Evaluating the Impact of Conservation Agriculture on Soil Structure and Glyphosate Degradation

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i. Abstract

Conservation agriculture (CA) is a set of practices used by farmers with the aim of building soil health and reducing inputs. A key tenant of CA is the reduction of tillage; however, this can leave CA practitioners vulnerable to heavy weed burdens. As such, many rely on glyphosate, the most used herbicide on the planet, to control weeds. While considered to pose a low risk to human, animal and environmental health, concerns about glyphosates long term, chronic impacts are mounting and interest into the factors affecting the molecules persistence is growing. This research uses X-ray Computed Tomography (XRCT) of intact soil cores coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) along a post-application time series, as well as a complimentary laboratory-based incubation experiment to investigate how the soil structure of a cultivated and uncultivated field affects glyphosate degradation. XRCT data showed total porosity increased two-fold after cultivation, and quantifiable levels of glyphosate dropping to zero after cultivation while appreciable levels continued to be detected in the uncultivated soil for several weeks longer. Also discussed are two other experiments, one comparing the effects of strip-tillage (ST) on soil structure; the other a pilot for a protocol to evaluate the effects of arbuscular mycorrhizal fungi (AMF) on soil structural genesis and evolution.

ii. Acknowledgements

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AMF	Arbuscular mycorrhizal fungi
AMPA	Aminomethylphosphonic acid
CA	Conservation agriculture
CT	Conventional tillage
DT-50	Half-life
EPS	Extracellular polymeric substances
ER	Euler characteristic
EU	European Union
GBH	Glyphosate- based herbicides
IARC	International Agency for Research on Cancer
K_{sat}	Saturated hydraulic conductivity
LC- MS/MS	Liquid chromatography tandem mass spectrometry
LOQ	Limit of quantification
LS	Loamy sand
MT	Minimum tillage
MWD	Mean weight diameter
N	Nitrogen
OM	Organic matter
PP	Permanent pasture
PSD	Pore size distribution
ROI	Region of interest
SL	Sandy loam
SOC	Soil organic carbon
POM	Particulate organic matter
SOM	Soil organic matter
ST	Strip tillage
STIR	Strip tillage in row
STOR	Strip tillage out row
TP	Timepoint
USDA	United States Department for Agriculture
USEPA	United States Environmental Protection Agency
XRCT	X-ray computed tomography
ZT	Zero tillage

1 Introduction

1.1 Rationale

Agriculture is critically important to support human life while also being one of the most damaging activities we undertake as a species. From a climate change perspective, agriculture is responsible for about 30% of anthropogenic green-house gas emissions (Tubiello et al., 2021) with ruminant livestock being the biggest contributor (Opio et al., 2013). However, as damaging as agriculture is to the environment, it also has the potential to play a part in climate change mitigation. To this end, many farmers are adopting conservation agriculture (CA) practices, which use minimum-tillage (MT) or zero-tillage (ZT), direct drilling, cover cropping and green manures to improve soil health whilst simultaneously enhancing SOC (Adhikari and Hartemink, 2016). However, without tillage to control weeds farmers using ZT rely on heavy herbicide use instead (Kudsk and Mathiassen, 2020). Glyphosate is the most used herbicide globally, and while it is generally thought to be low risk, there are questions emerging around its environmental and human health impacts (Andreotti et al., 2018; Meftaul et al., 2020; Romano-Armada et al., 2017). This research aims to better understand the fate of glyphosate when it enters the soil, how farmers may be able to increase its efficacy and how long it remains active under different conditions and adjuvants. If farmers were able to reduce the amount, and negative impacts, of glyphosate they use then this may enable them to continue using it and managing their land without tillage.

2 Literature Review

2.1 Overview

Soil is recognised as the “most complicated biomaterial on the planet” (Young and Crawford, 2004). It is comprised of a mixture of minerals, organic matter, liquids, gasses and organisms and is crucial for sustaining life on Earth. Soil is home to 59% of species on earth (Anthony et al., 2023) and is the growing medium for 98.8 % of calories consumed by humans every day (Kopittke et al., 2019), as well as for fuel and fibre crops which humans rely upon. In addition, soil provides vital ecosystem services such as carbon storage and sequestration, water storage and purification and flood and drought protection (Gao et al., 2016; Stolte et al., 2016). However, rapid population growth and the subsequent need to increase food production has led to an extensification and intensification of agriculture land. As such, soils are increasingly vulnerable to degradation due to the unsustainable practices employed by modern intensive agriculture. This decline in quality and fertility means that soils are less able to provide the food, fibre, fuel and services on which we rely.

2.2 Soil health

The term ‘soil health’ started to gain popularity in the 1980s and 1990s (Michel et al., 2011; Powlson, 2020) as a metaphor around which to discuss the negative impacts of conventional agriculture. It is broadly defined as “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health” (Doran, et al., 1996). An agricultural soil in ‘bad health’ is seen as one that has deteriorated,

due to unsustainable agricultural practices, to a point where it is unable to support production and provide broader ecosystem services (Kibblewhite et al., 2007).

Restoring agricultural soils to, or maintaining them in, 'good health' is the focus of much research (Chang et al., 2022; Lal, 2016; Lehman et al., 2015; Pearsons et al., 2023). While agricultural management strategies and best practices have been developed for soil health in general (Doran, 1996; Miner et al., 2020; Toor et al., 2021), due to the complexity of the soil system and the different scales on which agriculture affects it, there remain many questions around the mechanisms of interaction between and within biological, chemical and physical aspects of soil and how they pertain to soil health.

2.2.1 Soil carbon sink

Of particular interest in recent years, and a principal focus of this research, is the important role that soil organic carbon (SOC) plays in both soil health and carbon cycling. From a soil health perspective, SOC is crucial in soil structural development and stability (Oades, 1984); serves as an energy source for soil organisms (Kristiansen et al., 2004; Nguyen and Marschner, 2016) and improves nutrient (Kushwah et al., 2016) and water retention (Lal, 2020; Werner et al., 2020).

Furthermore, soil is the largest pool of terrestrial carbon with around 2,400 Pg of organic carbon in the top 200 cm (Batjes, 1996). Conversion of land to agriculture typically leads to a loss of SOC (Guo and Gifford, 2002; Wei et al., 2014). Globally, this loss is estimated to be around 133 Pg from the top 2m of soil (Sanderman et al., 2017) (mechanisms for this loss are discussed further in section 2.5.3).

Although the exact figure is debated due to the complexity and scale of the assessment (Crowther et al., 2016), there is consensus that carbon lost from soil is a significant contributor to anthropogenic climate change. However, this loss represents a carbon deficit that holds huge potential for sequestering atmospheric carbon (Freibauer et al., 2004).

2.3 Soil texture and structure

Soil texture, the proportion of sand, silt and clay in a soil, is a fundamental property of a soil which change slowly over thousands to millions of years (Tugel et al., 2005). Soil texture is agronomically important as it affects nutrient availability and retention, water movement and root penetration; as well as influencing how prone a soil will be to compaction and erosion. Furthermore, the texture of a soil plays a significant role in determining soil structure.

Soil structure describes the spatial arrangement of soil particles and their aggregates and the pore space between them. Like texture, structure is a critical component of soil health, and in turn, crop performance (Miedema, 1997). From an agricultural perspective, a well-structured soil is one that allows infiltration and retention of water without becoming saturated and allows gasses to move through the soil. These properties are determined primarily by the size distribution of aggregates and pore spaces (Ciric et al., 2012; Guber et al., 2003). Agriculturally desirable soil structure is associated with high organic matter, many plant roots and rich and diverse microbiology.

2.3.1 Aggregate formation and stabilisation

Aggregation is the process by which soil particles (sand, silt and clay) are joined to form distinct structural units. While there is lack of agreement between classification systems (Totsche et

al., 2018), soil aggregates are generally grouped according to their size as either microaggregates (<250 µm) or macroaggregates (>250 µm) (Edwards and Bremner, 1964).

While microaggregates are considered the basic structural elements of aggregates, they are comprised of flocculated organo-mineral particles. The surface charges of clay particles mean they adhere to other clay particles, as well as organic particles, to form micro-structured compound particles (Kleber et al., 2015). Due to their abundance and surface chemistry, these small (< 0.2 – 2.0 µm) composite particles act as strong binding agents between organic matter and larger mineral particles to form microaggregates (Asano and Wagai, 2014). The organic matter in microaggregates is mostly of microbial origin rather than plant derived (Oades and Waters, 1991). At the small microaggregate (<20 µm) scale persistent microbial products, chiefly extracellular polysaccharides (EPS) and proteins, interact with clay minerals to make very stable particles (Six and Jastrow, 2002). This stability means the organic matter is resistant to microbial breakdown and is less susceptible to changes in land management (Cambardella and Elliott, 1993).

Microaggregates are bound together by OM to form macroaggregates by temporary microbial binding agents such as EPS and EPS biomolecules, including polysaccharides and proteins (Kleber et al., 2007). Plant roots and fungal hyphae play an important role in macroaggregate formation and stabilisation, both through physical enmeshment of particles (discussed below) and through exudation of polysaccharides and proteins (Nichols and Halvorson, 2013).

Macroaggregates do not form one continuous mass of soil, rather physical processes break macroaggregates along lines of weakness. The mechanical forces of water shrinking and swelling caused by changes in water content of a soil, freezing and thawing, by the movement of macro-biota and by the growth of roots all act to alter the arrangement and size of

aggregates. As well as the physical forces acting to break up aggregates, the binding agents holding macroaggregates together will degrade over time by microbial and chemical activity, releasing the stable microaggregates to continue the cycle of aggregation (Tisdall and Oades, 1982).

In soils of varying textures, the development of aggregate stability will occur at different rates and through different mechanisms. The stability of sandier soils will be more reliant on organic matter and will develop more slowly than clay soils. Due to the reduced surface area available for binding, the macroaggregates of sandy soils tend to be less stable than those of clay soils (Regelink et al., 2015; Rivera and Bonilla, 2020).

As well as affecting agronomically important factors including nutrient adsorption, aeration, microbial community structure and root penetration (Anderson and Kemper, 1964; Gallardo-Carrera et al., 2007; Karami et al., 2012; Mehra et al., 2018); greater stability of aggregates also affects a wide range of environmentally important processes such as soil organic matter stabilization and organic carbon protection from erosion or decomposition (Goebel et al., 2005; Guo et al., 2020; Wiesmeier et al., 2012).

Oades (1984) suggested that "the majority of macroaggregates should have diameters in the range 1 to 10mm" in an agricultural soil. Adding organic matter and cover cropping can help maintain this structure, but disturbance of the soil by tillage and compaction can lead to a breakdown of aggregates and degradation of soil structure that will significantly influence soil health and productivity. Tillage is discussed further in section 2.5.

2.3.2 Porosity

Soil pores, the water and gas filled spaces within and between soil aggregates, reflect a different but closely related aspect of soil structure. The amount, size distribution and connectedness of pores determines the movement and storage of water and the aeration in a soil, this movement of water and air allows nutrients, heat, pollutants and even microbes and microfauna to move through the soil structure (Hao et al., 2019).

Porous architecture develops as a result of soil aggregation, organic matter decomposition, root growth and decay, microbial activity faunal burrowing and earthworm activity, wet-dry and freeze-thaw cycles (Gargiulo et al., 2013; Hao et al., 2019; Xia et al., 2022).

While no universal classification is agreed, soil pores are commonly defined by their hydraulic function, as described in Table 2.1. Water in the largest pores, those above 50 µm, drain freely. These pores allow the rapid movement of water and gas through the soil and are important for root growth. Mesopores between 0.5 and 50 µm are particularly important for storing water available to plants as surface tension holds water against the force of gravity. Water in micropores less than 0.5 µm is bound tightly by cohesive forces between water molecules and

Table 2.1
Pore-size classification with relation to hydraulic function. Adapted from Ashman and Puri (2013).

Class	Pore size	Function
Transmission	Macropores (> 50 µm)	Drainage, aeration and plant-root extension
Storage	Mesopores (0.5-50 µm)	Storage of plant-available water
Residual	Micropores (< 0.5 µm)	Bound water

adhesive forces between water molecules and mineral particles and is unavailable to plants (Tuller and Or, 2022). Micropore water plays a role in soil chemistry and micropores larger than 0.2 µm are still accessible to microorganisms (Thomsen et al., 1999)

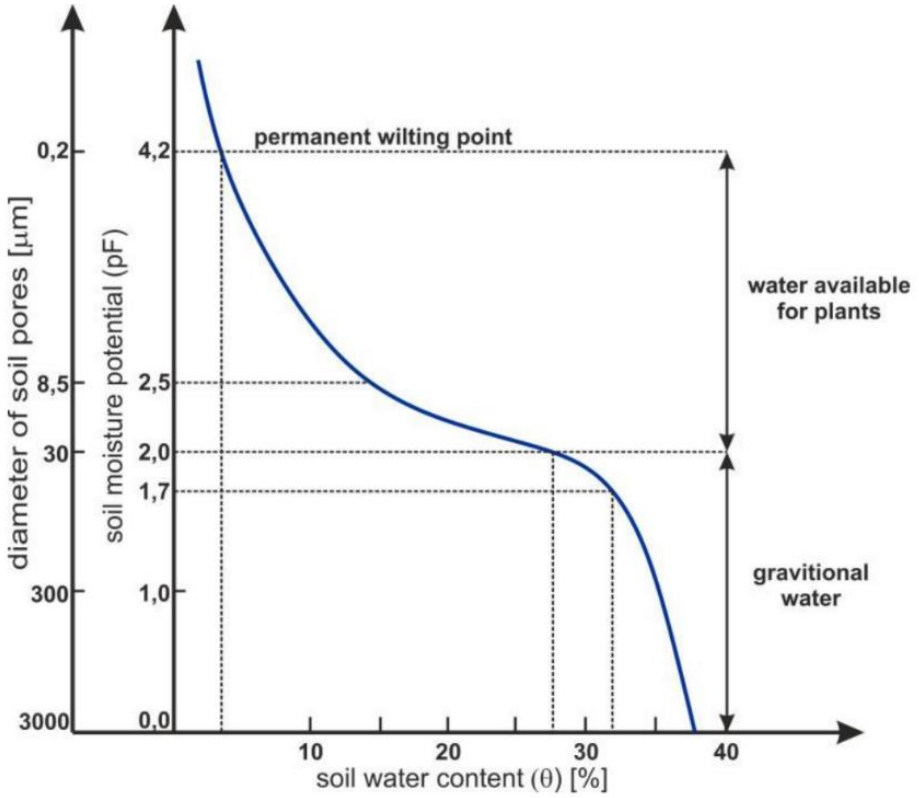

Fig. 2.1 Illustration of the relationship between soil water content and soil matric potential in relation to the size of soil pores, from Baziak, 2019.

As illustrated in Fig. 2.1, when a soil is saturated all the pores are filled with water and the soil will have a low or zero matric potential. Gravitational water is water that freely drains under gravity and 'field capacity' describes a state where the water is held against gravity after free drainage. 'Permanent wilting point' refers to plant-unavailable micropore water. The difference between field capacity and permanent wilting point is the water available to plants (Ashman and Puri, 2013; Baziak, 2019). The shape of this curve in Fig. 2.1 will be different for every soil and will be affected by a soil's structure and texture.

