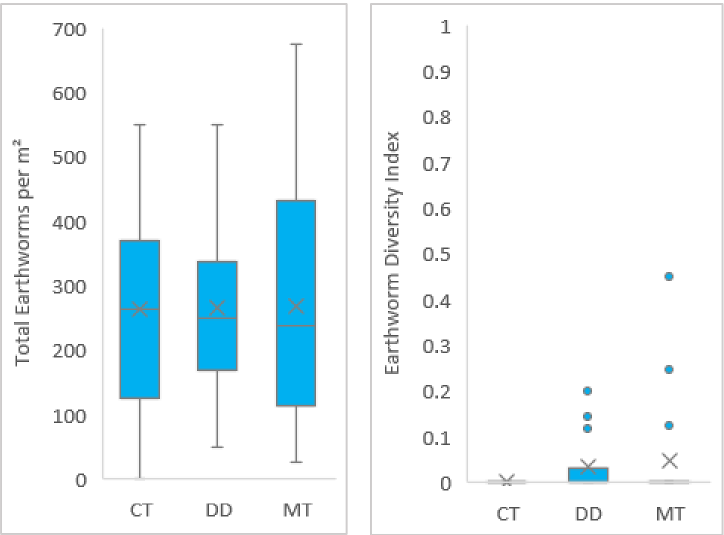


Figure 27: Winter recordings for total earthworms per m² (a) and Simpsons Diversity Index for earthworms (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Macrofauna – Other

There were no significant differences in total macrofauna ($F_{2, 10} = 0.74$, $P = 0.501$) (figure 28a) or macrofauna diversity indices ($F_{2, 10} = 1.19$, $P = 0.344$) between cultivations (figure 28b).

(a) (b)

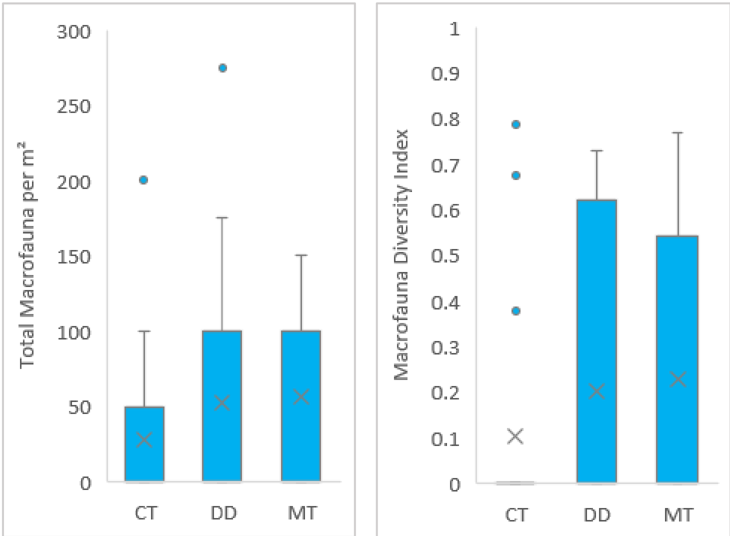


Figure 28: Winter recordings for total macrofauna per m² (a) and Simpsons Diversity Index for macrofauna (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Mesofauna

There were no significant differences in total collembola ($F_{2, 10} = 2.37$, $P = 0.143$) or some collembola orders (Entomobryomorpha: $F_{2, 10} = 1.23$, $P = 0.333$; Symphlypleona: $F_{2, 10} = 2.00$, $P = 0.186$) between cultivations. There were however significant differences in collembola order Poduromorpha ($F_{2, 10} = 7.54$, $P = 0.010$). CT plots on average had 189 less Podurmorpha individuals compared to DD plots ($P = 0.002$) and 123 less individuals compared to MT plots ($P = 0.023$) (figure 29).

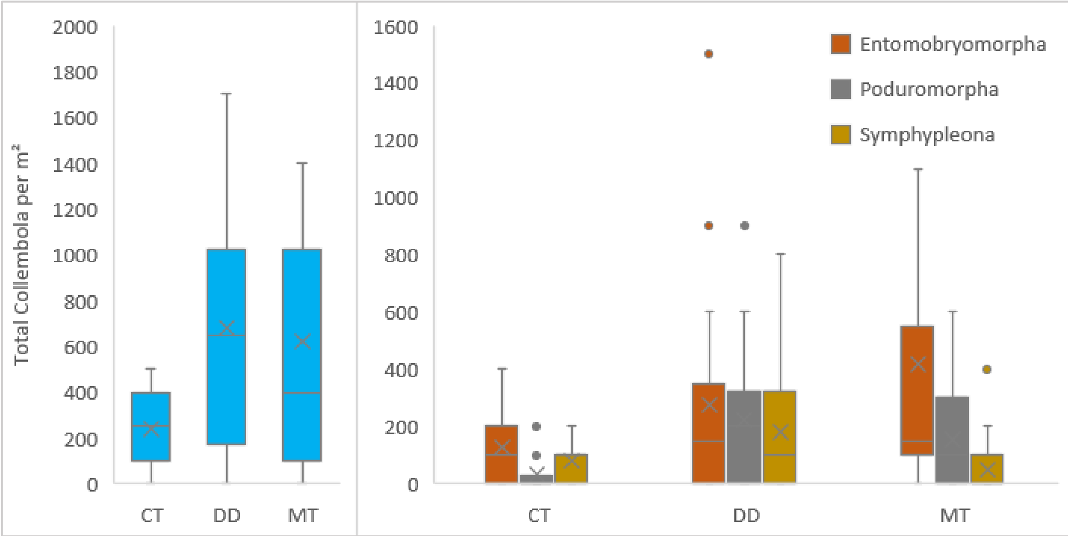


Figure 29: Winter recordings for total collembola per m² as well as by taxonomic order: Entomobryomorpha, Poduramorpha and Symphypleona using a Tullgren extraction method showing means (X), medians (-) and outliers (o). There were no significant differences in cultivations except for Poduramorpha (P = 0.006) which were lower in conventional tillage (CT) compared to minimal tillage (MT) and direct drill (DD).

There were no significant differences in total acari ($F_{2,\,10}$ = 2.64, P = 0.120) (figure 30a), total mesofauna ($F_{2,\,10}$ = 3.26, P = 0.081) (figure 30b), collembola diversity indices ($F_{2,\,10}$ = 0.13, P = 0.879) (figure 31a), or acari to collembola ratio ($F_{2,\,10}$ = 0.12, P = 0.891) (figure 31b) between cultivations.

(a) (b)

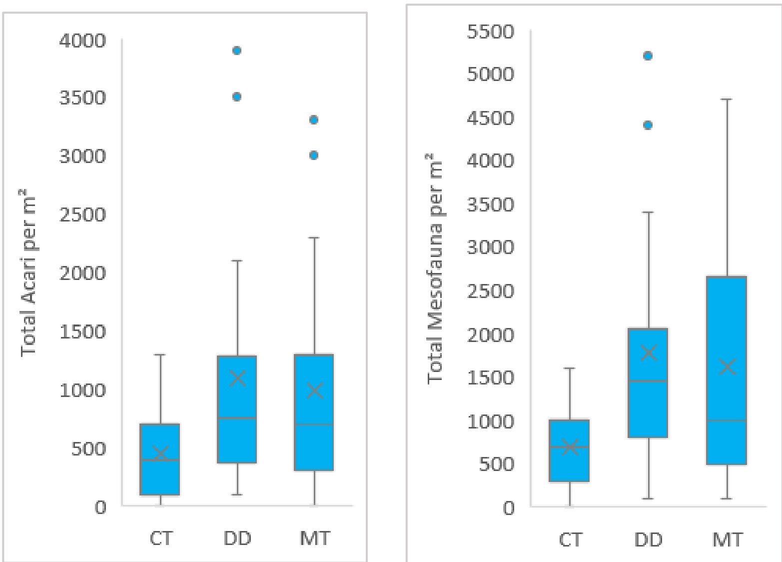


Figure 30: Winter recordings for total acari per m² (a) and total mesofauna per m² (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

(a) (b)

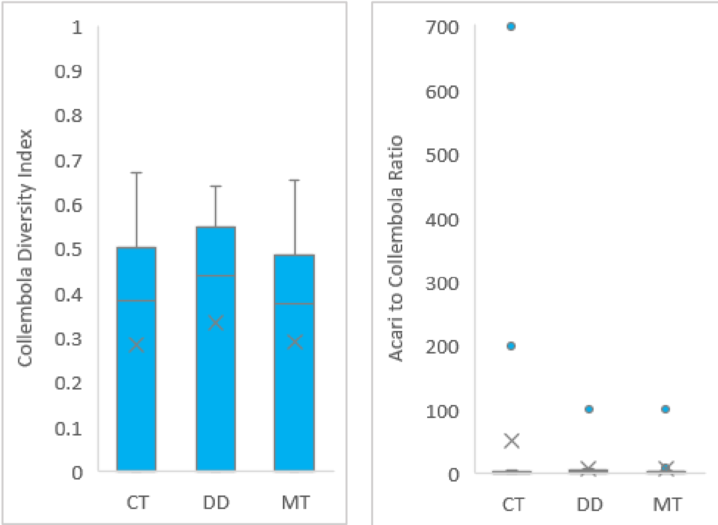


Figure 31: Winter recordings for Simpson's Diversity Index for collembola (a) and acari to collembola ratio (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD) for either measurement.

Microfauna

There were significant differences in total nematodes between cultivations ($F_{2, 10}$ = 11.35, P = 0.003). CT plots on average had 795 less individuals compared to DD plots (P = 0.001) and 830 less individuals compared to MT plots (P = 0.002) (figure32).

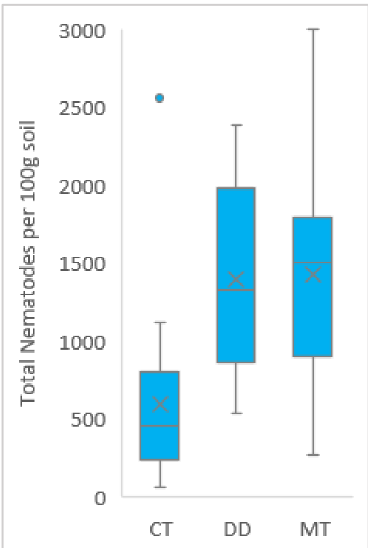


Figure 32: Winter recordings for total nematodes per 100g soil showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

Stage 4 – Spring

Soil Physical Properties

Moisture Content

There were no significant differences in gravitational- ($F_{2,\,10}$ = 3.51, P = 0.070) (figure 33a), volumetric- ($F_{2,\,10}$ = 0.97, P = 0.413) (figure 33b) or visual evaluation of moisture content ($F_{2,\,10}$ = 3.02, P = 0.094) (figure 33c) between cultivations.

(a) (b) (c)

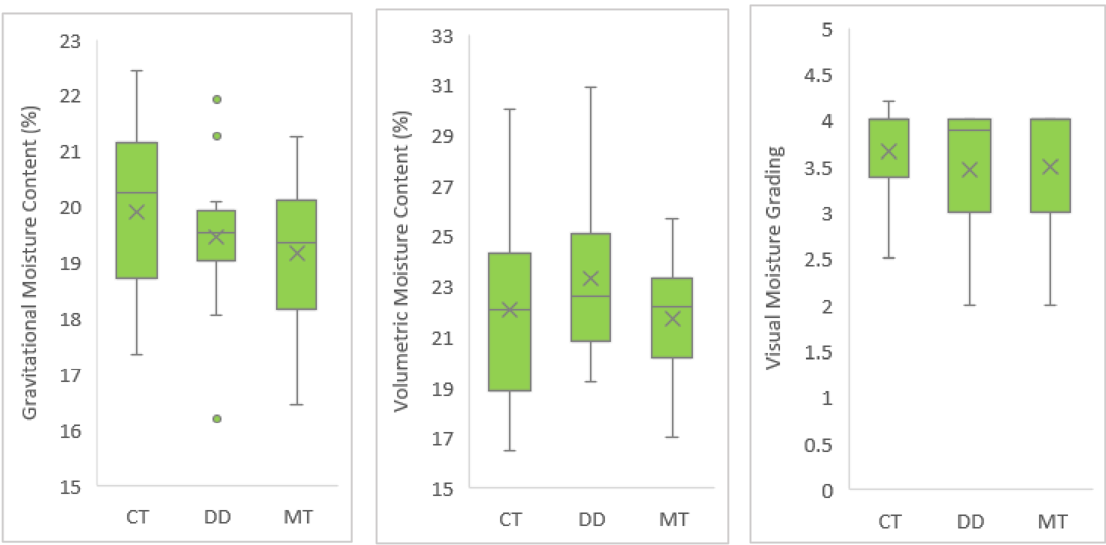


Figure 33: Spring recordings for gravitational moisture content (%) (a), volumetric moisture content (b) using moisture meter readings and visual assessment (c) grading soil profile 1 (very dry soil) to 5 (saturated soil), showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT or direct drill (DD).