2.3.3 Visualising soil structure

Due to the importance of soil structure for many processes, there has long been a drive to quantify the arrangement of mineral and organic particles, water and air within a soil. Soil

hydraulic properties such as infiltration rate and water retention characteristics can suggest the nature of a soil's porosity and pore connectivity (Kutílek, 2004; Lipiec et al., 2006; Mohammadi et al., 2009). However, methods are hard to standardise, and they only give a very crude indication of the pore architecture. Resin impregnation and production of thin sections for microscopy offers a look 'inside' an intact soil sample and aspects of porosity and structure can be quantified at high resolution depending on the slicing and microscopy techniques used (Bendle et al., 2015; Papadopoulos et al., 2009). However, resin impregnation and thin sectioning is extremely time consuming and destructive, and samples cannot be used for other analysis nor time series analysis.

X-ray Computed Tomography (XRCT) offers a rapid, non-destructive approach to visualise the interior of solid objects. XRCT was originally a medical tool developed for non-destructive

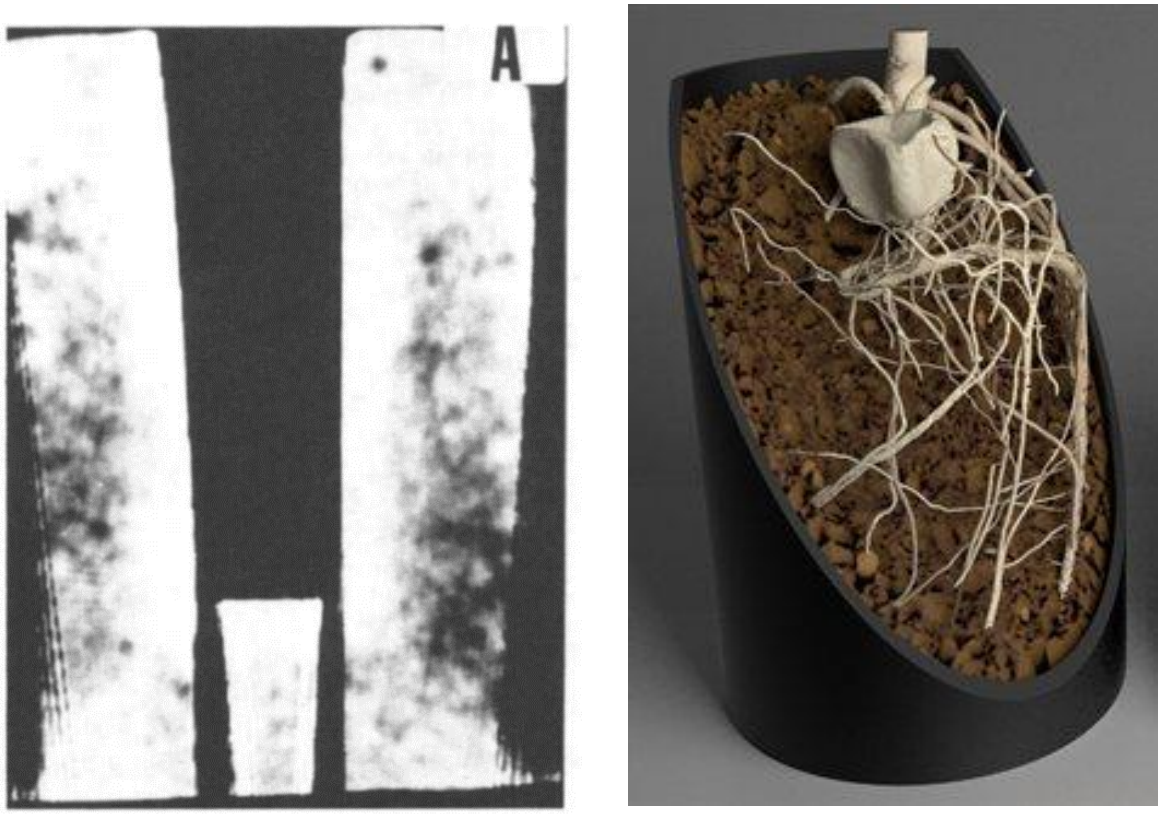

Fig. 2.2 The first X-ray CT image of soil. A sandy loam soil with a 76mm tapered hole through it (left) by Petrovik et al. (1982) and a modern X-ray CT image of a maize seedling (right) (courtesy of the Hounsfield Facility, University of Nottingham)

imaging soft tissue and bone (Hounsfield, 1973) and has been used in soil science since Petrovik et al. (1982) used it to assess bulk density of a soil in the early eighties (Fig. 2.2a). The following 40 years have seen huge improvements in scanner resolution and image processing power with XRCT becoming an accessible technology used to visualise and quantify soil structural characteristics in a wide variety of studies (Liu et al., 2021; Teramoto et al., 2020; Trusler et al., 2023). Current XRCT scanners are capable of sub-micrometre resolutions and fast scan times. The non-destructive nature of XRCT enables longitudinal studies of, for example, the development of soil structure, root-soil interactions and pest behaviour over time (Booth et al., 2020; Burr-Hersey et al., 2017; Helliwell et al., 2014).

XRCT allows measurement of the X-ray attenuation of a material in 3D. The process uses differences in the attenuation of electromagnetic waves by constituent materials in a sample to create a radiograph. Cone beam XRCT scanners, the type most used for soil investigation, comprise three main components: An X-ray source, a sample stage and a detector. X-rays are generated by an X-ray tube, in which a tungsten filament (cathode) is heated to cause the release of electrons which are accelerated towards a metal target (anode) by a high voltage. The X-ray tube is maintained at a high vacuum to prevent electrons striking air molecules and losing energy. When the high energy electrons strike the anode, they are slowed subsequently releasing their energy as heat and electromagnetic radiation (X-rays), which are focused towards the sample. The stage, on which the sample is secured, manipulates the sample so a series of radiographs can be obtained at incremental angular positions, usually over 360^{o}. The sample causes the beam to be attenuated by absorption and/or scattering of the X-rays. The degree of attenuation is proportional to the density, atomic number and thickness of the material. The detector panel, which is stationary in typical in CT scanners used in soil science,

converts the X-rays that have passed through or around the sample into a 2D digital image with pixel greyscale values relative to the attenuation. Radiographs are reconstructed to create a volumetric dataset of attenuation values for the sample which can be rendered and viewed in 3D (Helliwell et al., 2013; Mooney et al., 2012; Sturrock, 2022).

The different phases of material present in the sample can be differentiated and grouped by their image greyscale values – a process known as segmentation. Soil is typically grouped into three main phases: mineral grains, organic matter and pore space, so proper segmentation is essential to ensure the accuracy and comparability of results obtained from CT image analysis. Highly attenuating mineral grains are represented by bright voxels and air-filled pores are seen as dark voxels due to their low attenuation. Due to the heterogeneity of the soil matrix, and the multiple phases of matter contained within it, soil presents a particular challenge to thresholding (Helliwell et al., 2013). As illustrated in Fig. 2.3, the grayscale values for gaseous, organic and solid phases often overlap. Organic matter is particularly hard to segment due to the presence of water in both soil and roots. Finding an appropriate threshold that accommodates this overlap while avoiding over or underestimating pore space is a subjective process with a diversity of approaches and lack of standardisation (Iassonov et al., 2009; Ramesh and Thyagaraj, 2021; Schnaar and Brusseau, 2005; Wirjadi, 2007). Added to this, choosing an appropriate threshold will differ depending on soil type, image quality and resolution and what exactly a researcher is interested in.

There are many approaches to thresholding that broadly fit into three categories: global, local and machine-learning based thresholding. Global thresholding is the simplest and most widely used segmentation method whereby the threshold value is chosen and applied to the whole image. The threshold value is commonly chosen using a model appropriate for the

image based on the histogram shape, examples of which include Li (Li and Tam, 1998), Otsu (Otsu, 1979) and Isodata (Ridler and Calvard, 1978). Global thresholding tends to work well for images with a bi-modal grayscale distribution with well-defined separated peaks and low noise. Edge definition and noise can be improved with filtering of the image, but this can be at the expense of losing smaller features of interest (Iassonov et al., 2009). Local thresholding methods compute local thresholds based on the characteristics of neighbouring voxels or pixels. These methods tend to be better at dealing with overlapping peaks and brightness variations over an image (Wirjadi, 2007). However, they are more computationally demanding and rely on the user to define several parameters which can greatly affect the thresholding value if set inappropriately. The recent advent of machine learning and artificial intelligence has enabled automated, and data driven thresholding approaches. In machine learning-based thresholding, algorithms learn from labelled training data to determine optimal threshold values that best separate foreground and background pixels (Ferreira et al., 2022). This approach is particularly useful when intensity distributions are complex or when different image regions require different threshold values. The availability of quality, labelled training data can be limited, and once a model is trained it will not necessarily perform well on another image set and may need to be retrained (Kan, 2017).

Appropriate thresholding allows for replicable quantitative analysis of aspects of the soil pore architecture such as pore volume, number, connectivity, shape and thickness (Beck-Broichsitter et al., 2022; Bendle et al., 2015; Burr-Hersey et al., 2017; Liu et al., 2021; Teramoto et al., 2020).

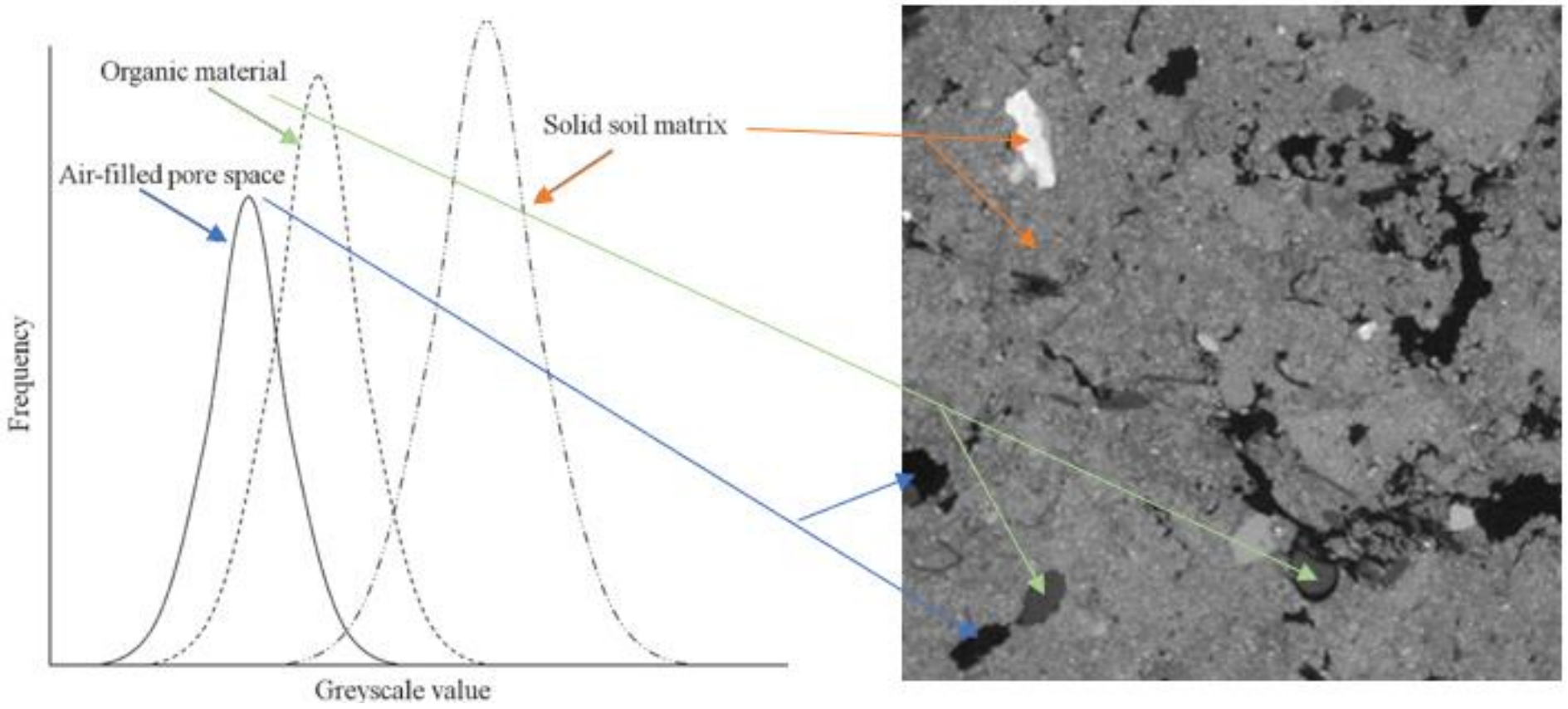

Fig. 2.3 An illustrative histogram showing the phases typically present in soil and the overlap between them which makes thresholding challenging adapted from Helliwell et al. 2013.

2.4 Conservation agriculture

Carbon loss from soil can be reduced, and more carbon can be drawn down in to soils through a suite of improved soil management practices often referred to as conservation agriculture (CA) (FAO, 2014; Kassam et al., 2009; Schreefel et al., 2020).

2.4.1 General principles

The main principles of CA are minimising soil disturbance, maintaining permanent soil cover and crop diversification and rotation. CA practises such as ZT or MT, direct drilling, cover cropping and green manures have been shown to reduce soil erosion (Seitz et al., 2018), nutrient leaching (Abdalla et al., 2019), soil organic carbon mineralisation (He et al., 2023). They also increase the activity of beneficial soil micro- and macrofauna (Ayuke et al., 2019), and can improve soil structure and water retention (Zhang et al., 2021) and reduce labour and have economic benefits (Giannitsopoulos et al., 2019). CA is often touted as a panacea (Indoria et al., 2017) and has been practiced by many farmers with positive agroeconomic results for decades. Globally, CA is practised on an estimated 180M hectares, 12.5 % of total global cropland (Kassam et al., 2019). However, there is still disinclination towards adoption from some farmers (Corbeels et al., 2014). The main barriers to adoption seem to be the perceived risk involved and lack of knowledge around its implication (Findlater et al., 2019; Rodriguez et al., 2009). Conventional farming, with full inversion tillage and a heavier reliance on chemical inputs for nutrition and pest management, is seen as more predictable, and secure in terms of yield with CA a perceived to be less consistent across soil types. More research and more effective practitioner outreach are important steps to increase uptake.