Soil Structure

There were significant differences in the visual evaluation of the soil structure between cultivations ($F_{2,\,10}$ = 8.88, P = 0.006). CT plots on average had a 0.372 points higher score than DD plots (P = 0.006) and on average had a 0.419 points higher score than MT plots (P = 0.003) (figure 34a). In contrast, there were no significant differences in soil compaction between cultivations ($F_{2,\,10}$ = 0.08, P = 0.920) (figure 34b).

(a) (b)

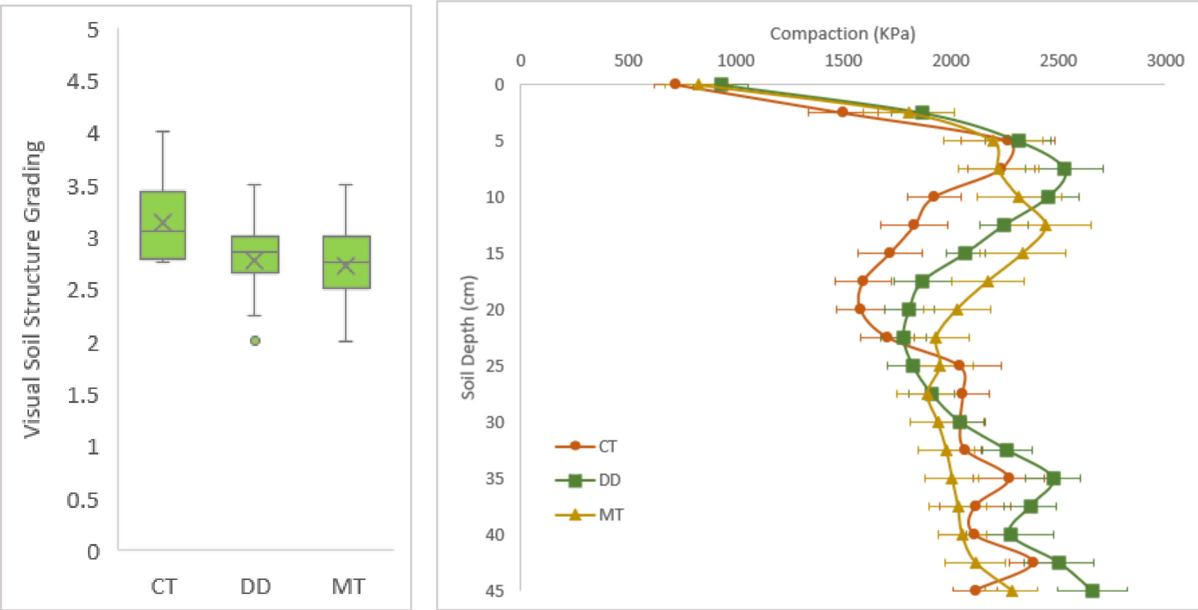


Figure 34: Spring recordings for Visual Evaluation of Soil Structure (VESS) (a) using a grading system to assess structure of soil profile between 1 (loose, crumbly) to 5 (compact, dense) showing means (X), medians (-) and outliers (o). Minimal tillage (MT) and direct drill (DD) were significantly lower compared to conventional tillage (CT) (P = 0.006). Graph (b) shows spring recordings for soil compaction by depth (b)with error bars using a penetrometer. There were no significant differences between cultivations (P = 0.920).

Soil Temperature

There were no significant differences in soil temperature between cultivations ($F_{2, 10}$ = 0.10, P = 0.910) (figure 35).

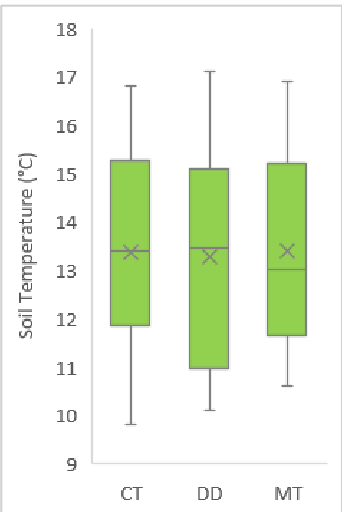


Figure 35: Spring recordings for soil temperature using a thermometer showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

Soil Biological Properties

Soil Organic Matter

There was a trending significance in SOM between cultivations at 550°C ($F_{2, 10}$ = 3.49, P = 0.071). DD plots were on average 0.70% higher in SOM compared to CT plots (P = 0.024) (figure 36b). There were however significant differences in SOM between cultivations at 400°C ($F_{2, 10}$ = 6.09, P = 0.019). CT plots were on average 0.66% lower in SOM compared to DD plots (P = 0.008) and on average 0.53% lower in SOM compared to MT plots (P = 0.024) (figure 36b). Additionally, there were no significant differences in the visual evaluation of SOM between cultivations ($F_{2, 10}$ = 0.33, P = 0.728) (figure 36a).

(a) (b)

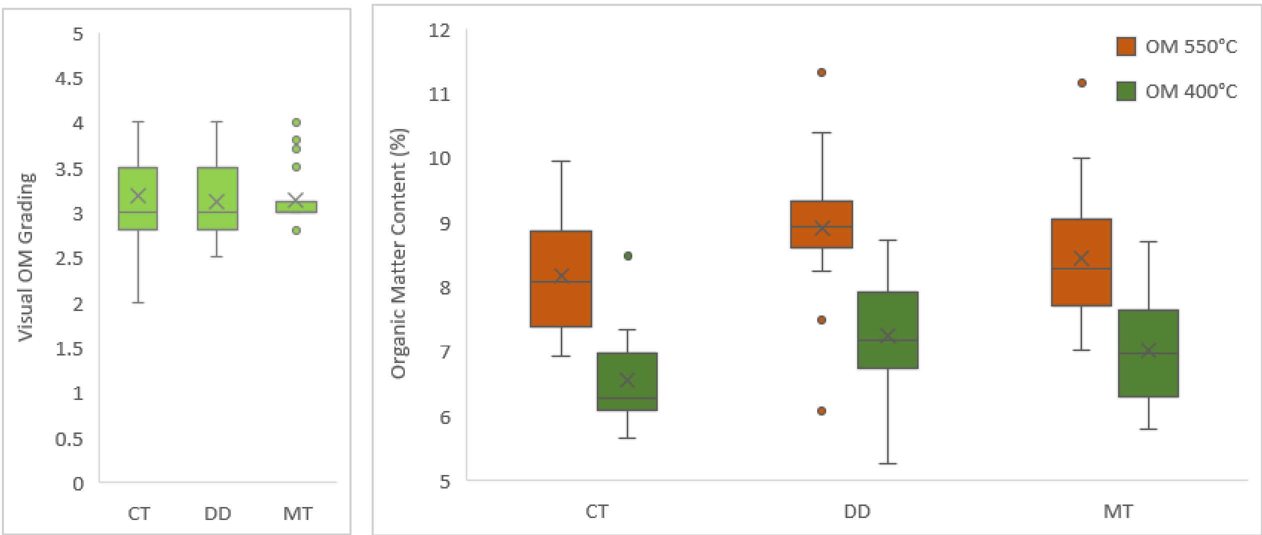


Figure 36: Spring cultivation recordings for soil organic matter content (%) (SOM) using an adapted visual grading method (a) with 1 indicating low organic matter and 5 indicating high organic matter, showing means (X), medians (-) and outliers (o). There were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD). Second graph (b) depicts loss on ignition rates of 550°C for 4 (LOI 550°C) and modified method of 400°C for 16 hours showing means (X), medians (-) and outliers (o) with DD showing a trending significance in higher SOM compared to CT at 550°C (P = 0.071), however at 400°C, both DD and MT were significantly higher compared to CT (P = 0.019).

Macrofauna – Earthworms

There were no significant differences in the total amount of earthworms ($F_{2, 10}$ = 3.15, P = 0.087) (figure38a), or ecological function groups; endogeic worms ($F_{2, 10}$ = 1.48, P = 0.272) or epigeic worms ($F_{2, 10}$ = 0.22, P = 0.808) (figure 37) between cultivations. There were however significance differences in anecic worms between cultivations ($F_{2,\,10}$ = 3.94, P = 0.055). MT plots on average had 9.7 more anecic earthworms compared to CT plots (P = 0.019) (figure 37). There was also a trending significant difference in overall diversity indices ($F_{2,\,10}$ = 3.59, P = 0.067). MT plots were on average 10.9% more likely to have 2 individuals from different ecological function groups compared to CT plots (P = 0.026) (figure 38b).

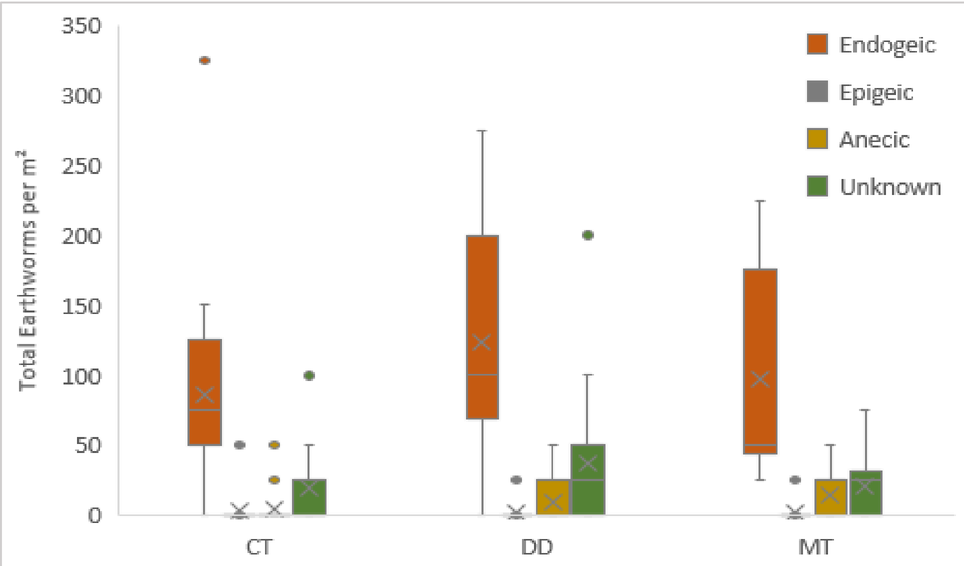


Figure 37: Spring recordings for total earthworms per m² by ecological function groups: endogeic, epigeic, anecic and unknown earthworms using a combination of hand-sorting and mustard extraction method showing means (X), medians (-) and outliers (o). Minimal tillage (TT) on average had significantly higher counts of anecic earthworms compared to conventional tillage (CT) (P = 0.055). No other significance differences were found in CT, MT, or direct drill (DD).