However, there is also a lack of consensus in the literature about many of the claims of CA. Considering differences in soil texture, climate, cropping systems, access to capital, land tenure, cultural considerations and market conditions it would be surprising if the broad-brush

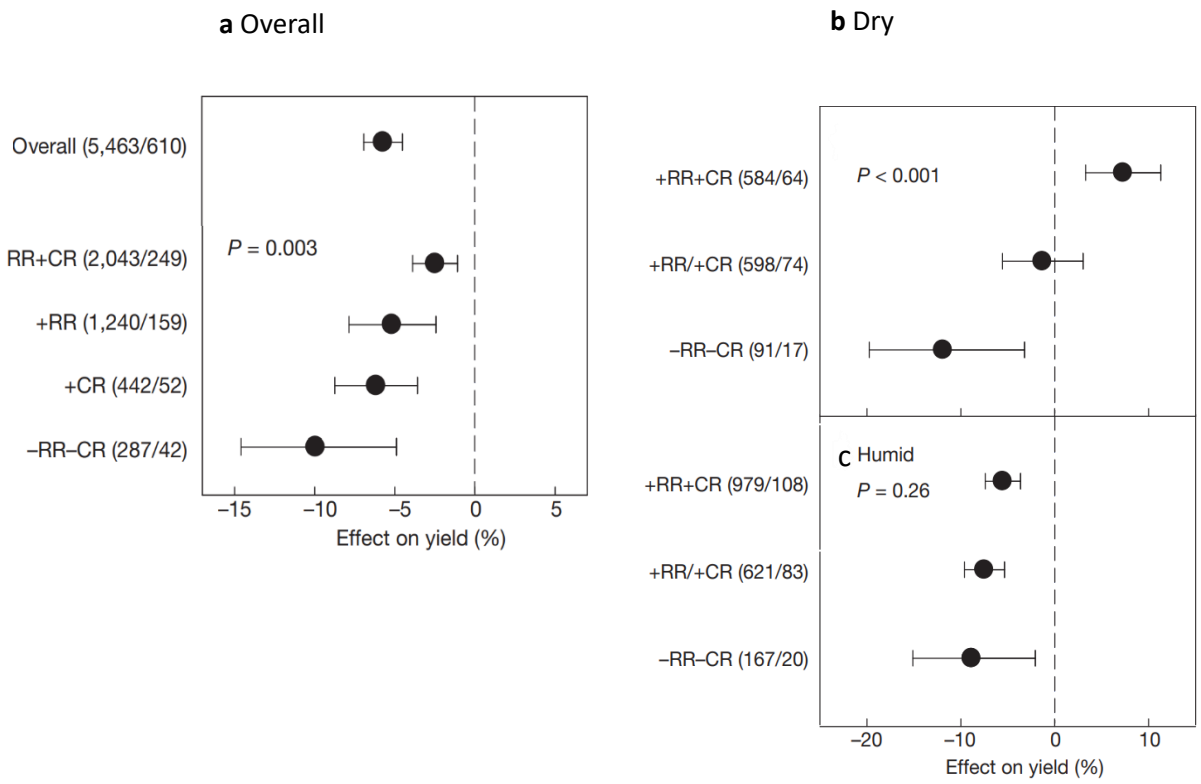

Fig. 2.4 Comparison of yield in ZT versus CT systems. Results are shown for the entire data set (a) and in relation to dry (b) and wet (c) climates. Categories represent presence or absence of residue retention (RR) and crop rotation (CR). The number of observations and total number of studies included in each category are displayed in parentheses. From Pittlekow (2015)

principles of CA were the 'panacea' that Indoria et al. (2017) claim. Seemingly contrasting reports regarding yields are a case in point. Several meta-analyses describe increased crop yield under CA (Farooq et al., 2011; Pittelkow et al., 2015; Rusinamhodzi et al., 2011). Conversely, several reviews seem to conclude the opposite (Alvarez and Steinbach, 2009; Ogle et al., 2012; Van den Putte et al., 2010). Under detailed examination they are not contradictory, but in fact are crop specific or based on regional conclusions; with yields increasing under water limited conditions and decreasing in cool climates where waterlogging

and compactions are concerns (Fig 2.4). The complexity of agriculture and soil in general, and the temptation to simplify and to draw big conclusions muddies the waters of CA research. However, despite this, by tailoring the overarching principals of CA to local conditions land managers should be able to reduce the impact of their agricultural practices.

2.5 Tillage

Tillage refers to the agricultural practice of disturbing soil to control weeds and improve conditions for crop establishment. The term 'conventional tillage' (CT) refers to the inversion of the top 15-30 cm of soil followed by secondary tillage operations (harrowing and/or discing) to break up clods to form a fine tilth (Blevins and Frye, 1993). CT loosens, aerates and warms the soil as well as removing residues of the previous crop by burying it, all of this aids crop germination and establishment (Lamichhane et al., 2018). It also acts to terminate weed plants and buries weed propagules (Yenish et al., 1992).

However, there are several accepted downsides to CT. CT leaves soil susceptible to erosion, can lead to subsurface compaction and is very energy and labour intensive and thus costly. Furthermore, and of most interest to this review, it damages soil structure, alters microbial communities and functioning and expedites the mineralisation of SOC (Janusauskaite et al., 2013; Mehra et al., 2018; Munkholm et al., 2008).

2.5.1 Effect of tillage practices on soil structure

The primary purpose of tillage operations is to modify the structure of the topsoil to create a homogenous seedbed. Tillage loosens soil and break up clods, increases total porosity and decreases bulk density (Mondal and Chakraborty, 2022) to improve condition for crop seed germination and establishment. Tillage is also an important tool for combatting the negative

effects of traffic associated with field operations. However, excessive or poorly timed tillage can damage soil and threaten productivity. Tillage breaks apart macropores and exposes the SOM previously protected within to microbial mineralisation. This breakdown of soil aggregates speeds erosion and impedes hydraulic function (Mikha and Rice, 2004). While the action of tillage can form aggregates, they are formed by different mechanisms and tend to be weaker and will coalesce upon wetting leading to the collapse of microporosity and (Or et al., 2021). Tillage also disrupts bio-pores created by plant roots and soil fauna. Biopores contribute to aeration and nutrient water flow as well as stabilising soil structure.

2.5.2 Effect of tillage practices on the soil microbiome

Tillage has been shown to alter the microbial communities, as well as the biological pathways present in in the soil (Mbuthia et al., 2015a; M. R. Nunes et al., 2020; Schmidt et al., 2018a). Tillage influences the structure of the soil and therefore the habitat of soil microorganisms. Microclimates are generally cooler and moister in ZT soils, and these more favourable conditions were reported to increase microbial abundance in reviews by Johnson and Hoyt (1999) and Martens (2001). As discussed further in section 2.6.3, organic matter dynamics, a key driver of microbial abundance and activity, will also differ with management practices (Jastrow et al., 2007). Smith et al. (2016) showed that tillage had a greater effect on microbial community structure and functioning that the crop that was planted. Their work showed that tillage decreased the abundance of nitrogen cycling associated genes in the microbial communities. Other work has shown that microbial biomass, diversity, and community stability is decreased under CT as compared to ZT (Wang et al., 2017; B. Zhang et al., 2012). Schmidt et al. (2018) reason that as bacterial diversity generally reflects the diversity of microhabitats and tillage tends to homogenise available microhabitats and destabilise

aggregates, microbial diversity will inevitably be lower in tilled soils. This is of concern because, as with all terrestrial ecosystems, decreased species diversity leads to instability and a loss of ability to perform important ecosystem services (Maron et al., 2018; Wagg et al., 2021).

2.5.3 Effect of tillage practices on soil carbon

The physical action of ploughing tends to accelerate the decomposition of organic matter and reduce soil carbon content as tillage exposes organic material to increased oxygen levels, promoting microbial activity that breaks down carbon-rich compounds (Gao et al., 2016). Additionally, soil disturbances from tillage can lead to the physical breakdown of macroaggregates that protect organic matter from microbial degradation (Zhang et al., 2012). Thus, as agricultural soils generally contain less C than native soil (Six et al., 1998), it stands to reason that ZT agricultural soils would contain more C than tilled soils. There are many examples in the literature where this is the case showing that ZT has a positive effect on SOC protection and sequestration (Almajmaie et al., 2017; Cooper et al., 2018; Mangalassery et al., 2014; Sun et al., 2020). However, some researchers have reported no effects or contradictory results (Cai et al., 2022; Porwollik et al., 2019; Powlson et al., 2014). These disparity in the literature are associated with local climate conditions, whether crop residue is retained, sampling depth and soil texture.

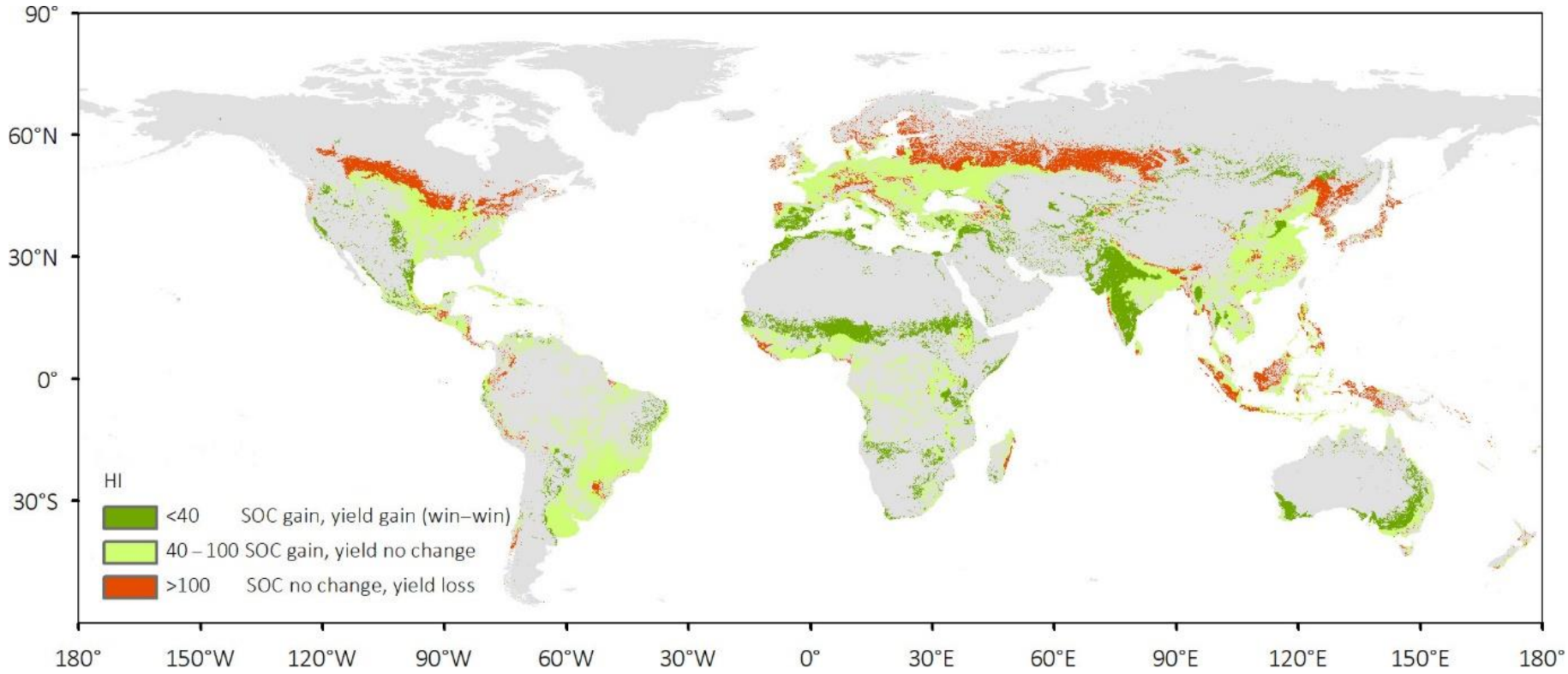

Fig. 2.5 Global patterns of change in SOC and crop yield after adopting conservation agriculture. From Sun et al. (2020)

A meta-analysis by Sun et al. (2020) showed the importance of considering local conditions when assessing the impact of ZT on SOC. Fig. 2.5 shows how, in cool humid and hot humid climates, ZT may result in a double negative of reduced yields and reduced SOC whereas in warm and arid regions ZT probably results in the win-win of increased yields and SOC. Therefore, CA, and ZT in particular, must be adopted with pragmatism taking into account local conditions rather than adopted blindly (Giller et al., 2015; Kirkegaard et al., 2014).

2.5.4 Tillage practices and weed management

The inversion of the topsoil acts to terminate and bury weed propagules, as well as any germinated weeds. Further, shallower cultivation can then to terminate weed seedlings before planting, this is known as the 'stale seed bed' technique which can be effective as chemical weed management (Lampkin, 1990; Riemens et al., 2007). Tillage is considered necessary for weed control in organic systems where synthetic herbicides are not permitted (Gruber and Claupein, 2009).

ZT tends to have greater surface weed seed concentrations as farmers are unable to use cultivation and stale seed bed techniques to control weeds (Yenish et al., 1992). Some studies have shown lower weed emergence under ZT due to the retention of crop residues suppressing germination (Hendrix et al., 2004; Trevini et al., 2013). However, as residues may also hinder crop emergence, it is not a suitable weed management strategy for many crops. Therefore, chemical-based weed management is relied upon for most ZT fields (Buhler, 1995).

2.6 Glyphosate

Glyphosate (N-(phosphonomethyl)glycine, $C_3H_8NO_5P$), is a broad-spectrum post emergence herbicide, first patented by Monsanto in 1974 under the trade name “Roundup” and is the most commonly used weedkiller in the world (Myers et al., 2016). Despite the increase in glyphosate resistant weeds, their usage continues to rise especially with the use of genetically modified glyphosate resistant crops and use for pre-harvest desiccation. Glyphosate-based herbicides (GBHs) are regarded as having low mammalian toxicity, low mobility in the environment and a short half-life (expressed as DT-50, the time take for the concentration to reduce by 50%) (Lange et al., 1973; Myers et al., 2016; Williams et al., 2000). However, a growing body of evidence is casting doubt on this, and it seems GBHs may be more harmful to human health and the environment than previously thought, causing some to call for GBHs to be more heavily regulated or even banned (Peng et al., 2020; Reynolds, 2016; Tosun and Varone, 2021).

2.6.1 The role of glyphosate in CA

As discussed above, other forms of weed control are crucial when tillage is not used. Glyphosate, being cheap, effective and relatively safe to use, is widely relied upon by farmers practicing CA and if its use is restricted or banned, many farmers may revert back to tillage (Böcker et al., 2018; Matousek et al., 2022), this issue is discussed further in section 2.7.5.

There is a delicate balance to strike between the two sides of the glyphosate debate. This potentially harmful weedkiller facilitates a farming practice with proven benefits to soil health and carbon sequestration. More research is essential to inform decisions about the future of GBHs and to minimise any harm done to people and planet.

2.6.2 Environmental impacts

Concerns over the environmental and human health impacts of glyphosate have been growing in recent years. While reports are often contradictory, as more long-term studies reveal their findings and more independent studies are carried out, it seems that glyphosate may not be as benign as earlier (industry funded) research has claimed (Meftaul et al., 2020; Myers et al., 2016; Zhang et al., 2019).