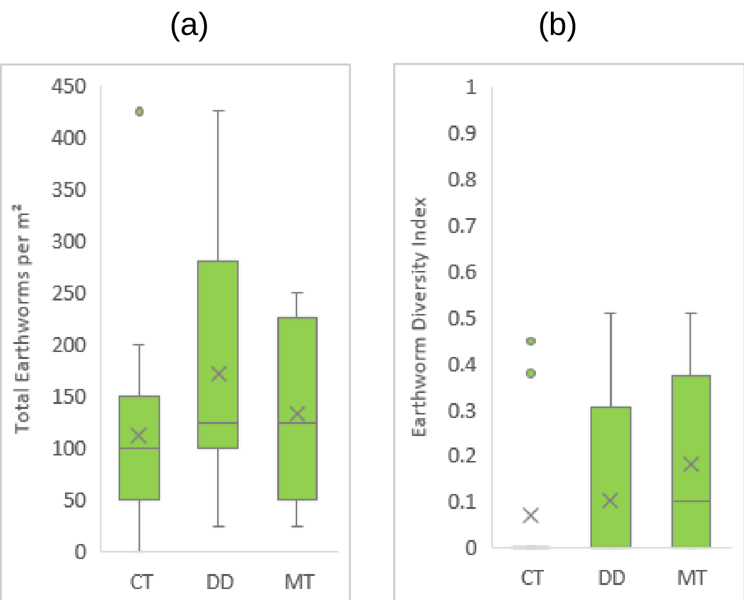


Figure 38: Spring recordings for total earthworms per m² (a) and Simpsons Diversity Index for earthworms (b) showing means (X), medians (-) and outliers (o). Minimal tillage (MT) was significantly higher in diversity indices compared to conventional tillage (CT) (P = 0.067). No other significance differences were found in CT, MT, or direct drill (DD).

There were no significant differences in total earthworm mass ($F_{2, 10}$ = 2.79, P = 0.109), mass of endogeic worms ($F_{2, 10}$ = 1.03, P = 0.393), or mass of epigeic worms ($F_{2, 10}$ = 0.92, P = 0.431) between cultivations. There were however significant differences in anecic worm mass ($F_{2, 10}$ = 7.64, P = 0.010). MT plots on average had 19.2 g more anecic earthworms compared to CT plots (P = 0.002) and on average had 10.5 g more anecic earthworms compared to DD plots (P = 0.059) (figure 39).

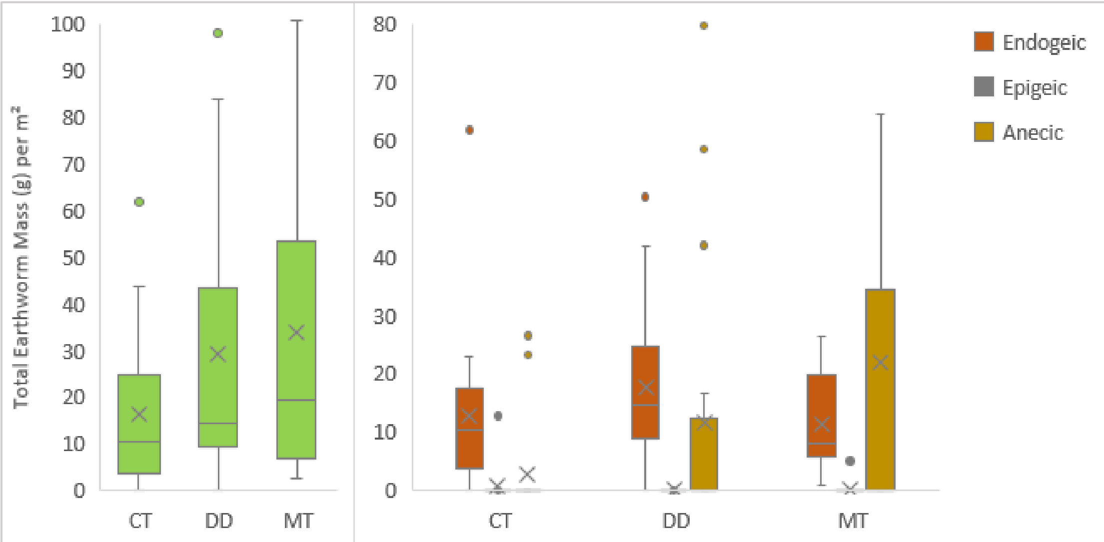


Figure 39:   Spring recordings for total earthworm mass per m² and mass by ecological function groups: endogeic, epigeic and anecic earthworms showing means (X), medians (-) and outliers (o). Minimal tillage (TT) and direct drill (DD) on average had significantly higher anecic earthworm masses compared to conventional tillage (CT) (P = 0.010).

Macrofauna – Other

There were no significant differences in total macrofauna ($F_{2, 10}$ = 0.33, P = 0.726) (figure 40a) or macrofauna diversity indices ($F_{2, 10}$ = 0.89, P = 0.442) (figure 40b) between cultivations.

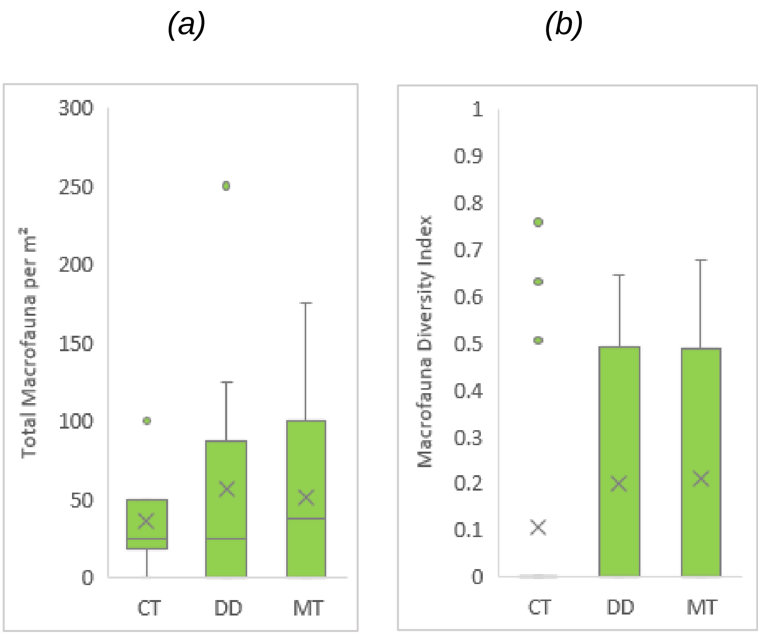


Figure 40: Spring recordings for total macrofauna per m² (a) and Simpsons Diversity Index for macrofauna (b) showing means (X), medians (-) and outliers (o). There were no significant differences between conventional tillage (CT), minimal tillage (MT and direct drill (DD).

Mesofauna

There were no significant differences in total collembola ($F_{2,\,10}$ = 0.64, P = 0.546) or collembola orders (Entomobryomorpha: $F_{2,\,10}$ = 0.26, P = 0.779; Poduromorpha: $F_{2,\,10}$ = 1.23, P = 0.334) between cultivations. There were however significant differences in collembola order Symphypleona ($F_{2,\,10}$ = 5.00, P = 0.031). DD plots on average had 50 more Symphypleona individuals compared to CT plots (P = 0.011) (figure 41).

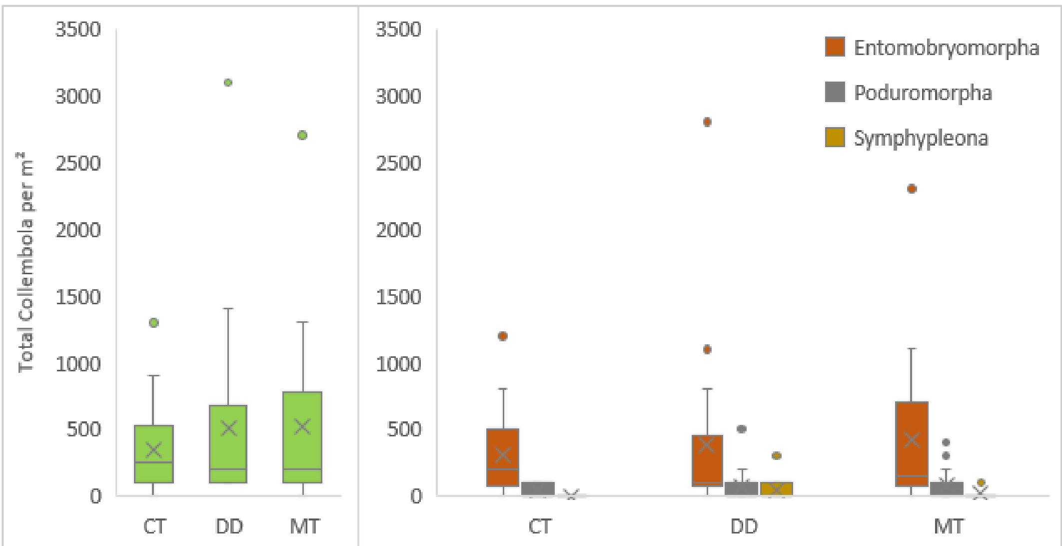


Figure 41: Spring recordings for total collembola per m² as well as by taxonomic order: Entomobryomorpha, Poduramorpha and Symphypleona using a Tullgren extraction method showing means (X), medians (-) and outliers (o). There were no significant differences in cultivations except for Symphypleona (P = 0.031) which were lower in conventional tillage (CT) compared to direct drill (DD). No difference in minimal tillage (MT).

There were no significant differences in total acari ($F_{2, 10}$ = 0.97, P = 0.411) or acari orders (Mesostigmata: $F_{2, 10}$ = 0.54, P = 0.596; Oribatida: $F_{2, 10}$ = 0.29, P = 0.751; Prostigmata: $F_{2, 10}$ = 1.87, P = 0.204; Astigmata: $F_{2, 10}$ = 2.98, P = 0.096) between cultivations (figure 42).

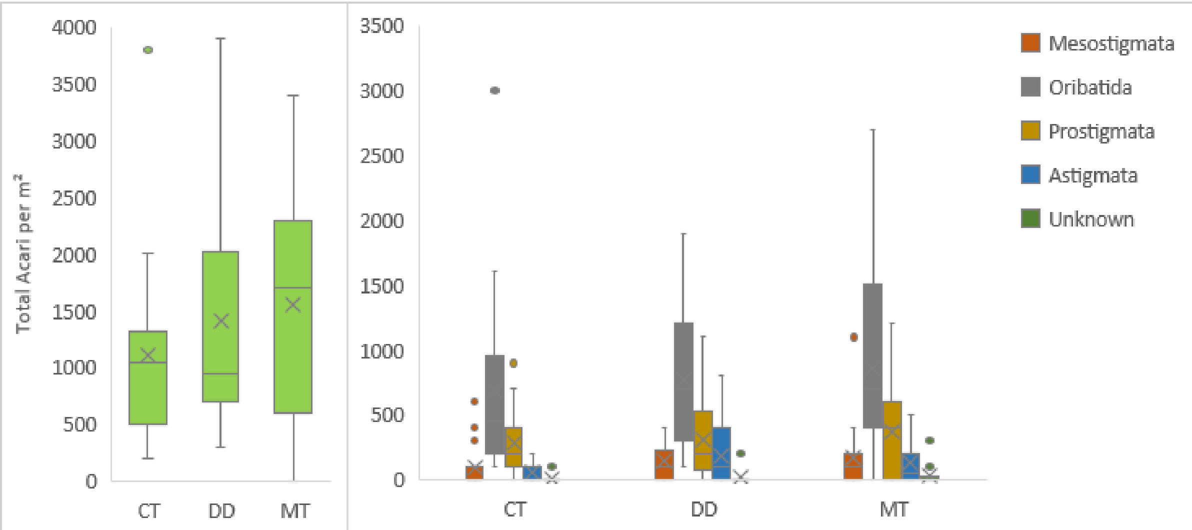


Figure 42: Spring recordings for total acari per m² as well as by taxonomic order: Mesostigmata, Oribatida, Prostigmata and Astigmata. using a Tullgren extraction method showing means (X), medians (-) and outliers (o). There were no significant differences in conventional tillage (CT), minimal tillage (MT) or direct drill (DD) in any of the counts.

There were no significant differences in total mesofauna ($F_{2, 10}$ = 0.94, P = 0.424) (figure 43a), collembola diversity indices ($F_{2, 10}$ = 1.11, P = 0.366) (figure 43b), acari diversity indices ($F_{2, 10}$ = 0.34, P = 0.721) (figure 44a) or acari to collembola ratios ($F_{2, 10}$ = 1.43, P = 0.284) (figure 44b) between cultivations.