2.6.2.1 Non-target organisms

Due to differences in formulations of GBHs, testing protocols and industry influence, the effects of glyphosate on non-target organisms are hard to ascertain. A few examples to illustrate this point are the effects on bees, earthworms and springtails. Contrary to earlier findings (Spurrier, 1973), it has now been shown that bees suffer high mortality if sprayed directly (Motta et al., 2018), and colony health can be impacted post application (Weidenmüller et al., 2022). Other negative sublethal effects are also being uncovered (Battisti et al., 2023, 2021). Giesy et al. (2000) reported that Monsanto's GBH 'Roundup' caused no mortality in earthworms, however, their study relied heavily on unpublished data from tests carried out by Monsanto. More recent independent work shows GBHs reduces activity and reproduction of earthworm (Gaupp-Berghausen et al., 2015; Pochron et al., 2020) and increases mortality (Correia and Moreira, 2010). Some of these studies referenced above point to the specific GBH formulations as being more harmful than others, suggesting that it may the adjuvants causing the negative effects (Székács et al., 2014). Research focused on the effect of GBH on springtails, a near ubiquitous soil arthropod sub-class which are commonly used as an indicator group for ecotoxicological studies and considered good bioindicators of soil quality and cultivation management (Chang et al., 2013), suggest GBHs have a small

positive affect on springtail density, richness and activity. Field trials by Chang et al. (2013), Fiera et al. (2020) and Maderthaner et al. (2020) all showed increased springtail activity after the application of GBH. However, Lins et al. (2007) and Bitzer et al. (2002) found no impact on springtail numbers in field trials.

2.6.2.2 Microbial community structure and functioning

Immediately after glyphosate application it is common to see an increase in microbial respiration as it is metabolised by microorganisms is the soil (Busse et al., 2001; Grossbard, 1985; Ratcliff et al., 2006; Stratton and Stewart, 1992). Due to the high bond strength of glyphosate's C-P bond, it is co-metabolised along with more readily available carbon sources (Kanissery et al., 2019) which could alter the carbon chemistry of a soil. Glyphosate's effect on microbial activity, biomass and community structure after a single application seem to be minimal at recommended application rates (Lancaster et al., 2010; Nguyen and Marschner, 2016; Schlatter et al., 2017) and transient even at higher application rates (Ratcliff et al., 2006; Vázquez et al., 2021). Although hard to unpick from the effects of cropping and tillage practices, there is evidence of changes in microbial community structure and functioning under longer term glyphosate application (Lancaster et al., 2010; Vázquez et al., 2021). Lancaster et al., (2010) showed the increase in microbial mineralisation was reduced upon repeated application, which suggests that long term glyphosate application does not lead to changes in community functioning that favours glyphosate mineralisation as one may expect. This is concerning as long-term glyphosate applications are very common and this may be impairing the soil microbial community's ability to degrade the herbicide.

2.6.3 Persistence and mobility in soil

Glyphosate is broken down by microbes in the soil. The exact DT-50 of glyphosate will be determined by several soil biotic and abiotic variables; of which SOC content, pH, texture, structure and microbial community structure have the greatest effect (Martins et al., 2023). The application rate and duration, as well as the specific formulation of the GBH also greatly affect glyphosate's persistence in soil. Glyphosate is most commonly reported as having a DT-50 of between seven and 60 days (Andréa et al., 2003; Giesy et al., 2000), but there are some reports of persistence in soil of hundreds and thousands of days (Feng and Thompson, 1990; Heinonen-Tanski, 1989). Early studies suggest that despite glyphosates potential ability to persist in soils past the usual 60 days, its phytotoxic effects are lost on contact with soil due to its low activity when made available to plant roots as well as its sorption to soil and degradation by microbes (Hance, 1976; Sprankle et al., 1975).

There are two microbial degradation routes of glyphosate, one that yields sarcosine (that will not be discussed more here) and one that produces the primary metabolite aminomethylphosphoric AMPA (Erban et al., 2018). From an environmental perspective, AMPA is phytotoxic but considerably less so than glyphosate (Gomes et al., 2014). AMPA is 3-6 times more persistent in the soil environment than glyphosate with a typical half-life of between 76 and 240 days (Domínguez et al., 2016; Sun et al., 2019) and more prone to leaching (Landry et al., 2005) than glyphosate. Despite it being classified as "not of toxicological concern" in the EU and USA (EU, 2002; USEPA 1993) there are several studies showing it is harmful to some soil organisms, fish and other aquatic life (Domínguez et al., 2016; Guilherme et al., 2014; Mottier et al., 2013). This toxicity coupled with the increased residence time and environmental mobility make AMPA as much of an environmental concern

as glyphosate and quantifying AMPA present in a soil can offer insights into the fate of any glyphosate applied. While it is not a direct relationship due to the sarcosine breakdown pathway, increasing AMPA in soil can be used as a proxy for the microbial breakdown of glyphosate.

2.6.3.1 Potential for leaching

The mobility of a compound in soil is dictated by its sorption characteristics – a strongly sorbed molecule will be immobile and will not be readily leached. Large molecules with few polar groups will not sorb strongly to soil particles or organic matter, this is the case with many common herbicides (Oliveira Jr et al., 2001). Conversely, glyphosate is a small molecule with three polar functional groups which forms strong bonds to soil particles, particularly clay particles (Vereecken, 2005). At pH 4-8, the range found in most soils, glyphosate has a high affinity for trivalent cations such as Al^{3+} and Fe^{3+}. As such, it is strongly sorbed to soil minerals and is minimally mobile in soil and poses a low risk of leaching into ground and surface waters (Gimsing et al., 2007; Sheals et al., 2002).

However, there can be a risk of leaching of glyphosate applied on phosphorous rich land. Inorganic phosphate has similar sorption properties to glyphosate and will therefore exclude glyphosate from sorption sites (Hance, 1976; Jonge and de Jonge, 1999). Furthermore, inorganic phosphate has the ability to displace at least a limited amount of pre-sorbed glyphosate; therefore, phosphorous fertilisation can lead to mobilisation and phytotoxicity of previously inactive glyphosate (Bott et al., 2011; Gimsing et al., 2007; Gimsing and Borggaard, 2002; Rose et al., 2018).

Despite being almost immobile due to its sorption properties, glyphosate can still be transported through soil by colloid-facilitated mass transport through macropores. Glyphosate applied to soils with large vertical pores or cracks can be susceptible to rapid movement through the soil after heavy rainfall. de Jonge et al. (2000) simulated rainfall events to leach glyphosate though intact soil columns, the cores were taken from either a well-structured macroporous sandy loam, or a structureless sandy topsoil. They reported 50 - 150 times more glyphosate leached from well-structured macroporous cores compared to cores from unstructured soils concluding the differences in pore structure rather than sorption properties explained the results. Up to 52% of the glyphosate leached was of colloidally associated glyphosate, the rest moved by mass transport. The timing of the rainfall was also an important factor with 5 times less leaching if rainfall occurred 96 hours after application.

The leaching of glyphosate is to a large part governed by the presence of connected macropores as studies have shown (de Jonge et al., 2000; Kjaer et al., 2005; Stone and Wilson, 2006). Some have suggested tillage may lead to reduced leaching risk due to the reduction in connectivity of preferential flow paths (Petersen et al., 2004, 2001; Stone and Wilson, 2006). Petersen et al., (2004) showed slightly less bromine (used as a proxy for herbicides) leached in ZT soils but the rates were mostly governed by rainfall amount and timing. In their earlier work, Petersen et al., (2001) used a dye to trace mass flow of surface applied molecules. While not directly relevant to glyphosate transport due to the different sorption characteristics of the dye, they showed that in the tilled topsoil there was little vertical mass transport, but below disturbed depth earthworm channels were the predominant route for mass transport.

A meta-analysis evaluating the effect of ZT and CT on soil pore architecture by Wardak et al. (2022) summarised that while ZT soils saw reductions in microporosity, the pore network was

better connected due to the prevalence of biopores from burrowing fauna and decaying routes. A review by Jarvis (2020) pointed to the implication for pesticide transport by mass flow through these large, cylindrical often vertical pores. Little work has been done directly on the implications of these changes in pore architecture on the movement of glyphosate through a soil. Carretta et al., (2021) compared glyphosate dissipation and degradation in ZT and CT soils. They found while glyphosate dissipated faster in the ZT soil, it persisted in the soil for longer. However, they attributed this difference to differences in the soil textures between the sites. Other studies have shown no difference in degradation and movement between ZT and CT soils (Okada et al., 2019; Zablotowicz et al., 2009). Although more work needs to be done in the area, it seems the adoption of CA tillage practices will not necessarily increase the risk to the environment or non-target organisms from glyphosate and that application timings as well as inherent soil characteristics may be the more important factor in determining glyphosate translocation risk.

2.6.4 Human health

Glyphosate is considered one of the safest herbicides available as it has very low toxicity via oral and dermal routes and is not considered to show mutagenic, carcinogenic, or teratogenic activity (Andreotti et al., 2018; Orsi et al., 2009; WHO, 1994) and levels for acute toxicity are well established (Turkmen and Dogan, 2020). However, the effects of its long-term, low-level exposure are less clear. Several epidemiological studies have reported an increased risk of cancer, in particular non-Hodgkin's lymphoma, linked with glyphosate use (Hardell et al., 2002; Roos et al., 2003; Zhang et al., 2019). Also, in vitro and animal studies suggest links between GBH exposure and health impacts as well as mechanisms that were not previously considered (Agostini et al., 2020; Bukowska et al., 2022). The epidemiological studies finding causative

relationships between GBHs and cancer are from people frequently exposed to high levels of GBHs for long periods (e.g. backpack herbicide sprayers and boom sprayer operators). There is very little evidence of glyphosate exposure from food residue causing any harm (Vicini et al., 2021). The issue is further confused by the adjuvants added to many GBHs which are known to be toxic in their own right (Mesnage and Antoniou, 2018). Health risk assessments tend to ignore these and only assess the stated active ingredient of the herbicide. Even among national and international regulatory bodies there is no consensus on the risks posed by glyphosate and GBHs. The UN's International Agency for Research on Cancer (IARC) concluded that glyphosate is 'probably carcinogenic' (IARC, 2015) but the United States Environmental Protection Agency (EPA), as well as European regulators, concluded that it poses no significant risk (Benbrook, 2019; Meftaul et al., 2020). These opposing conclusions are primarily a result of the IARC using data from occupational exposure and the EPA and European regulators looking at dietary exposure of the general population. Clearly more, independently funded research is needed to clarify the impacts of exposure to GBHs on human health.

2.6.5 Implications of glyphosate regulation

Glyphosate has recently been granted approval for use for another ten years in the EU (Casassus, 2023) with regulations on its use as a pre-harvest desiccant. While the UK may follow suit, glyphosate is currently only approved for use as a plant protection product in the UK until 15th December 2025 when the approval will be reviewed. There is speculation that it may be banned or strictly regulated (Kudsk and Mathiassen, 2020), and concern that farmers practicing CA with ZT and MT management will be forced to return to conventional tillage practices (Matousek et al., 2022; Schulte et al., 2016). Although no studies have looked specifically at the impact on UK farming, several studies have modelled impacts of the ban on

EU agriculture. All conclude that a ban would reduce profits, increase mechanisation and increase carbon losses from soil with land currently under ZT being most affected (Böcker et al., 2018; Brookes et al., 2017; Wynn and Webb, 2022). The soil-carbon, aggregate stability and erosion susceptability benefits of ZT and CA would be lost. In their review of possible impacts, Wynn and Webb (2022) estimate increased tillage intensity could lead to greenhouse gas emissions from machinery increasing by 1.4–3.8 Mt CO_2-equivilant emissions values per year in the EU. The ongoing debate surrounding GBHs underscores the necessity of carefully weighing potential health concerns against the benefits it offers to sustainable agriculture. While apprehensions about its impact on human health and the environment are valid and warrant rigorous research, these must be balanced against its role in CA which can enhance yields, sequestering carbon and conserve soil health.

2.6.6 Quantifying glyphosate in soil

Considering how widely used glyphosate is, there is relatively little work quantifying it in soils. This is probably due to difficulties in detecting it and the absence of low tech, field-test protocols (Valle et al., 2019). Glyphosate does not absorb UV, has a low volatility, high hydrophobicity, poor solubility in common solvents, low mass and limited ionisation (Bernal et al., 2012; Simonetti et al., 2015). The complexity and variability of the soil matrix compounds the problem meaning the quantification of glyphosate in soil requires high-end equipment and high through-put analysis is difficult to achieve. There are many attempts in the literature to establish protocols using a variety of methods with varying success including liquid chromatography (Kaczyński and Łozowicka, 2015; Si et al., 2009; Thompson et al., 1989), gas chromatography (Kataoka et al., 1996), spectroscopy (Lee et al., 2013), NMR (Cartigny et al., 2004; Dickson et al., 1988), capillary electrophoresis (Chang and Liao, 2002; Cikalo et al.,

1996), enzyme-linked immunosorbent assays (Clegg et al., 1999; Rubio et al., 2003) and all have their benefits and drawback. It is chromatography-mass spectrometry (LC-MS) methods that are most suited and commonly used for glyphosate detection in environmental samples due to their sensitivity, selectivity and relatively easy sample preparation (Valle et al., 2019). The use of liquid chromatography (LC) with tandem mass spectrometry (LC-MS/MS) increases sensitivity by reducing noise and avoids the need for a derivatisation step. Briefly, LC-MS/MS works by introducing the compounds eluted by the LC column into a first mass spectrometer where ions are formed and selected based on their mass-to-charge ratio and fragmented. The fragments are then subjected to a second mass analysis in a second mass spectrometer. The secondary mass analysis offers more insight into the structures of compounds present and allows for better discrimination between similar compounds (Zimmer, 2003).

3 Experimental Work

The following sections describe experimental work undertaken over the course of this research. The first experiment investigates the practice of strip tillage (ST), a technique with potential to reduce the intensity and impact of tillage. Tillage practices are known to negatively affect AMF communities (Lu et al., 2018) which, in turn, play a large role in the development and stabilisation of soil aggregates (Bedini et al., 2009). The second experiment attempts to use XRCT to track the formation and development of soil aggregates with and without fungal hyphae to better understand how and to what extent fungal hyphae contribute to aggregation. Uncultivated, ZT fields are beneficial not only for AMF, but a host of other soil health indicators. However, farmers practicing ZT often rely on chemical methods of weed control, the most common of which is glyphosate and surprisingly little is known about its impact on soil health, in particular it's impact on the soil microbiome. The third experiment

aims to examine the fate and persistence of glyphosate in soils which have been managed conventionally and under ZT to assess the importance of, and impact on, the soil microbial community.

3.1 Strip tillage effect on soil structure

3.1.1 Introduction

ST also referred to as zonal or zone tillage (Pierce et al., 1992) is a conservation agriculture practice whereby narrow planting strips (referred to as strip-till, in row; STIR) are cultivated while the rest of the field is left undisturbed (referred to as strip-till, out row; STOR) and crop residue is either incorporated or placed between cultivated, STOR, rows (Potratz et al., 2020). The target crop will dictate the widths of the STOR with vegetable crops having wider spacing and cereal crops narrow; typically, 50 - 75% of the field will be left undisturbed. ST aims to combine the advantages of conventional tillage (CT) with the benefits of ZT.

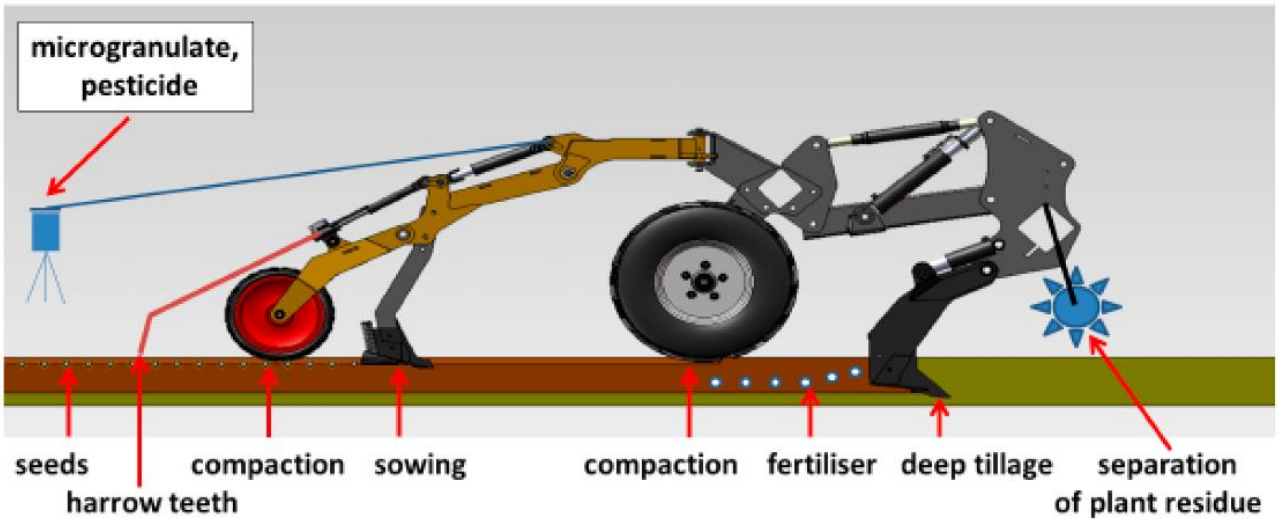

Fig. 3.1 Cross sectional diagram of a typical 'one-pass strip till' machinery showing the agrotechnical practices it performed. From Jaskulska and Jaskulski (2020).

3.1.1.1 Benefits of strip tillage

ST is a fairly new practice and recent advances in machinery enable tillage, drilling and fertilisation to be completed in one pass by machinery illustrated in Fig. 3.1, reducing labour costs (Jaskulska and Jaskulski, 2020). As less soil is disturbed compared to CT, and fewer field operations are required, smaller machines can be used, lowering fuel costs and compaction risk (Jabro et al., 2011; Saldukaitė et al., 2022). Less disturbed soil also reduces erosion risk, and the STOR strips act to catch soil run-off thus further reducing erosion (Procházková et al., 2020; Ryken et al., 2018).

The STOR, which often have the crop residue left on, increase in OM content (Mangalassery et al., 2015) and act as a soil moisture reservoir which plants are able to access once established (Johnson and Lowery, 1985). The STOR has also been shown to increase diversity of beneficial fauna such as spiders, predatory carabid beetles and earthworms (Jaskulska et al., 2020; Wenninger et al., 2020).

The tilled strip improves crop establishment by lifting and aerating the soil, terminating weed seedlings, speeding up early-season soil-warming (Licht and Al-Kaisi, 2005). The precision placement of granular or liquid fertilizer beneath the seed reduces fertiliser costs and runoff risk compared to broadcasting of fertiliser (Pan et al., 2017; Rahman and Zhang, 2018).

Studies across a variety of crop species have shown that strip till can be used to obtain comparable yields to other tillage systems. Tarkalson et al. (2012) maintained sugar beet yield with a reduction in tillage costs of between 53-76%. Saldukaitė et al. (2022) assessed the yield and energy efficiency of different tillage practices in winter oilseed rape and found that ST was the more energy efficient and higher yielding compared to CT and ZT. ST has also been shown

to be effective in maize (Ren et al., 2019) and is a widely adopted practice in the United States (Claassen et al., 2018). ST has also been used successfully for various field vegetable crops including pepper (Spieser, 1984), carrot (Brainard and Noyes, 2012) and broccoli (Jokela, 2016).

3.1.1.2 Limitations of strip tillage

Although the area of land under ST is rising (Zemlicka, 2018), it represents only a small proportion of agricultural land worldwide. The expense of specialist machinery required for ST is one of the main barriers to its adoption (Morris et al., 2010). Some soils are unsuitable for ST; the increased water retention may lead to water logging and delays in field operations in heavier soils, and the single tillage pass may not produce a suitably fine tilth in some soils (Laufer and Koch, 2017). Tilled strips are generally kept in the same place each season which can lead to patch depletion of nutrients (Zhang et al., 2023) and problems with compaction in machinery wheelings and vertical hard pans (Ren et al., 2019). Also, without field width cultivation to control weeds, they can be an issue under ST leading to a greater reliance on herbicides (Storr et al., 2019; Trevini et al., 2013)

3.1.1.3 Changes in soil physical properties under strip tillage

The STIR and STOR strips established under ST are largely comparable to CT and ZT respectively. Physical differences between STIR crop rows and STOR interrow area are, to a large extent, in line with differences expected for a field managed wholly under CT or ST (Tabatabaeekoloor, 2011). However, due to their proximity the rows interact to affect physical changes beyond what would be expected in a homogeneous managed field (Pöhlitz et al., 2018). Higher moisture content in the STORs act as a reservoir to maintain moisture in the

STIR (Hendrix et al., 2004; Jaskulska and Jaskulski, 2020). Fernández et al. (2015) showed increased SOM in the STOR strips compared to ZT and suggested this may be due to increased biomass production under ST, and/or increased decomposition of residues on the warmer ST soil. They also showed an 18% reduction in penetration resistance and 4% reduction in bulk density in the STOR compared to ZT after two years in under ST. It is worth noting however, this trend was not backed up by other short term strip tillage experiments (Ren et al., 2019). The effects appear to be highly soil-type dependent.

Pöhlitz et al. (2018) used X-ray Computed Tomography (X-ray CT) alongside traditional mechanical soil tests to explore the effects of ST on soil mechanical and morphometric properties. In addition to the changes described above, they observed that CT and ZT areas responded differently to mechanical loads. The annual disturbance of the WS soil hindered structural formation and created coarse, unstable pores which were destroyed by relatively low mechanical pressure. In contrast, the ZT soil was more mechanically stable with higher aggregate density and lower pore-connectivity. The STORs are subject to mechanical loads from field operations and structural development is not disrupted by tillage operations. Ren (2019) showed that while yields were unaffected by tillage practice, maize roots under ST were not able to cross the boundary between strips, and macro-porosity was decreased in the tilled strips compared to CT. ST is a promising agricultural practice offering several benefits. However, there is relatively little literature, and its effectiveness varies across different soil types and conditions, highlighting the need for more extensive research.

3.1.2 Experimental investigation of effect of strip tillage on soil structural properties

To investigate the influence of ST and other soil tillage strategies on soil properties, XRCT was used to examine intact soil cores from the STIRs and STORs of a ST field, and adjacent fields under CT and permanent pasture (PP).

The main objectives of this investigation are to:

1. Explore the differences in soil physical properties, between STIR and STOR strips in ST field. The first hypothesis that 'soil in the STIRs will have lower bulk density, a greater number of macropores which are less well connected than in the STORs' is based on previous work by Cooper et al. (2021) on ZT and CT fields which shows marked changes in soil structural characteristics.
2. Explore the differences in soil physical properties between spatially paired ST, CT and permanent pasture (PP) fields. The second hypothesis was 'STIRs and STORs will share physical properties with CT and ZT respectively.'

3.1.2.1 Methods

3.1.2.1.1 Sampling

Sampling was undertaken in South Devon, UK in Feburary 2022, each of the sites was under a different cropping regime and stage of growth aside from the PP sites, see Table 3.1. The two groups of sites, A and B, were selected for their similar soil characteristics. Group A is sandy loam and group B is loamy sand. The fields in group A were all adjacent to one another running up a south westerly slope whereas the fields in group B were more spread out with the CT field being 1.1 km away.

Intact cores (30 cm long, 5 cm diameter) were collected with a manual corer with clear PVC liners (Van Walt Ltd, Haslemere, UK) for XRCT. Topsoil samples were collected using stainless steel cylinders (4 cm long, seven cm diameter) for measuring bulk density. All samples were taken in triplicate, randomly from across treatments. Samples were stored at 4 °C and scanned within two days of collection.

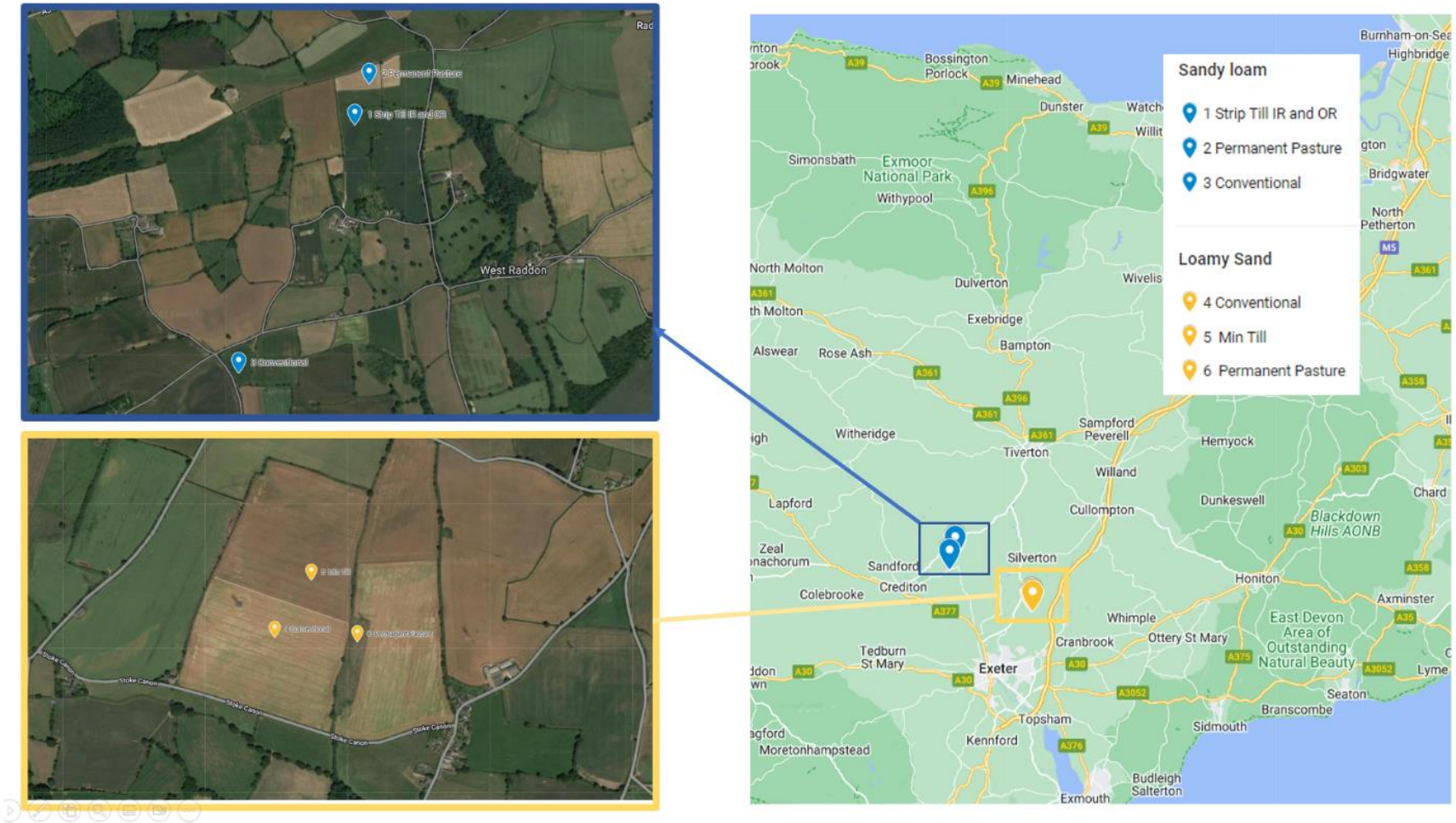

Fig. 3.2 Sampling locations. Yellow and blue markers indicate loamy sand and sandy loam sites respectively.

Table 3.1
Summary of sampling sites

Field	Group	Treatment	Management	UK Soil Class (sand:silt:clay)	Aspect
1	A	Strip till, untilled strip (STOR)	Direct drill with Claydon strip drill. Wheat into sprayed off crop with volunteer beans.	Sandy Loam (50:43:7)	Southerly, mid slope
1	A	Strip till, tilled strip (STIR)	Direct drill with Claydon 'strip drill'. Wheat into volunteer beans.	Sandy Loam (50:43:7)	Southerly, mid slope
2	A	Permanent pasture (PP)	Rough grass. 30-years continuous pasture not tilled. Cut once per year for hay. Some FYM annually	Sandy Loam (57:41:2)	Southerly, top slope
3	A	Conventionally tilled (CT)	Spring barley recently, ploughed regularly. Had cows on it after SB harvest, veg, potatoes on before that.	Sandy Loam (51:43:6)	Level
4	B	Conventionally tilled (CT)	Young oil seed rape crop on it	Loamy Sand (82:13:5)	Slight southerly slope
5	B	Minimally tilled (same tillage as STIR) (MT)	Established cover crop w/mustard, radish, linseed, OSR. Winter barley following OSR, following WB following Maize before that.	Loamy Sand (82:15:3)	Level, top slope
6	B	Permanent pasture (PP)	Rough grass. 30 years continuous pasture not tilled. Cut once per year for hay. Some FYM annually	Loamy Sand (78:20:2)	Slight Southerly slope

Scanning was performed at the Hounsfield Facility, University of Nottingham, Sutton Bonnington using a Phoenix V|Tome|X m x-ray scanner 240 kV (GE Measurement & Control Solutions, Wunstrof, Germany). Resolution was set to 85 µm, with a potential energy of 180 kV and a current of 210 µA. Soil cores were scanned in two 10 cm sections (i.e., depths) to allow greater resolution, with a total scan time of 20 minutes per core. A total of 2400 image projections were captured for each core.

Projections were reconstructed at 16-bit using Phoenix Datos x2 reconstruction software. Images were optimized to correct for any movement of the sample during the scan and noise was reduced using the beam hardening algorithm in Datos × 2, set at level 8. Scan sections were converted from 32-bit to 16-bit, normalised for minimum and maximum grey values and

then aligned and stitched together using Volume Graphics VGStudio Max 2023.1 software before exporting as TIFF stacks. Image stacks were processed using ImageJ version 1.54f where they were filtered with a 0.2 x 0.2 x 0.2 3D median filter to reduce noise while maintaining edge detail. Thresholding used the triangle algorithm to separate pixels into either solid or airspace, binarised images were analysed using the particle analysis function within Fiji image analysis software (Schindelin et al., 2012). For this analysis XRCT images were treated as a stack of 2D slices, rather than a 3D volume. As such, pore size is calculated as total porosity as a percentage of the total image area, and pore size distribution is expresses as the percentage of the total pore area in the images in each pore size bin. Bulk density cores were oven dried at 105 °C for 24 hours and weighed to determine bulk density and gravimetric water content, stones were not removed for this analysis. Particle size analysis was performed using the hydrometer method (Day, 1965). pH was determined using a pH probe with a 5 : 1 water : soil suspension (Day, 1965). Soil organic matter was estimated by loss on ignition for 30 minutes at 850 ^{o}C (Ball, 1964).