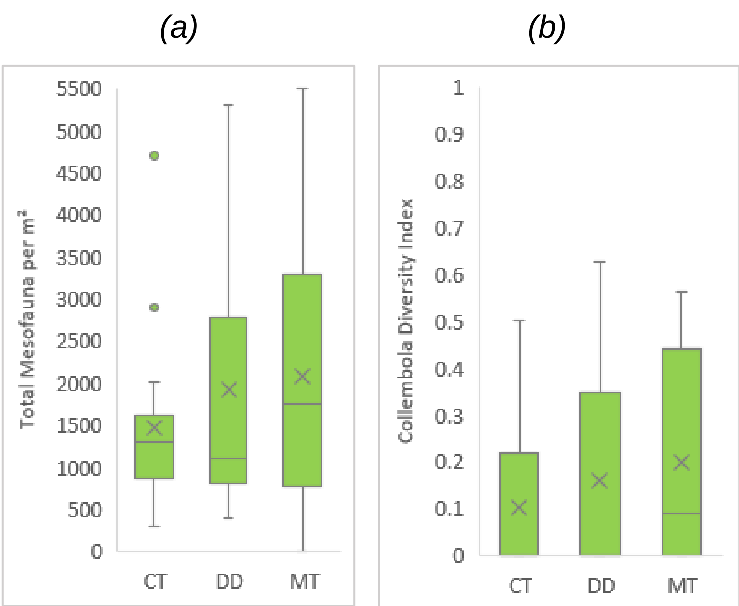


Figure 43: Spring recordings for total mesofauna per m² (a) and Simpson's Diversity Index for collembola (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

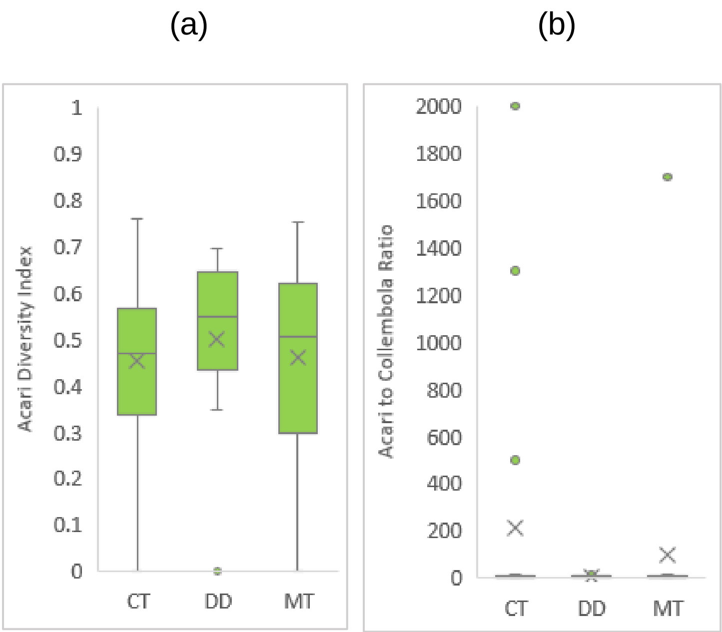


Figure 44: Spring recordings for Simpson's Diversity Index for acari (a) and acari to collembola ratio (b) showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

Microfauna

There were no significant differences in total nematodes between cultivations ($F_{2, 10}$ = 3.06, P = 0.092) (figure 45).

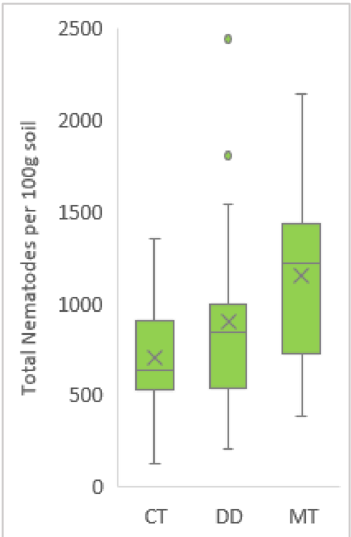


Figure 45: Spring recordings for total nematodes per 100g soil showing means (X), medians (-) and outliers (o). There no were significant differences between conventional tillage (CT), minimal tillage (MT) or direct drill (DD).

Summary Results

Table 6 lists all significant values along with pairwise t-test results for cultivation comparisons for each variable at each stage, values highlighted in red are either highly significant or trending significance.

Table 6: Signficant results summary of all stages

Highest and lowest means, as well as p-values listed for variables nitrate (NO3), gravitational moisture (GM), volumetric moisture - gravimetric method (VM_gm), visual evaluation of moisture content (MC_ve), bulk density (BD), visual evaluation of soil structure (VESS), soil organic matter at 550°C (SOM_550), collembola abundance (COL_t), Entomobryomorpha collembola (COL_ento), total mesofauna (MES_t), visual assessment of soil organic matter (SOM_ve), Poduromorpha collembola (COL_podu), nematode abundance (NEM_t), soil organic matter at 400°C (SOM_400), anecic earthworm abundance (EARTH_anec) and mass (EARTH_anec(g)), Simpson's Diversity Index for earthworms (EARTH_SDI) and Symphypleona collembola (COL_symph)

Type	Stage	Variable	Highest Mean	Lowest Mean	ANOVA P Value	T-Test P Value	
Chemical	1	NO_3	DD: 4.82	CT: 3.25	**P = 0.055**	DD/CT = 0.019	**DD/MT = 0.058**
Physical	2	GM	MT: 24.12	CT: 23.11	P = 0.025	MT/CT = 0.007	
	3	GM	MT: 27.1	CT: 25.69	P = 0.027	MT/CT = 0.011	DD/CT = 0.034
		VM_{gm}	DD: 39.36	CT: 34.88	P = 0.009	DD/CT = 0.005	MT/CT = 0.007
		MC_{ve}	CT: 4.033	DD: 3.561	P = 0.009	CT/DD = 0.002	DD/MT = 0.054
		BD	CT: 0.995	DD: 0.933	P = 0.054	CT/DD = 0.019	
		VESS	CT:3.539	DD: 2.934	P = 0.009	CT/DD = 0.004	CT/MT = 0.008
	4	VESS	CT: 3.141	MT: 2.722	P = 0.006	CT/MT = 0.003	CT/DD = 0.006
Biological	2	SOM_{550}	DD: 8.869	CT: 7.872	**P < 0.001**	DD/CT < 0.001	MT/CT = 0.002
		COL_t	MT: 461	CT: 195	P = 0.017	MT/CT = 0.005	MT/DD = 0.052
		COL_{ento}	MT: 356	CT: 89	P = 0.018	MT/CT = 0.008	**MT/DD = 0.023**
		$MESO_t$	MT: 1662	CT: 684	P = 0.028	MT/CT = 0.015	DD/CT = 0.023
	3	SOM_{550}	DD: 8.814	CT: 7.89	P = 0.009	DD/CT = 0.003	MT/CT = 0.026
		SOM_{ve}	DD: 2.956	CT: 2.294	P = 0.029	DD/CT = 0.016	MT/CT = 0.023
		COL_{podu}	DD: 222	CT: 33	P = 0.006	**MT/CT = 0.002**	**DD/CT = 0.023**
		NEM_t	MT: 1429	CT: 599	P = 0.018	**MT/CT = 0.002**	**DD/CT = 0.001**
	4	SOM_{550}	DD: 8.51	CT: 8.17	**P = 0.071**	DD/CT = 0.024	
		SOM_{400}	DD: 7.2	CT: 6.54	P = 0.019	DD/CT = 0.008	MT/CT = 0.024
		$EARTH_{anec(g)}$	MT: 22	CT: 2.8	P = 0.010	MT/CT = 0.002	**MT/DD = 0.059**
		$EARTH_{anec}$	MT: 13.9	CT: 4.2	P = 0.055	MT/CT = 0.019	
		$EARTH_{SDI}$	MT: 0.18	CT: 0.071	**P = 0.067**	MT/CT = 0.026	
		COL_{symph}	DD: 50	CT: 0	P = 0.031	DD/CT = 0.011	

Soil Organic Matter

Overall, when all stages were collectively considered, there was a highly significant differences in SOM between cultivations ($F_{2, 30} = 21.87$, $P < 0.001$). DD plots were on average higher in SOM compared to CT plots (figure 46).

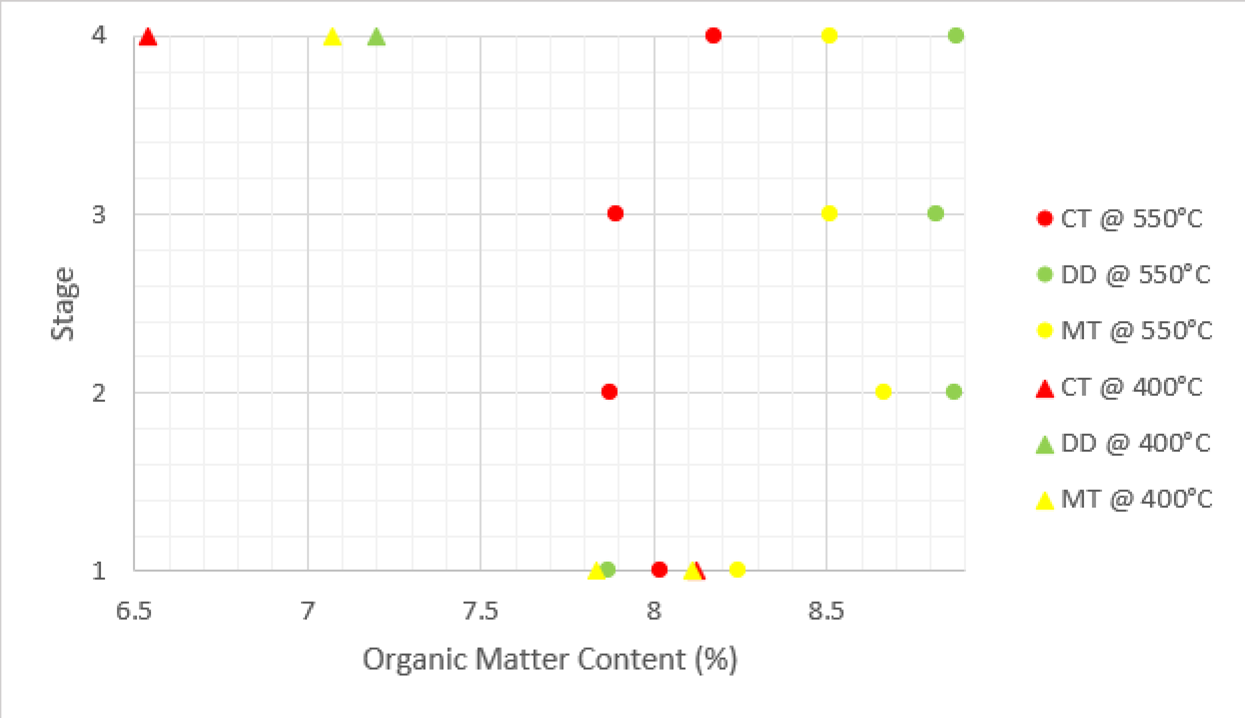


Figure 46: Soil organic matter content means from stages 1-4 for conventional tillage (CT), minimal tillage (MT) and direct drill (DD) plotting loss on ignition results at 550°C for 4 hours as well as 400°C for 16 hours. Overall significance was recorded in DD plots compared to CT plots (P < 0.001).

A significant difference in SOM was recorded for DD plots between stages ($F_{3, 67} = 8.153$, $P < 0.001$). Autumn, winter and spring were all on average higher in organic matter content than summer, with autumn on average 1% higher ($P < 0.001$), winter on average 0.95% higher ($P = 0.002$) and spring on average 1.03% higher in SOM ($P = 0.001$). SOM recordings within cultivations are fairly similar across the stages with a visible increase in CT (figure 46) between winter and spring during crop establishment period.

Moisture Content

Gravimetric moisture content was assessed by stage (see sections above) as well as by month (figure 47) as replicates in stage 2 were taken over a period of 6 weeks where total rainfall had increased substantially. September had 1 replicate and October had 2 over a 2-week period. There were no significant differences between cultivations in September means ($F_{2, 15} = 2.39$, $P = 0.126$) when total rainfall for the week prior to sampling was 8.9 mm, however there were highly significant differences between cultivations in October means ($F_{2, 15} = 16.7$, $P < 0.001$) when total rainfall for the week prior to sampling was 16.8 And 40.7 mm respectively. CT plots on average was 1.97% lower in soil moisture content when compared to MT plots ($P < 0.001$) and 1.49% lower when compared to DD plots ($P < 0.001$).

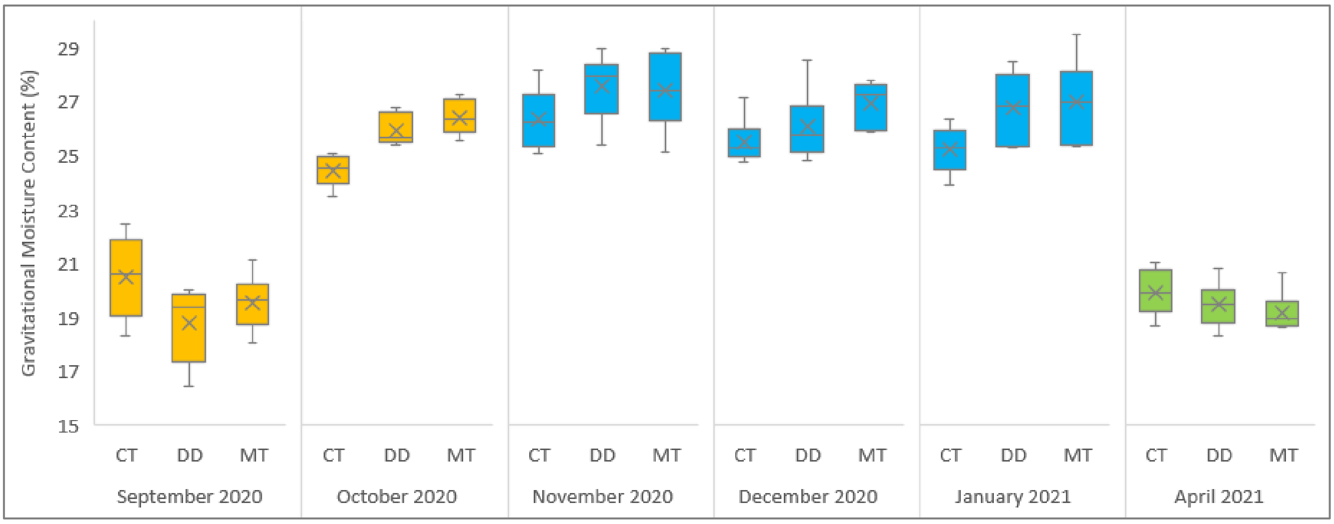


Figure 47: Gravitational moisture content recordings by month using oven dry method highlighting differences in water content within stage 2 (September and October) as well as differences in water content between stage 3 and stage 4. Significant differences were recorded in October measurements (P < 0.001) but not September (P = 0.126). Conventional tillage (CT) was significantly lower compared to minimal tillage (MT) and direct drill (DD) in October 2020.

Mesofauna

Collembola communities overall showed significant differences in both DD and MT when compared to CT in Entomobryomorpha in autumn (P = 0.018), Poduramorpha in winter (P = 0.006) and Symphlypleona in spring (P = 0.031) with overall collembola totals and total mesofauna being significantly affected by Entomobryomorpha results in autumn due to their overall higher abundance levels compared to the other two orders (figure 48).

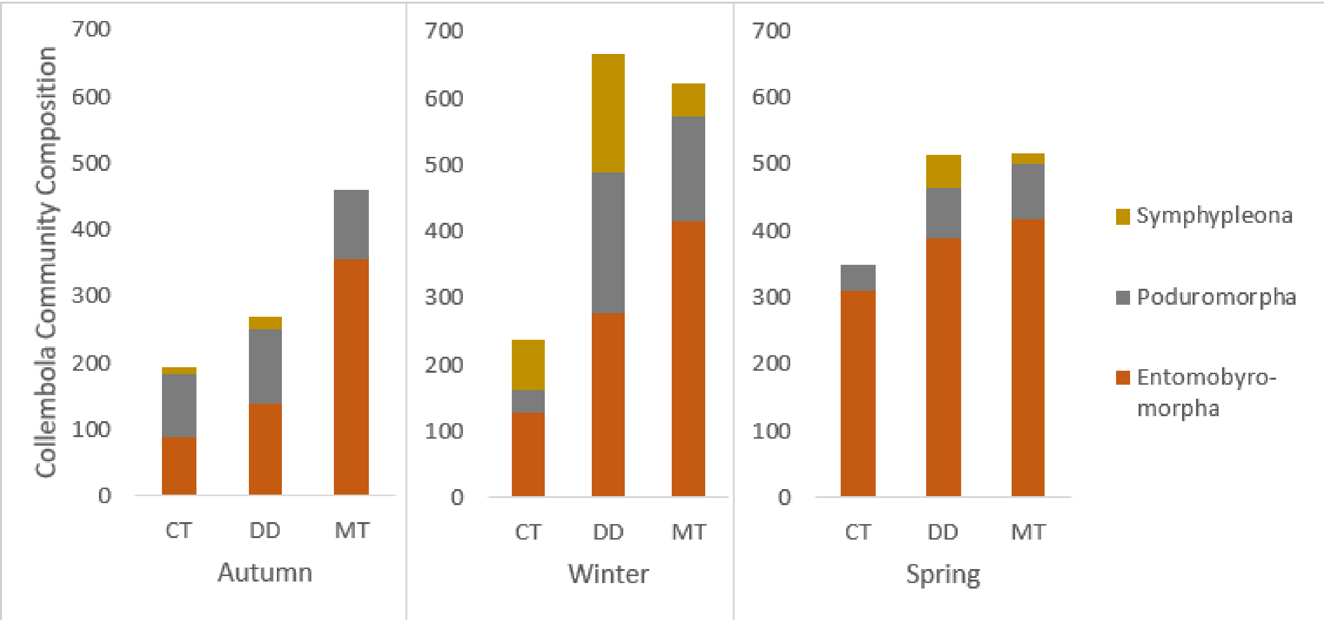


Figure 48: Total collembola community composition by taxonomic orders: Entomobryomorpha, Poduromorpha and Symphlypleona by cultivation methods: conventional tillage (CT), minimal tillage (MT) and direct drill (DD) as well as by stages 2 (autumn), stage 3 (winter) and stage 4 (spring). Significant differences were recorded in Entomobryomorhpa (P = 0.018) in autumn for MT and DD compared to CT, Poduromorpha (P = 0.006) in winter for DD & MT compared to CT, and Symplypleona (P = 0.031) in spring for DD compared to CT.

Discussion

Long-term tillage studies of varying intensity and crop rotations affect soil health (Congreves, Hayes, Verhallen, & Van Eerd, 2015), and thereby influence agricultural sustainability. However, quantifying soil health attributes is a complex process. In particular, the biological component of soil health is the least understood yet potentially the most important aspect of healthy soil because a functioning soil ecosystem is a dynamic living system that delivers multiple services such as water quality, plant productivity and soil nutrient recycling(Karlen et al., 2019; Menta & Remelli, 2020; Nunes et al., 2020). Soil fauna abundance, diversity and activity directly and indirectly affect a soil's ability to deliver on those services (Tahat, Alananbeh, Othman, & Leskovar, 2020). This experiment looks at a range of components within the physical, chemical and biological categorisations of soil health, over various stages of crop establishment. Below each stage is considered individually, followed by a discussion on the collective impact long term tillage has displayed.

Stage 1 – Summer

Chemical

Although a trending significance was found in nitrate DD means (4.82 mg/l) compared to CT means (3.25 mg/l), DD means were also higher compared to MT means (3.54 mg/l) showing less differentiation between MT and CT plots (figure 6a). Furthermore, the variation in means for DD plots were higher (SD ±2.05) compared to MT (SD ±1.07) and CT (SD ±0.82). It is worthwhile to note that significance was based on post log transformation of data and when raw data were assessed, there were multiple high readings in the DD plots which were not replicated in MT & CT plots. This potentially suggests that less soil disturbance allows for concentrated areas of higher microbial activity levels – specifically microbes that are involved in ammonium to ammonia oxidation, as well as nitrite and nitrate conversion which possibly intimates higher biodiversity levels in DD plots. Chemical analysis was only replicated once at the start of the experiment (stage 1), therefore congruency in results cannot be confidently determined as there are no repeated measures. Although these results are in contrast to a review paper conducted by Alvarez and Steinbach (2009), which found nitrate nitrogen significantly higher in CT plots when compared to NT, the experiments reviewed were all non-organically managed crops where nitrogen fertiliser was added as part of the crop management practice.

Significance was not replicated in ammonium results which represents the mineralisation stage of the nitrogen cycle whereby organic nitrogen is converted to ammonium. High levels of ammonium however were recorded in DD plots which had the highest mean (3.4 mg/l) compared to CT (2.16 mg/l) and MT (2.7 mg/l). Variation in DD plots were the highest (SD ±4.1) compared to MT (SD ±1.4) and CT (SD ±1.0) (figure 6b). As plants can absorb either nitrate or ammonium though their root hairs directly from soil or through the establishment of symbiotic microbial relationships, it is unclear in this experiment what the impact tillage has had on the biodiversity of microbes due to the inconsistencies of the results.

Plant available phosphorus (PO_4) showed no significant differences between cultivations, but on average had highest means in MT plots (0.09 mg/l), then CT means (0.06 mg/l) and lowest in DD plots (0.05 mg/l) (figure 6c). Phosphorus is cycled between several soil pools which leads to its limited availability to plants as weathering, mineralisation, dissolution and desorption are all processes that increase plant available P, whereas immobilisation, adsorption, precipitation and runoff/erosion decrease the plant available P (Ruttenberg, 2003). The factors that influence its availability are firstly organic matter which release plant available P through the decomposition process as well as molecule adsorption taking place. In this experiment, significant differences were found in organic matter consistently across the stages, except for stage 1, however potentially the reasons for this are not due to comparative OM levels which are discussed in more detail under Soil Organic Matter Content section below. Other factors that influence P values are clay content, soil mineralogy (metal content of soil) soil pH, temperature, moisture, and soil aeration. Although it may be deduced that soil tillage does not have a major influence on plant available P content of soil, measurements were not consistently taken across the sample interludes to confidently confirm such a relationship within the parameters of this experimental environment. However, as the plant availability content of P is reliant on environmental factors such as clay and mineral content of soil, precipitation and atmospheric temperatures; if abiotic factors are prone to naturally reducing the plant availability of P, tillage practices would be important to consider as tillage has be shown to reduce organic matter content of soils which is the leading contributing factor to plant available P (Zibilske et al., 2002).

Soil Organic Matter

In stage 1, samples were processed at an alternative site due to equipment availability. The muffle furnace used was 27 years old with a large cavity that enabled all 52 samples to be ignited at the same time. The means of each cultivation method however, were not only very similar to one another, but they were also very similar at different burn rates (550°C: CT – 8.018, DD – 7.867, MT – 8.247; 400°C: CT: 8.113, DD – 7.831, MT – 8.110) (figure 7). This is not congruent with the other stage results, nor is it in alignment with other studies (Heiri et al., 2001; Nelson & Sommers, 1996; Salehi, Beni, Harchegani, Borujeni, & Motaghian, 2011). It is therefore highly likely that the efficiency of the muffle furnace has substantially degraded over its lifespan and potentially not fit for purpose.

Loss on ignition is one of the most widely used methods for determining SOM in soils but does not have a uniform protocol. A wide range of factors may influence its accuracy such as type of furnace used, the mass of the sample, the positioning of the sample within the furnace, the duration and temperature of ignition as well as clay content of soil samples. Many authors (Heiri et al., 2001; Hoogsteen, Lantinga, Bakker, Groot, & Tittonell, 2015) have conducted a series of experiments to quantify these effects in pursuit of more structured guidelines. Three confounding factors predominate when deciding on their protocol, which is incomplete combustion of SOM at low temperatures (Ball, 1964); the removal of structural water from clay minerals (Sun, Nelson, Chen, & Husch, 2009) and the decomposition of soil carbonates at higher temperatures (Kasozi, Nkedi-Kizza, & Harris, 2009).