3.1.2.1.2 Statistical analysis

Normal data distribution was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variance was tested using the Brown-Forsythe test (Brown and Forsythe, 1974). When these assumptions required for analysis of variance (ANOVA) were met, two-factor analysis of variance (ANOVA) with tillage treatment and soil type as factors was used, where these assumptions were not met (total porosity and soil organic matter) Kruskal-Wallis one-factor ANOVA on ranks (Kruskal and Wallis, 1952) was completed. Pairwise post-hoc analysis was completed using the Holm-Sidak (Holm, 1979) method. All statistical tests were completed using SigmaPlot 15.0 software (Systat Software Inc., San Jose, CA).

3.1.2.2 Results

The sandy loam (SL, blue bars) had significantly higher ($p < 0.001$) soil organic matter (SOM) as measured by loss on ignition than loamy sand (LS, orange bars) for each equivalent soil management practice (Fig 3.3a). The SL PP had a SOM content (Fig. 3.3a, 9.5 g cm^{-3}) significantly higher ($p < 0.01$) than any other tillage on either soil type. The PP soil was also the most acidic (Fig. 3.3b, pH = 5.6) and had the lowest bulk density (Fig. 3.3c). The bulk density of the PP on the LS was also lowest than the other tillage treatments on the LS however the differences were not significant. Any changes in soil structure were not seen in the quantitative image analysis with no significant differences found between soil types or treatments (Fig 3.3d). The STIR and STOR rows were not significantly different in any of the analysis. All tillage treatments had higher porosity in the top 10 cm, particularly in LS (Fig. 3.4a and b). Soil from CT plots was more consistent as depth increased in both soils. The differences between the treatments in the LS were only apparent in the top 10 cm, below which the treatments were similar at around 4%. In contrast, the SL STIR and STOR and PP soils varied greatly below 10 cm.

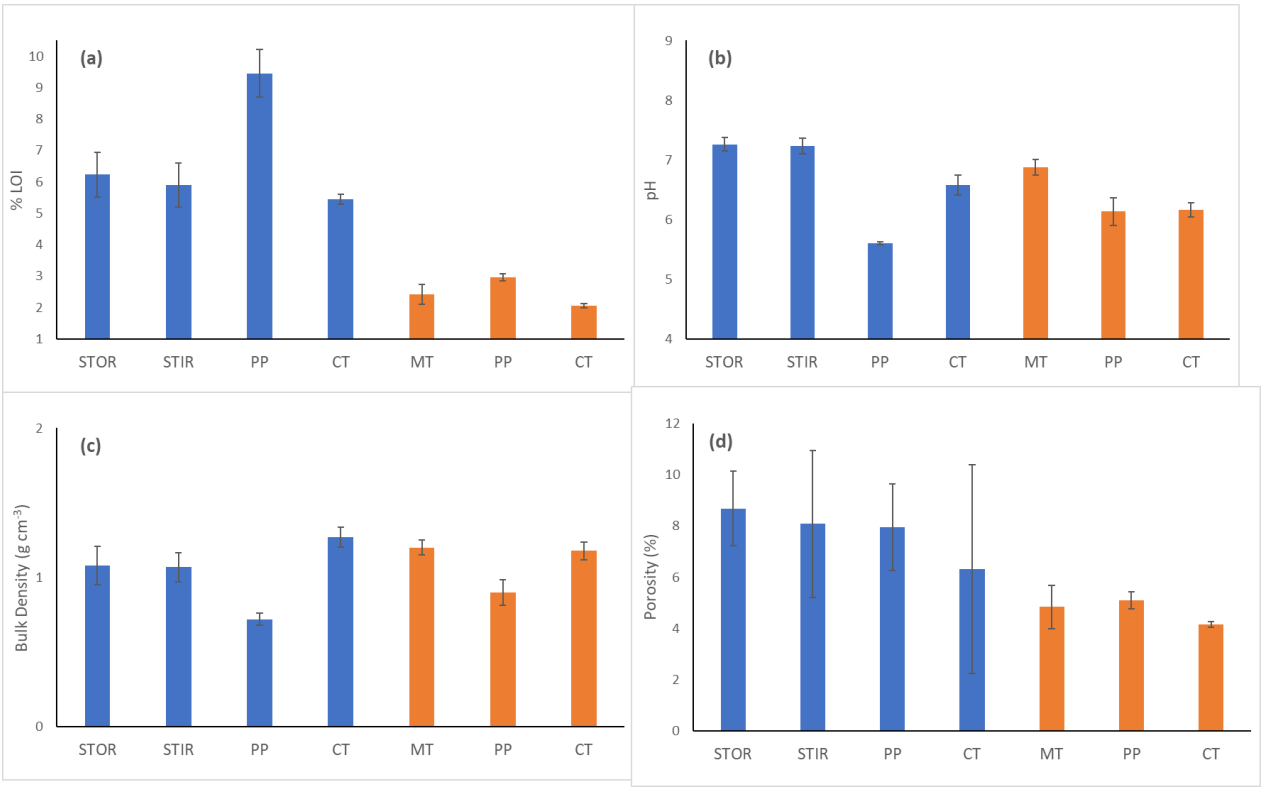

Fig. 3.3. Organic matter content measured by loss on ignition (a), pH (b), bulk density (c), and percentage porosity from 2D image analysis (d) of a sandy loam (blue bars) and loamy sandy (orange bars) under conventional tillage (CT), minimum tillage (MT), permanent pasture (PP), strip tillage in-row (STIR) and strip tillage out row (STOR). Vertical bars denote standard errors.

Pore size distributions (PSD) in the two soil types followed distinct patterns. In the SL soil (Fig. 3.5a), all treatments (except CT) had a higher percentage of the pore space in larger pore sizes. SL CT had a similar percentage of pores in each pore size class above than 0.316 ≤ 1 mm^2 except for the 100 ≤ 316 mm^2 size class which contained four times the pore area of any other pore size class. SL PP had a large proportion of the pore area in large pores above 316 mm^2. There were many small (<0.1 mm^2) pores in all treatments and across both soil textures which, while numerous, contributed little to the total pore space. The small pore size classes, < 3.16 mm^2, in all treatments on LS followed the same pattern as the SL soil. However, the pore size classes between 3.16 and 100 mm^2 contained most of the pore area and the largest two pore

size classes represented very little pore space and PP and CT had no pores in the 316 ≤ 1000 mm^2 pore size class. The differences between treatments were less pronounced in the LS soil with all the treatments following similar patterns.

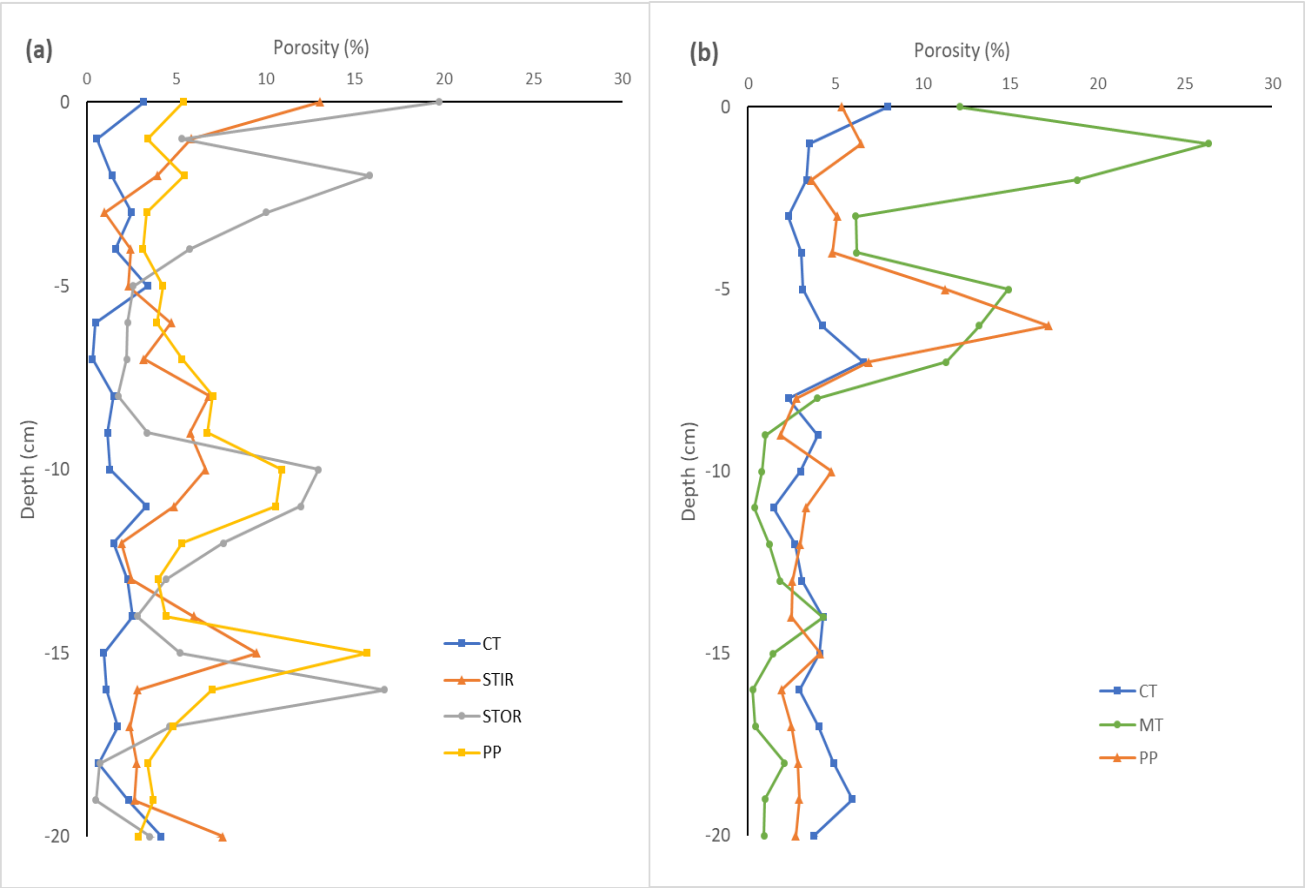

Fig. 3.4. Percentage porosity change along a depth profile for sandy loam (a) and loamy sandy (b) under conventional tillage (CT), minimum tillage (MT), permanent pasture (PP), strip tillage in-row (STIR) and strip tillage out row (STOR). Pore size calculated on a 2D basis as a percentage of total image area.

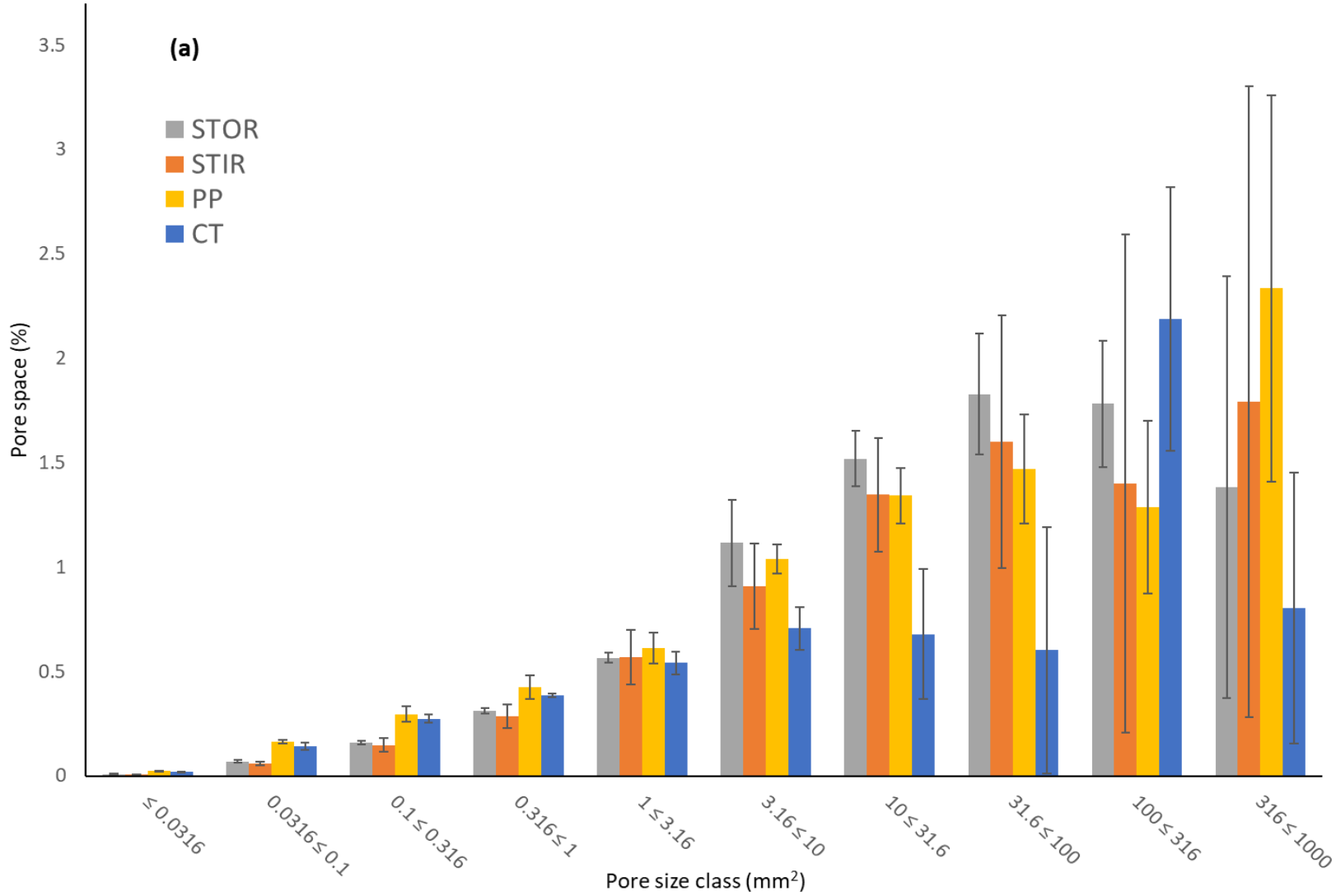

Fig. 3.5a Percentage pore space in pores of each pore size class in sandy loam under conventional tillage (CT), minimum tillage (MT), permanent pasture (PP), strip tillage in-row (STIR) and strip tillage out row (STOR). Pore size calculated on a 2D basis as a percentage of total image area. Vertical bars denote standard errors.