In a literature review conducted by Hoogsteen et al. (2015), 40 publications were found spanning 1960 – 2011 where LOI was used as a method for determining SOM in either sediments, compost or soils. Little consistency was found in that literature as to temperature and duration combinations that should be employed for accurate determination, with a wide variety in both temperatures (300-1000 °C) and ignition duration (30 minutes to 32 hours). Many studies were characterised by the lack of information regarding the details of LOI application with very diverse analytical conditions. The type of furnace was only reported in 35% of the studies, of which there were eight different makes of furnaces. Sample mass was only reported in 62% which varied between 0.2 g and 20g averaging at 5g. However, as this experiment is focussed on comparative analysis rather than accurate determination of SOM, two methods were settled on and clay correlation factors – suggested by Ball (1964) were not incorporated for ease: one that will account for structural water loss by lower temperature and higher duration (400°C for 16 hours) and the most commonly used method (550°C for 4 hours), referenced from Ball (1964) and Radojevic and Bashkin (2007) respectively.

When comparing stage 1 results to the stages 2, 3 & 4, a difference in at least the different methods would be expected, therefore the results achieved point to an instrumental influence rather than a treatment influence.

Stage 2 – Autumn

Soil Moisture Content

Soil moisture content was significantly higher in MT plots alone when compared to CT plots. DD plots showed no significance in variation compared to MT or CT plots (figure 11). Replication of sampling did not take place within close repetitions of one another but rather over a 6-week period. The first repetition took place on the 21st of September where daily rainfall in the week prior to sampling was 0 mm except for one day (19th of September) recording 8.9 mm of rain. When this dataset was analysed, there were no significant differences present. The second and third repetition took place on the 22nd and 28th of October where rainfall had increased to 16.8 mm and 40.7 mm respectively. When this dataset was analysed, there was a highly significant difference present. In addition to separate month analysis, an average of all three samples were used to determine overall stage significance, which highlighted significant variation between plot treatments (figure 11).

The variation in rainfall appears to have influenced the soil's water holding capacity when wet, and an increase in saturation levels in DD & MT plots in wet conditions may have a contributing effect on plant growth and microbial activity (Alvarez & Steinbach, 2009). Clay soils naturally have higher water holding capacity than coarse soils as texture influences porosity and soil aggregate structure (Brady, Weil, & Weil, 2008). Organic matter also influences a soil's water holding capacity as it acts like a sponge not only alleviating compaction but absorption. Soil cultivation increases evaporation of water by exposing the soil profile to atmospheric temperatures therefore will have a compounding affect in dry hot weather conditions, but an alleviating affect during cold and wet weather conditions. Constant soil saturation creates an anaerobic environment which in itself promotes greater populations of aquatic biota such as nematodes, protozoa and amoebas which may lead to an imbalanced soil food web as their predators (micro-arthropods) require air pockets within and between soil aggregates for their habitats (Barrios, 2007). Within the soil food web, microflora (bacteria, fungi and mycorrhizae) are predominately responsible for organic matter turnover, whereas microfauna (bacteria- and fungal feeding protozoa and nematodes) are predominantly responsible for nutrient transfer. Mesofauna (collembola and mites) improve soil structure and disease transmission whereas macrofauna (earthworms, and macro-arthropods) degrade pollutants (Fortuna, 2012). Although each trophic level overlaps functions in many instances, each group fulfils an ecosystem function and requires favourable conditions for their expansion or depletion. Where the water content of the soil is in imbalance, it impacts the oxygen levels within the soil environment and thus influences the energy flow within the soil food web which impacts plant growth. Most organisms on earth are dependent on six elements: carbon, oxygen, hydrogen, nitrogen, phosphorus, and sulphur, which are transformed by soil organisms based on the environmental factors favouring their growth or decline. It could therefore be argued that a strategic approach to cultivation would have a potentially positive effect on both plant growth and soil biota. The amount of water to air-filled pore space within soil aggregates plays a key role in population composition on a microscopic scale which has a compounding affect up the energy chain.

Mesofauna

In autumn, Entomobryomorpha, total collembola and total mesofauna means were all significantly higher in MT compared to both CT and DD means (figure 18). A dataset analysis reveals this is due to the high means of Entomobrymorpha in MT plots (355.56) compared to DD (138.89) and CT (88.89) plots as well as higher (NS) means in total acari, as Poduromorpha and Symmplypleona means were higher in DD plots, but consistently lower in CT plots across the board. Although transformation requirements were met, variability in Entomobryomorpha MT means were greater (SD ± 547.95) when compared to DD (SD ±125.34) and CT (SD ± 140.98). Variability with two instances of particularly high quantities (2200 & 1200) in individual plots were recorded at the same time (2nd replicate – 21st October 2020) shortly after first rainfall (8.9 mm).

Entomobryomorhpa is one of three main taxonomic classifications of collembola orders which is primarily based on their morphological features, specifically their body shape; long and slim. They can either have short or long legs, antennae as well as well-developed or under-developed furculae (jumping organs). Their body shape however is not an indication of their feeding strategy and an alternative classification in discussed in many studies (FAO et al., 2020; Potapov, Semenina, Korotkevich, Kuznetsova, & Tiunov, 2016; Rusek, 1998).

Collembola often display a suite of morphological characteristics correlated to their habitats known as their ecomorphological life forms. Collembola have four ecomorphological life forms based on their trophic niches (Potapov, Semenina, Korotkevich, Kuznetsova, & Tiunov, 2016). Atmobiotic life forms relate to collembola found on litter surfaces as well as on vegetation. They are generally large with round or elongated pigmented bodies and well developed furca (jumping organs) and full sets of ocelli (8 eyes). Epedaphic life forms inhabit the upper layers of litter or soil surface, they have medium to large size bodies, well developed furca but smaller legs and antenna. Hemiedaphic life forms are medium to small in size, have less pigmentation, shortened legs and reduced number of ocelli and inhabit decomposing vegetation. Euedaphic life forms have medium or small elongated bodies, no ocelli or pigmentation, reduced or absent furca, and inhabit the upper soil horizons and or upper mineral layers. The atmobiotic and epedaphic species collectively referred to as the epigeic group, while the hemiedaphic and euedaphic species are collectively referred to as the edaphic group. According to a study conducted by Christiansen et al. (2009) on species typically found temperate forest communities, Entomobyromorpha consisted of 49% epedaphic, 24% atmobiotic, 22% hemiedaphic, and 5% euedaphic species. This order constitutes the largest superfamily of collembola: Entomobryoidea along with Tomoceroidea and Isotomoidea which are usually very active above ground and rarely euedaphic (Zhang, Bellini, & Soto-Adames, 2019).

Although no distinction between life forms were made in this study, based on Christiansen et al.'s research, it is likely to not be atmobiotic species as new vegetative cover was minimal at this stage of the experiment, however visible residue was present in DD and MT plots which may account for the higher recordings in Entomobryomorpha species in MT plots, but not why MT plots were significantly higher compared to DD plots. Both DD and MT plots were cultivated at 7.5 cm depths between the 24th of August 2020 and 5th September 2020, albeit twice for MT plots compared to once for DD plots. Total rainfall for the period between cultivation and sampling was low in September (19 mm) and high for October (114 mm) with more moisture contained in MT plots where GM was significantly higher in MT but not DD, therefore it could be argued moisture levels played an important role in the abundance levels of mesofauna with an assumption this order of collembola is particularly active at this stage (autumn/post germination prior to plant maturation).

In stable isotope analyses ^{13}C and ^{15}N values in collembolans were found to relate to their life forms reflecting a movement in available food sources across different habitat layers matching vertical isotopic gradients of SOM (Potapov et al., 2016). When Potapov et al. linked these isotope compositions with taxonomic identity and life form groupings, they were able to identify four functional guilds that access different types of food and perform specific ecosystem functions. As detailed below in stages 3 & 4, a specific collembola order was highlighted as significantly higher in either DD and or MT plots when compared to CT plots which had distinctly different vegetative cover as well as temporal stages.

Stage 3 – Winter

Soil Water Content

In the winter results, a significant difference in soil water content was recorded, similar to autumn results, however this stage recorded a significance in both MT ($P = 0.011$) and DD ($P = 0.034$) plots when compared to CT plots in both mass recordings as well as volumetric recordings (figure 22). The latter however showed slightly more significance in DD plots ($P = 0.005$) than MT plots ($P = 0.007$) when compared to CT plots. As per the October moisture measurements, consistent high rainfall was recorded throughout winter sampling (22 November to 11 December 2021) with a total rainfall of 56.8 mm with only 2 days of no rain.

The visual evaluation of moisture content produced conflicting results whereby CT plots were shown to be significantly higher in moisture content when compared to DD and MT plots. These findings were not repeated in either autumn results or oven dry method therefore it would be a fair estimation to conclude the method is not a reliable way to assess moisture content. Furthermore, moisture meter readings did not result in similar significance for either autumn or winter sampling, in line with oven dry method thus bringing its reliability into further question.

This experiment focused on soil water content however soil water potential should also be considered when assessing impact on soil health as this will provide a better understanding of available water to both plants and soil biota. Water that is functionally available to plants and soil organisms is frequently viewed simply as incoming precipitation (Schwinning & Sala, 2004), however the process of precipitation's transformation into water uptake is complex. Soil depth, texture, organic matter content, porosity, intensity and frequency of rainfall, atmospheric temperature, vegetation cover, compaction, tillage, animal burrows, ground slope etc. all play a role in the amount of water that will infiltrate the soil surface and when or at what pace it will drain away (Loik, Breshears, Lauenroth, & Belnap, 2004). These factors all impact what plants and soil organisms are able to utilise. This in turn impacts population sizes, community compositions as well as plant shoot vs root growth, parasitic susceptibility etc.

Soil Structure

Soil compaction is a continuous concern for farmers. Conventional tillage has traditionally been employed to alleviate topsoil compaction to aerate the soil, facilitate seed bedding and dislodge weeds thus increasing yields. This in turn has led to an increase in compaction due to aggregate displacement, heavy machinery loading - particularly in wet conditions - exacerbated by low soil organic matter content (Hamza & Anderson, 2005). In this experiment soil structure was assessed through bulk density in stages 2 & 3, VESS scoring in stages 2, 3 & 4 as well as compaction measurement in stage 4. Although no significant differences were noted in stage 2 for bulk density and VESS scoring, they were noted in stage 3. DD plots were significantly lower in bulk density compared to CT plots and both DD and MT plots were significantly lower in VESS scoring when compared to CT plots (figure 24). This is in contrast to other studies that proclaim no tillage increases soil compaction (Tormena, Barbosa, Costa, & Gonçalves, 2002).

The degree of compaction is influenced by various factors; soil texture, soil organic matter, water status, structure of tilling, axle load, tyre dimensions and velocity as well as soil-tyre interactions (Hamza & Anderson, 2005) and even in no till systems, heavy machinery will pass at least 30% of ground area (Tullberg, Hunter, Paull, & Smith, 1990). Furthermore, all of the plots in this experiment were subsoiled to at least 30cm depths after the previous year's harvest (T. Norris, personal communication, 14 September 2021).

Stage 2 bulk density results showed no significant differences between cultivations, even when split between post and pre-rainfall (figure 13). Stage 3 bulk density results in contrast did with lowest density in DD plots (P = 0.01) when compared to CT plots with no difference in MT plots. Since soil bulk density is the most frequently used method to characterise soil compactness and is conducted on dried soils, soil moisture may account for significant vs non-significant results. In a study conducted by Grant and Lafond (1993) on tillage impact in clay soils, no till plots had significantly higher soil bulk density when compared to CT plots in the top 10 cm soil layer, but had no difference in moisture content. The same study though found differences when comparing crop type and rotation variations.

Moisture levels have been shown to affect bulk density and compaction rates as per the review compiled by Hamza and Anderson (2005). Furthermore, type of soil (clay, loam or sand) influenced compaction rates. The effects of tillage on soil water content, SOM and soil compaction were investigated by Benito et al. (1999). They found that less intensified tillage methods conserved more water, particularly in dry years. Compaction was less and organic matter content increased which is comparative to this experiment. But this is in contrast to an experiment conducted on Mollisols which is a semi-arid region soil with high organic content (Hill & Cruse, 1985) and deep-loess soils which is a rich porous dust-like soil, both having higher compaction rates in reduced tillage compared to conventional tillage.