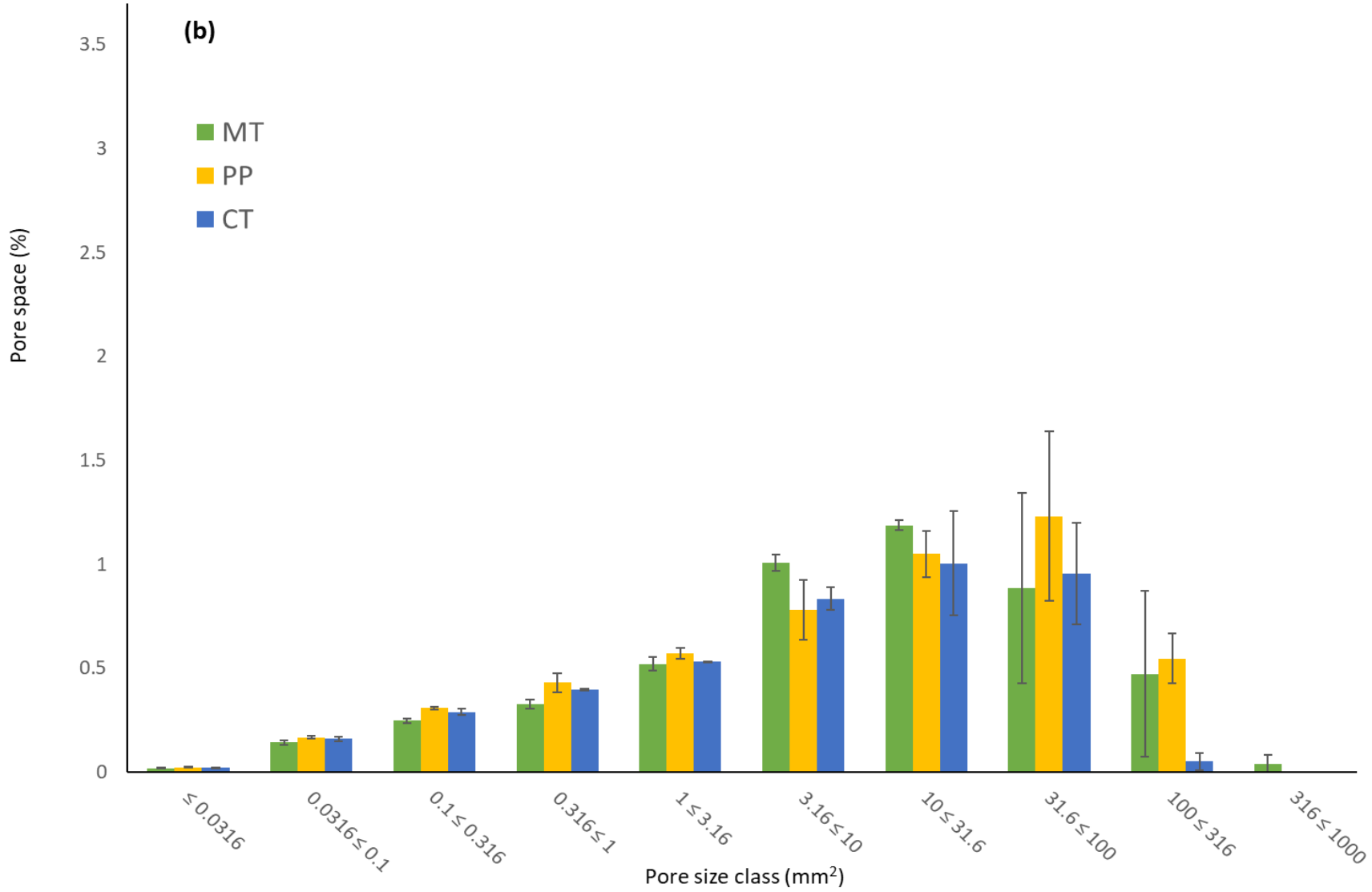

Fig. 3.5b Percentage pore space in pores of each pore size class in loamy sand under conventional tillage (CT), minimum tillage (MT), permanent pasture (PP), strip tillage in-row (STIR) and strip tillage out row (STOR). Pore size calculated on a 2D basis as a percentage of total image area. Vertical bars denote standard errors.

3.1.2.3 Discussion

This experiment was undertaken with the aim of examining the differences in structure between STIR and STOR strips in fields under ST. However, it was not clear at the time of sampling that although the ST field had been under ST for several years, the position of the tilled strips was changed each season to avoid compaction and heterogenous nutrient enrichment. This practice means that any effect of ST was effectively nullified each year and differences in soil structure were not able to develop between the STIR and STOR rows. As others have reported (Jaskulska et al., 2020; Mangalassery et al., 2015), if strips were established in the same position each year, we would expect the tilled and planted strip to become more similar to the CT soil, with more homogenous structure and lower bulk density; and the STOR to become increasingly similar to the PP fields with more SOM. The PP sites on both soil types have a thick layer of organic matter-rich soil at the top of the soil profile, consisting of roots and decomposing above ground biomass (visible in the radiographs, Fig. 3.6a) which would explain the lower bulk densities and higher LOI figures. Also, the lower pH in the PP fields may be due to the higher organic matter deposition (Cox and Koenig, 2010). Any differences due to the tillage history of the sampling locations are likely obscured by the different soil textures between the LS and SL sampling areas (~80% and ~53% sand content respectively). Soils with high sand content and low soil organic matter, as is the case with the LS soil, have lower aggregate (and therefore pore) stability (Durner, 1994; Zhou et al., 2017). The PSD of the LS soil shows this, with very little of the total porosity comprised of larger pores (larger than 100mm^2) and most of the porosity of the SL soil is represented by pores above 100mm^2. All soils were very stoney. This interfered with the quality of the cores collected (Fig

3.6b) and therefore the quality of the data. Collecting intact soil cores from stoney sites poses challenges as stones will either be pushed out of the way by the corer, creating a void in the sample; or they will be included leading to localised compaction and disruption of the soils' structure. Collecting more cores per site can increase the representativeness of the data, however, due to logistical constraints only two cores were collected per site.

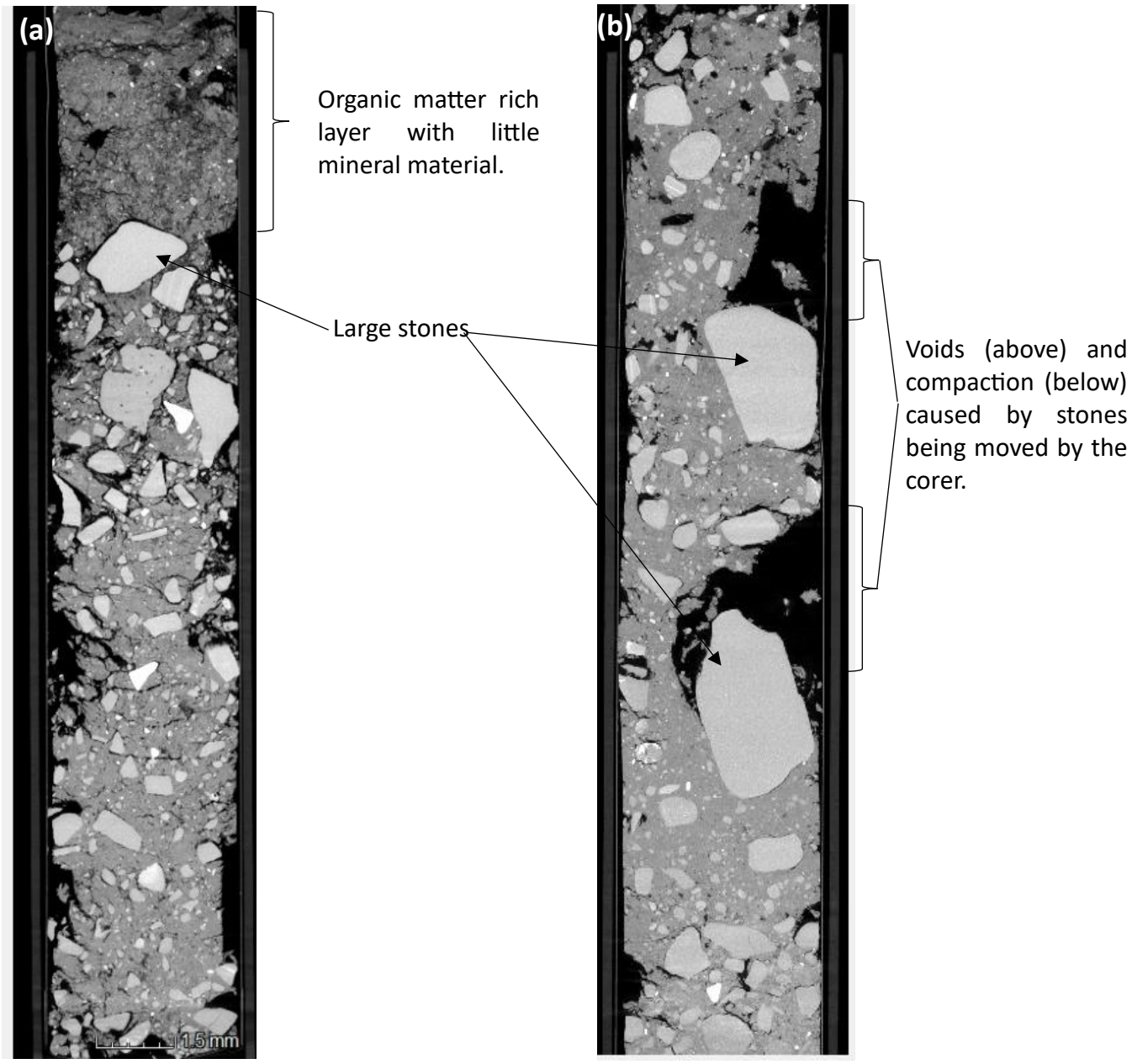

Fig. 3.6 X-ray CT images showing the organic matter rich layer at the top of the permanent pasture fields (a) and the effect of stones on the quality of the images and representativeness of the data (b).

3.1.2.4 Conclusions

No differences between the STOR and STIR strip were ascertainable by any of the methods used in this study. Equally, the STOR and STIR strips had not developed any similarities in physical properties with CT and ZT respectively as was hypothesised. The practice of changing the position of tilled strips each season effectively nullified any long-term effects of strip tillage. As a result, differences in soil structure between the STIR and STOR did not develop.

The textural differences between the sampling areas are the biggest driver of differences seen between most measured parameters. Texture is often overlooked in studies of soil structure despite it having a large impact on chemical, biological and physical characteristics of soil (Ben-Hur et al., 2009; Fatichi et al., 2020). While these results show no differences between strip till strips, this lack of differences was due to experimental and methodological limitations, and it would be incorrect to conclude that ST does not cause structural differences.

3.2 Arbuscular mycorrhizal fungi contribution to soil aggregation

3.2.1 Introduction

The development of soil aggregates and associated pores is crucial for healthy soils that can support agriculture and ecosystem services. The activity of soil microorganisms play an important role in the development and stabilisation of soil structure chiefly through the production of extracellular polymeric substances (EPS). Microbes produce EPS to serve several functions including nutrient acquisition and protection against predation and environmental stresses (Sandhya and Ali, 2015). Due to their gelling characteristics and ionic charges, microbial EPS can enhance the aggregation of soil particles and benefit plants by maintaining the moisture of the environment and trapping nutrients (Chenu, 1995).

Arbuscular mycorrhizal fungi (AMF) contribute to soil aggregation through a complex and interrelated variety of biochemical, biophysical and biological mechanisms (Rillig and Mummey, 2006). Biophysical mechanisms are the direct physical interaction of the fungal hyphae with soil particles and includes enmeshment, exertion of pressure and changes in local water potential and are affected chiefly by the architecture of the mycelium. Direct experimental evidence for these mechanisms in AMF is scant but work by Miller and Jastrow (1990) and Tisdall and Oades (1980) mycelium length is positively correlated with aggregate stability as measure by mean weight diameter (MWD) and there is causative evidence from non-mycorrhizal fungi that hyphal enmeshment increases water stable aggregation of soil particles (Daynes et al., 2012).

Biological mechanisms are more indirect and include the AMF's influence on bacteria which are known to be much more diverse and numerous in the hyphosphere than in the bulk soil (Warmink and Van Elsas, 2008). AMF have complex interactions with the plants they are

associated with and are able to influence the amount and composition of root exudates as well as root architecture (Azaizeh et al., 1995; Harris, 2008). The change in exudate quality and root branching will affect the compounds acting as glues and the soil area in proximity to roots thus influencing aggregation.

Biochemical mechanisms are primarily linked to fungal products such as such as polysaccharides, glycoproteins, and hydrophobins which are released from both living and decomposing hyphae. They have been seen to attract and align soil particles to form microaggregates, act as cements which fill gaps and stabilise aggregates and increase hydrophobicity of aggregate surfaces (Hooker et al., 2007; Lehmann et al., 2017). Glomalin, a glycoprotein EPS produced by AMF, has gained particular attention for its role in stabilizing soil structure and facilitating nutrient cycling (Rillig, 2004). It has been shown to increase aggregate water stability (Rillig et al., 2002) and has been shown to be a primary contributor to aggregate stability in the rhizosphere (Wu et al., 2014). Glomalin is thermostable and highly recalcitrant to microbial degradation with a turnover time measured in years (Halvorson and Gonzalez, 2006; Marschner et al., 2008). Although not unique to AMF, majority of glomalin and glomalin related soil proteins (GRSP)[1] is of fungal origin (González-Chávez et al., 2008).

[1] Glomalin refers solely to the microbial product. GRSP is used to refer to the operationally defined pool of SOM extractable from soil by hot citrate buffer which will contain compounds of other origin and function. For more see Holátko (2021).