Mesofauna

In the winter sampling results, Poduromorpha were on average significantly higher in both DD and MT plots compared to CT plots (figure 29), along with nematode abundance which also showed significance in both MT and DD when compared to CT plots (figure 32). While most soil inhabiting collembola primarily feed on decomposing vegetation and fungi, experimental studies (Christiansen et al., 2009) have shown that, given a choice, collembola display selectiveness to both the state of decay and nature of vegetation as well as fungi species. A number of collembola are primarily or exclusively carnivores, feeding on a variety of organisms, ranging from other collembola to rotifers and most commonly, nematodes (Hopkin, 1997). In a study referred to in stage 2, Poduromorpha orders in temperate forests were found to consist of 48% euedaphic species and 38% epedaphic.species (Potapov et al., 2016). Euedaphic species have been shown to feed on fungi as well as nematodes (Kaneda & Kaneko, 2011). Furthermore Malcicka, Berg, and Ellers (2017) identified in their experiment that all predator/scavenger species of collembola were from the Poduromorpha order as their diet could be associated with mouthpart morphology independently of the phylogenetic relationships even though all three orders spanned all feeding guilds (bacterivores, fungivores, herbivores, scavengers, predators). Specifically, they found several species from the Neanuridae family have adjusted to a scavenger/carnivorous lifestyle by structural modifications of maxillae and have evolved scratching/piercing mandibulae.

In a recent study conducted by Moos, Schrader, and Paulsen (2020), consideration was given to conventional vs reduced tillage practices that had been implemented for over a decade which specifically looked at the impact on collembolan communities. Although no significant differences were found in species of community compositions, species were reclassified into life forms which varied substantially based on which literature was referred to. According to their classification, euedaphic individuals consisted predominantly of Poduramorhpa collembola and their proportion can be used as an indicator for stable soil habitat conditions. They also found, as arguably this study has, that collembolan abundance and species composition reacted to intermingled effects of different crops cultivated with interannual variability.

Microfauna

In this experiment, winter sampling resulted in significantly higher abundance counts of nematodes in both MT and DD plots when compared to CT plots (figure 32), with no other significance recorded at any other stage. Stage 3 also experienced the most continuous rainfall (see appendix 1 for full rainfall chart), which is important to note as nematodes are aquatic species and are likely to increase in population size when conditions are most ideal (Neher, 2001). Investigations that focus on this microfauna group can provide highly valuable information relating to the functional changes within arable soils (Freckman, 1988) as they show clear preferences for certain food sources (Ruess, Zapata, & Dighton, 2000), as well as being highly sensitive to soil moisture content (Kaya & Gaugler, 1993). Therefore cultivation methods that alter these soil conditions would likely impact nematode community compositions, and may result in promoting or inhibiting specific soil processes and functions (van Capelle, Schrader, & Brunotte, 2012).

Although nematode diversity was not explored in this experiment, samples were taken at different times of the year which were correlated with different stages of the crop growth cycle. Significance in one of these stages may intimate functional diversity which would warrant further exploration. Other studies have found varying results; van Capelle et al. (2012) found no difference in nematode abundance among tillage systems, which was in contrast to Fu, Coleman, Hendrix, and Crossley Jr (2000) and Treonis et al. (2010) who found abundance to decrease when intensity of tillage was decreased, whereas Parmelee and Alston (1986) found abundance was dependent on when in the year samples were taken. The latter experiment found CT abundance was in general greater than NT, except in summer months when plant parasitic nematodes were in greater abundance. It is worth noting their sampling depth was 20cm, their extraction method was not detailed, and their soil was a well-drained sandy/loan/clay soil. In a recent European review paper compiled by D'Hose et al. (2018), only 2 studies were found relating to non-inversion tillage impact on nematode abundance and community composition, and in both those experiments, no significant differences were reported on bacterivorous, fungivorous and plant-parasitic nematodes. This is however in contrast to what would be expected; it is generally considered that decomposition rates are faster in conventional tillage systems compared to minimal and no till systems (Six, Elliott, & Paustian, 1999). This is potentially because the soil is exposed to oxygenation and litter is buried four times faster (Parmelee & Alston, 1986) in CT systems when compared to NT systems. This in turn favours higher densities of bacteria and metabolic activity (Gołębiowska & Ryszkowski, 1977) whereas crop residue accumulation in NT systems favours increased abundance in fungal communities.

Research into nematode community composition has traditionally focussed around plant parasitic behaviour (Thomas, 1978) and impact on tillage on nematode communities is less well-known; therefore it is clear more studies are required that would be able to provide robust evidence on tillage impact.

Stage 4 - Spring

Soil Structure

The Visual Evaluation of Soil Structure (VESS) showed CT plot means (Sq 3.539) on average graded higher when compared to MT means (Sq 2.99) and DD means (Sq 2.934) in winter (24a), and similarly in spring where CT means (Sq 3.141) were significantly higher than MT means (Sq 2.722) and DD means (Sq 2.769) (34a). Treatment means were all lower in spring compared to winter, which crop establishment would have potentially played an important role in. This is not particularly reflected in the SOM results which although do show a remarkable increase in SOM for CT plots from winter to spring, this improvement is not replicated in DD and MT plots (figure 46). Although this experiment's results do not correlate with the findings of Celik et al. (2020) who found an increase in VESS score correlated with the reduction of mechanical manipulation of the soil, it is important to note that there were marked differences in tillage definitions. CT plots in Celik et al's experiment were subjected to a mouldboard plough reaching depths of 30-33cm, whereas in this experiment a five-furrow plough reaching depths of 18cm was used. In addition, their RT treatment plots were aggressive in their depths (18-20 cm) and applications, whereas this experiment used the same disk harrow (7.5 cm depth) for MT and DD, with a differentiation of passes (1 for DD, 2 for MT).

As VESS scoring provides a scale measure of quality of soil structure - Sq5 being poor and Sq1 being good – this experiment was not able to determine that any of the treatments rendered the structure of the soil as poor. CT has however shown to degrade the soil structure which directly impacts not only root penetration, but may also indicate a reduction in biota activity, specifically the species that burrow (macro- and mesofauna) and break up aggregates in the soil (Oades, 1993).

In addition to a visual evaluation of the soils, soil compaction was measured to assess tillage impact. The assessment took place at the final stage of the experiment where below ground vegetation was at its highest (full crop establishment) and moisture levels at their lowest thereby rendering a non-significant result. All plots, regardless of treatment, were subsoiled post 2019 harvest due to compaction concerns at the time. A subsoiler consists of three or more heavy vertical shanks that can be operated at depths of 35–75 cm deep.

It has been proposed by Johannes et al. (2017) that the soil organic carbon (SOC) to clay ratio of soil is a pertinent criterion when considering structural quality of soils due to the contribution of SOC to resistance and resilience of soil structure. When comparing SOC (based on an accepted standard of estimation: 58% of SOM (Nelson & Sommers, 1996)) ratio to clay content by treatment, CT mean (1:10.6), MT mean (1:10) and DD mean (1:9.7) all fall within the reasonable (1:10) to optimal (1:8) range. SOC estimations were taken from spring samples results based on 400°C temperatures.

Earthworms

At the turn of the century, there were conflicting reports on the impact tillage had on earthworms (Chan, 2001). Tillage was reported to impact abundance and community composition of populations in different ways which were all reported to be dependent on soil and climatic conditions as well as land management practices. Reduction in tillage did not and does not automatically equate to increase of earthworm populations. However, the report does point out that the reason for confliction is the lack of accurately describing both tillage practices as well as species affected. In the studies that do describe species, there is a congruency in identifying that deep burrowing species such as anecic earthworms generally experience a decline with increased tillage intensity, and in contrast increases endogeic species which are r-strategists and show more resilience to disturbances.

Factors affecting earthworm abundance and diversity are temperature, moisture, carbon to nitrogen ratios (i.e. quality of organic matter), predation and competition (Edwards, 1997). Earthworms do not have the ability to maintain their internal water content and are thus reliant on the water potential of their surrounding environment in determining their feeding and fecundity behaviour (Chan, 2001; Edwards, 1997). It was also found that particularly in anecic species, activity ceased at water potential values much higher than plant wilting points (Baker, Barrett, Carter, Williams & Buckerfield, 1993; Kretzschmar & Bruchou, 1991).

Although water potential was not measured as part of this experiment and higher abundance of anecic earthworms were found in spring when rainfall temperatures were at the lowest. Gravitational moisture content consistently recorded higher means in MT alone at stage 2, and both MT & DD at stage 3. High rainfall was recorded in October and consistent rainfall recorded during winter sampling. Soil organic matter content recorded higher means in DD & MT consistently throughout the experiment. It can therefore be likely that higher water content transpired to higher water potential in reduced tillage plots which provided more favourable conditions for anecic earthworms that were able to inhabit higher levels of the soil profile.

Changing regimes of soil water content and temperature affect different species of earthworms in terms of their migration activity up and down the soil profile therefore “active hotspots” may vary at different times of the year (Gerard, 1967). Volumetric moisture content did not explain differences as moisture conditions ranged from poor (<20% & >40%) in 5 CT plots, 2 DD plots and 4 MT plots to optimum (25% - 30%) in 3 CT plots, 5 DD plots and 2 MT plots according to Onrust, Wymenga, Piersma, and Olff (2019) categorisation. Temperatures remained optimum (Curry, 1998) for earthworms throughout stage 4 (10-20°C).

Conventional tillage does however have the obvious impact on larger, long living k-strategists such as anecic earthworms due to the mechanical manipulation required to alter the soil. Such action not only increases the chance of physical destruction of earthworms, but also the destruction of burrows, redistribution of litter outside of changes to SOM and soil moisture content. That being said, the intensity of plough (depth, axle load), the time of tillage (spring or autumn i.e. dry or wet conditions), soil types and crops all affect the impact load under the land management practices factor (Gerard & Hay, 1979).

In one of the most recent national surveys carried out on tillage impact on earthworm communities, conducted in the spring (15^{th} March – 30^{th} of April) all epigeic, anecic and total abundance earthworms were all negatively affected ($p < 0.05$) by conventional tillage except for endogeic earthworms. No details or descriptions of tillage types were given by participants other than “plough”, “min till”, “no till” or “pasture”, however crop type and organic amendment was described (Stroud, 2019).

Outside of ecological functional segregation of earthworms, functional attributes can be assigned to certain species for instance compacting vs de-compacting (soil loosening) earthworms as discussed in a study conducted by Guéi Jr, Baidai, Tondoh, and Huising (2012). The study showed that the presence of both types of earthworms had a positive correlation with stable soil structure rather than one type on its own regardless of whether they were compacting or de-compacting the soil.

Collembola

Symplypleona species were significantly higher in DD plots (not MT) when compared to CT plots at stage 4 (figure 41). This is also when organic ley had reached maturity and livestock (sheep) had been introduced to field to graze (pre-sampling). These are the third major group of collembola and include the spherical or globular species. It is a small group with much more uniformed habits (DeWalt & Resh, 2019) such as being active jumpers, superficial litter layer and vegetation dwellers, as well as in forest canopies with a predominant diet of fungi and plant tissue (Malcicka et al., 2017).

Potapov et al. (2016) described Symphlypleona species as 57% atmobiotic, 36% epedaphic and 7% hemiadaphic specis, which was identified in temperate forest environments, however Moos et al. (2020) identified Symphlypleona species found in tilled organic agricultural fields as 100% hemiadaphic. Collembola are morphologically and ecologically categorised into feeding guilds. Sucking mouth parts feed mainly on bacteria, yeast and other organisms from fermenting fluids and water films covering solid soil particles while predators have modified mandibles and maxillae and feed on nematodes, rotifers, tardigrades, enchytraeids or even on other Collembola. The feeding guilds can be recognised by the morphology of their mouth parts (Wolter, 1963) and gut content analysis (J Rusek, 1986). The life-form structure is an important aspect of community compositions and reflect the state of soil development during succession. It can also provide an indication of soil moisture content and humus formation as collembola species belonging to different life forms distributed calcium in different soil layers (Rusek, 1998). Epigeic species deposited calcium at the soil surface whereas euedaphic species deposited at the bulk soil layers. Many atmobiotic species are phytophagous and feed on live plant tissues with large bodies, long limbs, full sets of eyes and brightly coloured (Rusek, 1998). Due to the proliferation of above ground and indeed below ground vegetation at stage 4, it can be argued the life forms present are atmobiotic;, however in contrast, if disturbance is affecting biodiversity abundance it is also possible the life forms of species found are hemiadaphic as Song, Liu, Yan, Chang, and Wu (2016) showed these to be sensitive to environmental stresses.

Using higher taxonomic classifications is widespread in soil biota distribution studies, but this is only useful in revealing general trophic groups (Malcicka et al., 2017). Collembolans occupy at least three trophic levels and although order of collembola can account for the majority of trophic variability, similarities in niches is likely based on complex inherited traits share within various taxonomic groups that could be better explored through ecomorphological classifications.

Isotopic composition is closely linked to collembola order however as Symplypleona have the most depleted ^{15}N values compared to the other two orders, this may be a strong indication that they are closely linked to primary producers (Malcicka et al., 2017). Although ^{15}N can be closely linked to order, ^{13}C values can vary greatly, suggesting a variation in carbon feeding source (Malcicka et al., 2017). Therefore overall, it seems likely, according to the above study, that collembola order can approximately predict trophic level, but family identity will likely indicate type of resource consumed. This in turn will provide better insight when assessing environmental impact – in this case land management practices – on diversity and abundance.

Collective Stages

Soil Moisture

Gravitational moisture was repeatedly higher in MT plots compared to CT plots, particularly in Autumn recordings where average rainfall was highest (4.03 mm per day) compared to all stages. Winter results showed GM significantly higher in both MT and DD readings compared to CT, MT more so than DD where rainfall averages were 2.9 mm per day, however volumetric moisture content – plant available water - was highest in DD plots compared to CT plots, however MT plots showed significant difference as well when compared to CT plots (figure 47).

This would intimate that DD and MT plots retained more moisture in high rainfall periods which may be linked to SOM content and its ability to improve a soil's water holding capacity. Furthermore, when assessing methodology, the gravimetric method resulted in significant differences whereas the moisture meter did not. As water molecules in soil profiles provide habitats for soil biota as well as the ability for nutrient transfer, we may potentially correlate the significant higher numbers of nematodes to GM content and mesofauna to VM content. Studies comparing tension meters, gravimetric measures and moisture meters show discrepancies in the accuracies of moisture meters when compared to volumetric moisture levels based on gravimetric methods which are viewed as the standard in measuring moisture levels in soil (Majhi & Sarkar, 2019).

The visual evaluation of moisture content showed contrasting significance indicating higher levels of MC in CT plots as well as MT plots compared to DD therefore it would be deduced that this method is not reliable when compared to standardised practice.

Soil Organic Matter Content

Soil organic matter was consistently higher in DD and MT plots at stages 2, 3 & 4, except for stage 1 which has been discussed below. Stage 4 results showed significance for DD only when ignition temperature was at 550°C, however both MT and DD were significantly higher when ignition rates were 400°C (figure 46).

Loss on Ignition is a method used to oxidise organic matter to CO^2 at an elevated temperature and has been popularised due to its simplicity, cost effectiveness and avoidance of chemical waste. Temperatures higher or lower than 450°C provide estimates of acceptable accuracies, however accuracy is greater for lower ignitions (between 375 and 450) which eliminates variations due to weight loss of structural water and destruction of carbonates from clay minerals. Accuracies are not applicable to all soils and all forms of organic matter and substantial viariances have been shown in OM/C ratios (Ball, 1964) , however it is generally accepted to use such a simple method for ecological studies where a high level of accuracy is not required. Nevertheless, a temperature around 375 – 450°C was identified as optimum by several authors. Salehi et al. (2011) for example evidenced the aforementioned temperatures burnt most organic carbon, destroyed less inorganic carbon and caused less clay structural water loss. Additionally Keeling (1962) demonstrated that more than 90% of the carboneaceous material was removed at a controlled temperature of 375°C for 16 hours without loss of structural water.

In this experiment, it is evident that a signicant difference in SOM was recorded at 550 °C compared to 400°C. Apart from summer samples, results showed that DD plots were consistently higher in SOM compared to CT plots both in overall averages as well as at individual stages (figure 46). Due to the age (27 years) of the muffle furnace used for the summer samples, it is highly likely that its efficiency has degraded and not fit for purpose. The means of each cultivation method were not only very similar to one another, but they were also very similar at different burn rates (550°C: CT – 8.018, DD – 7.867, MT – 8.247; 400°C: CT: 8.113, DD – 7.831, MT – 8.110). This is not congruent with the other stage results, nor is it in alignment with other studies (Heiri et al., 2001; Nelson & Sommers, 1996; Salehi et al., 2011). This experiment initially focused on following the standardised methods described by Radojevic and Bashkin (2007) which recommends a temperature of 550°C for 4 hours, however other studies (Heiri et al., 2001; Nelson & Sommers, 1996; Salehi et al., 2011) show that for clay soils, a lower temperature for a longer period is required for greater accuracy when comparing tillage impacts of varied intensity.

Furthermore, significance levels were greater in 400°C (table 6) ignition rates compared to 550°C as well as showing significance levels in MT compared to CT which were not evident at 550°C. Although this experiment shows that SOM is reduced by land cultivation on average, it has also highlighted that ignition temperatures and exposure durations are crucial as they can skew significance in results.

Discarding the results of stage 1, all SOM recordings showed no significant increase or decrease in any cultivation over the year, however there was a remarkable increase in SOM for CT plots from winter to spring which may indicate that a fertility building ley may provide a higher level of remediation to disturbed soils.   Crop rotations such as leguminous “fertility building” leys are a pivotal phase within organic farming practices. It is a nutrient building strategy usually incorporated from one to five years in length whereby atmospheric nitrogen is fixed by the legume-rhizobium symbiosis, and made available to proceeding crops when the ley is incorporated and nitrogen mineralised by micro-organisms (Watson, Atkinson, Gosling, Jackson, & Rayns, 2002).

A visual evaluation method of assessing SOM is not a reliable form of determination despite showing statistical significance in mean differences as the results do not correlate with LOI results.

Organic matter helps to retain nutrients and water in soil. Benefits include reduced compaction and surface crusting, plus improved water infiltration into the soil. The long-term stabilisation of OM is mediated by soil biota, soil structure (i.e. aggregation) and their interactions (Six et al., 2002). Tillage introduces oxygen into the soil and raises its temperature which increases decay rate of SOM. Evidence has been reported that soil disturbances influence OM dynamics, therefore SOM losses in CT may be attributed to reduced aggregation and increased decompistion due to aggregate disruption (Six, Elliott & Paustian, 1999).

In 2021, only 38% of farmers kept track of soil organic matter on their farm (figure 49). Of those not keeping track 36% provided the main reason as not important enough to monitor (DEFRA, 2021).

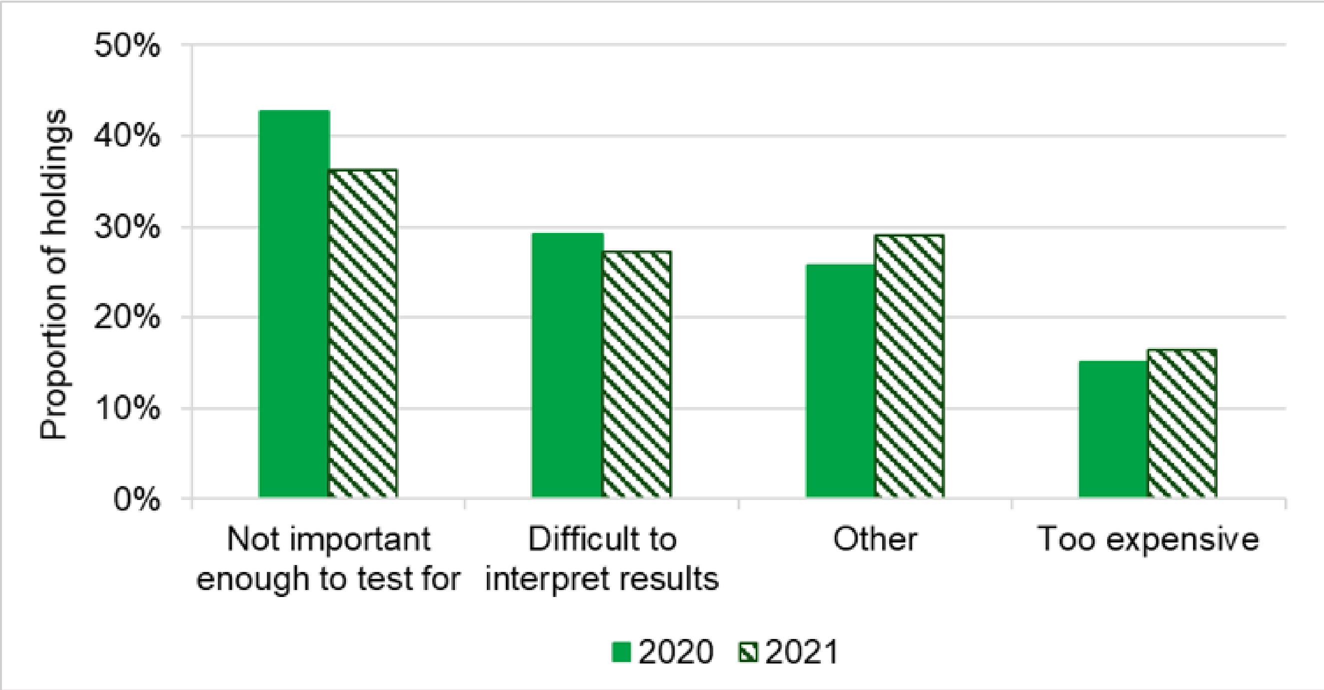


Figure 49: Reasons preventing farmers monitoring soil organic matter and proportion of farm holding: 2020 – 2021. Source: Greenhouse gas mitigation practices – Farm Practices Survey (DEFRA, 2021)

Variation in biota counts

From a diversity perspective, soil ecosystems are often seen as environments with high species diversity with similar dietary requirements that co-exist, therefore allowing for a high redundancy factor, making biota assessments challenging (Huisman & Weissing, 1999). Anderson (1975) described 'the enigma of soil animal species diversity' whereby high species diversity is often found in small spatial scales and the mechanisms influencing this were largely unknown. Today that enigma is still largely unsolved, although it is recognized that the high variations in soil heterogeneity that frequently exists at small spatial scales is likely to be the main contributor to this diversity. This would include micro-climates, nutrient availability, moisture content, soil temperatures as well as soil structure with variation existing both vertically and horizontally across soil profiles. Adding to this complexity is land management practices which include tillage, crop choices, rotations which all influence the biology and ecology of soil organisms which drive species and trait specialisations (Malcicka et al., 2017).

Conclusion

This study has clearly demonstrated that both direct drill and minimal tillage has consistently shown significant improvements on soil health indicators as well as soil biota abundance and diversity when compared to conventional tillage. It has also highlighted the importance of environmental factors such as rainfall and temperature when assessing impact. Mesofauna analysis proved most interesting when considering tillage impact and more detailed investigation is required to identify ecological function as different temporal scales. Analysis of soil organic matter raised important questions regarding quality of instruments used as well as methodology followed, highlighting a higher recovery rate in conventionally tilled plots when compared to reduced tillage. A recommendation would be made to assess water potential alongside soil water content due to its importance for soil biota and plant growth. This experiment also highlighted the high variability in soil biota abundance and diversity which is potentially related to the vast array of feeding strategies and microhabitat use which is less well studied.

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Appendices

Appendix 1: Temperature and rainfall data by month

Month	Temperature			Rainfall
	Min	Max	Mean	Total
Jun-20	4.3	30	15.815	79.9
Jul-20	5.7	25.2	15.7929762	60.1
Aug-20	6.5	34.6	18.3790323	106.9
Sep-20	8.75	28.4	16.7955049	21.3
Oct-20	2	17.7	9.78958333	161.4
Nov-20	-3.9	16	8.06296296	82.1
Dec-20	-8.5	12.5	4.65	143.8
Jan-21	-11	11.7	3.475	122.7
Feb-21	-5	16.1	4.92380952	70.6
Mar-21	-3	21.6	6.92580645	29.6
Apr-21	-5	20.4	6.1212963	14.05
May-21	-2	20	9.17788462	131.1
Jun-21	4	27.4	15.3448276	33.6