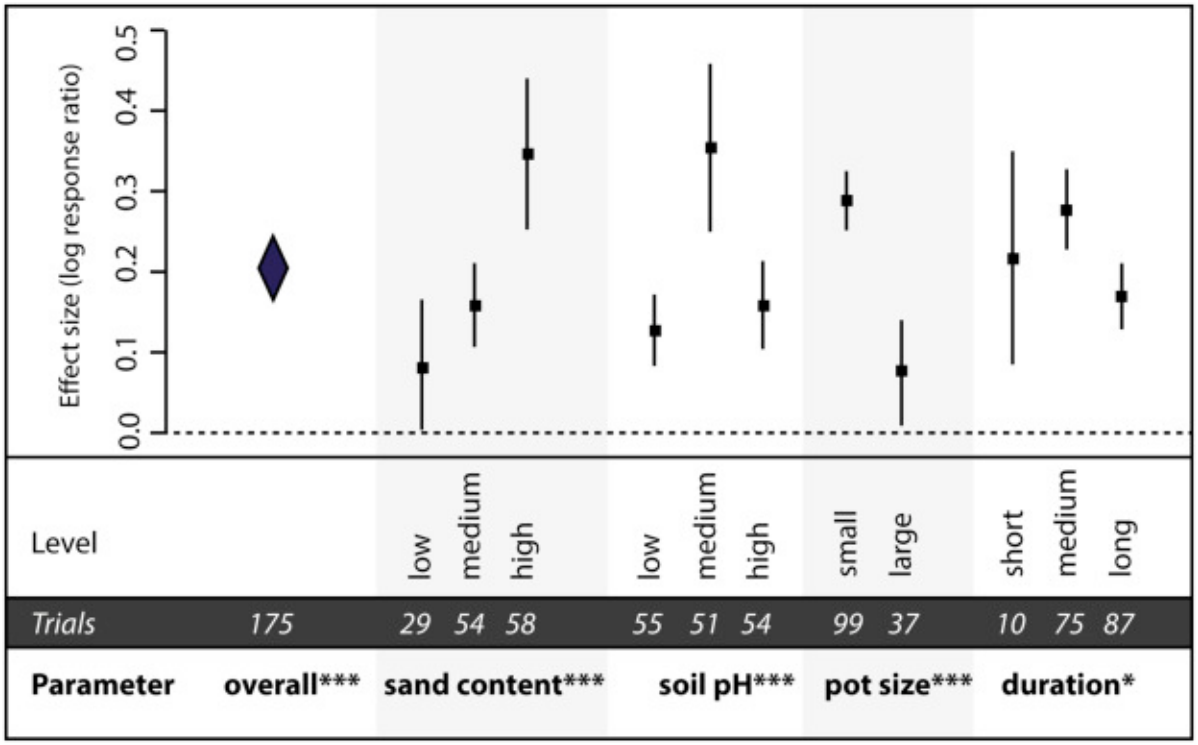

Fig. 3.7. Overall effect of arbuscular mycorrhizal fungi on soil aggregation and the impact of soil derived (sand content, soil pH) and experiment-related variables (pot size, experimental duration). Data from a meta-analysis by Leifheit et al. (2014) redrawn in Lehmann (2017)

The relative importance of AMF to aggregation is not well understood as it is very hard to study them in isolation as they are obligate symbionts, so the influence of AMF is almost always considered alongside roots. Some studies have excluded roots and found the soil aggregation ability of mycorrhizal fungi comparable to plant roots, but that this seems to be a non-additive effect (Andrade et al., 1998; Rillig et al., 2010; Thomas et al., 1993). In natural systems, AMF's contribution to aggregation will depend on fungal species composition, host plants and climate and soil conditions (Helgason and Fitter, 2009; Leifheit et al., 2014; Pietikäinen et al., 2009) and, as described in Leiftheit et al. (2014; Fig. 3.7), experimental variables will also influence the apparent aggregation ability of AMF being studied.

This experiment was devised to investigate the relative contributions of roots, mycorrhizal fungi and their synergistic interactions in soil aggregate formation and stabilisation.

3.2.2 Experimental design

3.2.2.1 Mesocosms

A method was devised to separate hyphae effects from root effects. A 39 µm nylon mesh, which has been shown to allow penetration of fungal hyphae but not plant roots (Thomas et al., 1993), was used to divide a column (7.9 cm inside diameter 14 cm tall). The base of the mesocosms was covered with a coarser mesh to retain soil but allow capillary rise of water. Mesocosms were packed to a bulk density of 1.2 g cm^{-3} with a sandy loam soil collected from a plot of a field trail that had been under ZT for ten years, dried and sieved to two mm. Mesocosms were sat in 4cm of water and left for six hours until saturated before draining for 24 hours to reach field capacity before planting. *Plantago lanceolata*, a widespread grassland species and agricultural weed, was chosen to grow in the mesocosms due to its known mycotrophy (Šmilauer, 2001), particularly with species in the glomalin producing *Glomus* genus (Staddon et al., 1998). Pilot studies showed it to be slow growing in comparison to *Zea mays* and *Triticum* species with no tap root which reduced the chance of plants becoming pot bound over the course of the experiment.

Preliminary XRCT scans of the drained soils revealed shrinkage of the soil away from the dividing mesh by 1-3mm (Fig. 3.8). There was concern that this may reduce the ability of the AMF to colonise the unplanted side of the mesocosm, however work by Sturrock et al. (2002) shows that fungi are unperturbed by air gaps and would be able to bridge the gap.

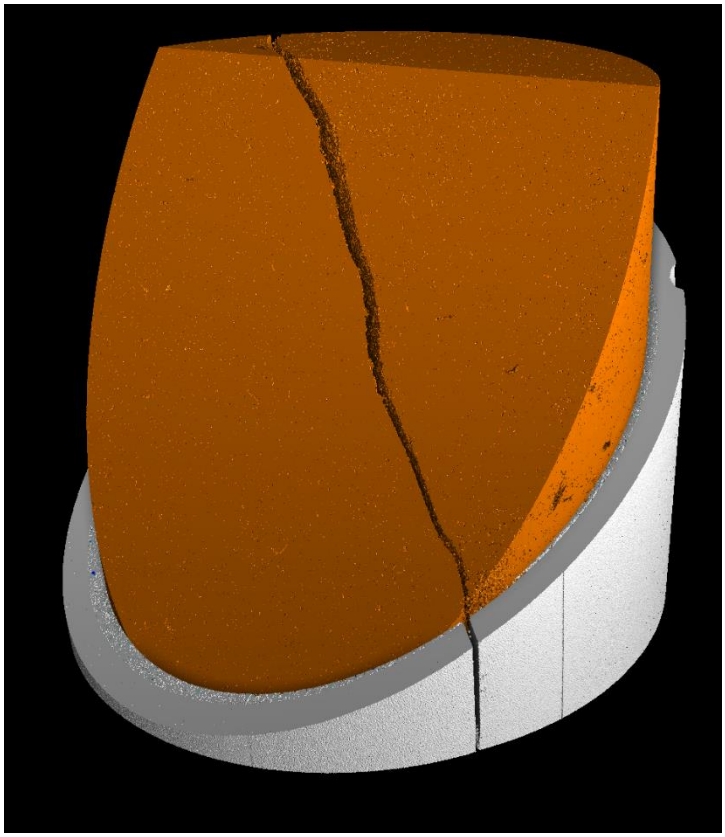

Fig. 3.8 Packed mesocosm (PVC tube in grey, soil in brown) showing a gap where the soil has shrunk away from the dividing mesh (mesh not visible in image).

3.2.2.2 Treatments

Six treatments were established in three pots, two per mesocosm as illustrated in Fig. 3.9. All treatments use the same sandy loam soil, air dried and sieved to 2mm. Maize starch was added to all the treatments (aside from one) at a rate equivalent to 18 g C kg^{-1} soil to trace Carbon-13 (^{13}C) into different aggregate size classes. Treatment A is unplanted on both sides, with one side free of starch to act as root free (A1), and starch free (A1) controls, any changes in aggregation on the starch free side (A2) will be the result of microbial activity, and any additional aggregation on the starch containing side (A1) can be attributed to an effect of the starch.

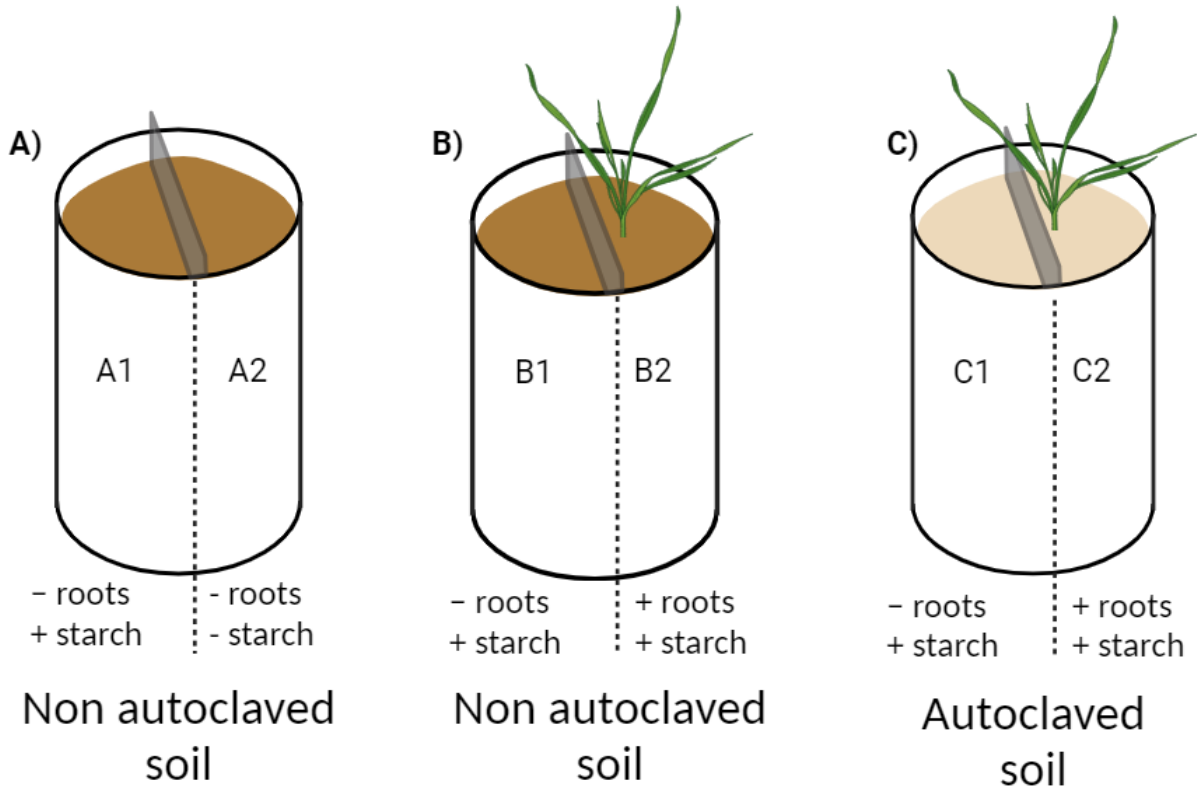

Fig. 3.9 Diagram of treatments used in the split pot experiment

Treatments B1 and B2 will show effects of microbial organisms, and microbial organisms and roots respectively. For treatments C1 and C2 the soil was autoclaved (Rodwell Phoenix 60, Rodwell Autoclave Company, UK) twice at 121°C for 10 minutes with a 10-minute vacuum pre- and post-heating with 24 hours between heating. The autoclaved soil, with greatly reduced microbial diversity and activity, will allow for the effect of the plant roots to be looked at in isolation on the planted side of the mesocosm (C2) and just the physical effects of wet-dry cycles on the other (C1).

Mesocosms were positioned at random in a greenhouse at the Hounsfield Facility, University of Nottingham and kept at 20 – 25°C with natural daylight (13–17-hour daylength). The plan was to run the experiment for two months starting on 24th March 2023 with harvests and scans at days 1, 30 and 60 where half the mesocosms would be non-destructively scanned

using XRCT and the other half destructively harvested and air dried before analysis. However, the experiment was aborted after the first harvest and scan time due to the issues outlined in section 3.2.3.

3.2.2.3 Image Analysis

XRCT scanning was done at the Hounsfield Facility, University of Nottingham, Sutton Bonnington, UK using a Phoneix v|tome|x M scanner (GE Measurement and Control Solution, Wunstorf, Germany). Voxel resolution was set to 45 µm, with a potential energy of 172 kV and a current of 200 µA. Soil cores were scanned in two sections (i.e., depths) to allow greater resolution, with a total scan time of 18 minutes per core. A total of 2,159 image projections were captured for each core. Scanned images were reconstructed at 16-bit using Phoneix Datos x2 reconstruction software. Images were optimized to correct for any movement of the sample during the scan and noise was reduced using the beam hardening algorithm in Datos × 2, set at level 8. Datos x2 Multi|Scan feature was used to automatically merge the two sections.

VGStudioMax v.2023.1 was used to standardise histograms across scans and create image stacks which were analysed using ImageJ c.1.54f (Schneider et al., 2012). Image stacks were filtered using a 0.1, 0.1, 0.1 3D median filter, thresholded using the Li algorithm (Li and Tam, 1998), binarised. A 900 x 900 x2500 pixel region of interest (ROI) was cropped from the centre of the core to avoid any cracking around the edge of the column. This ROI was used for analysis of total porosity, pore size distribution and pore connectivity using the particle analysis

3.2.2.4 Further analysis

As the experiment was not completed the following analysis was not completed, however the plan is outlined here to demonstrate an understanding of the methods which would have been used. The destructively harvested soils were to be air dried and gently crushed to break it along natural planes of weakness. A representative selection of aggregates from 3-10 mm were to be selected for analysis using a soil aggregate erosion chamber as in Park and Smucker (2005). Briefly, individual aggregates are placed inside a small, knurled chamber with a mesh base which is secured to a rotary shaker platform and rotated at 250 rpm to erode layers of the aggregate (Fig 3.10a). Each concentric layer peeled from the aggregate is a third of the aggregate by weight (Fig 3.10b). Each layer is then subsequently analysed for total C and ^{13}C using laser ablation isotope ratio mass spectrometry.

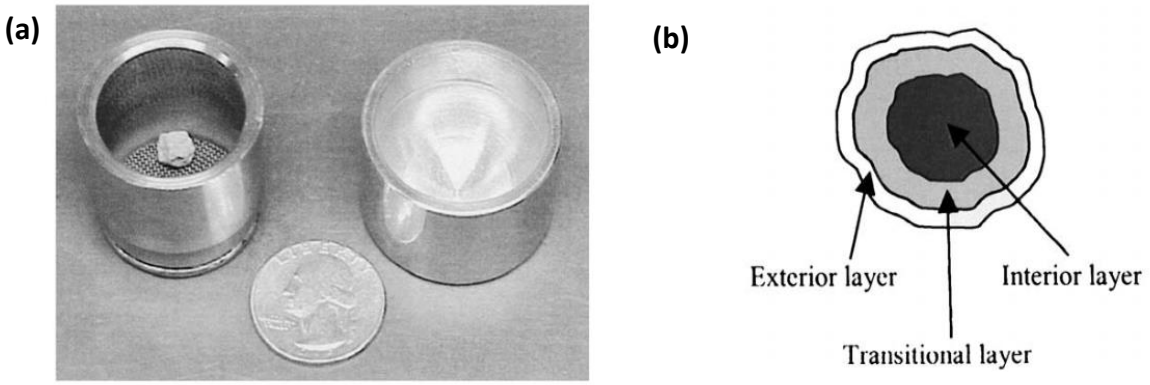

Fig. 3.10 Soil aggregate erosion chamber for mechanically abrading layers from a single aggregate (a). Concentric layers of an aggregate (b).

The water stability of a subsample of soil can be determined through wet sieving. After sieving, 50 g of aggregates between two and 8 mm are placed on top of a series of nested sieves (2000, 500, 250 and 53 µm) and oscillated in water for 10 mins at 30 oscillations per minute before drying for 48 hours at 105^{o}C and weighing.

3.2.3 Challenges

The experiment faced several challenges which ultimately led to the analysis not being conducted. The primary issue was the length of time needed for aggregates to develop and stabilise to a point where differences would be discernible using the techniques outlined above seems to be longer that the timescales used in this experiment. When using XRCT to image soils there is a balance to strike between sample size and resolution. From this experiment, ideally the mesocosms would be much larger to allow the experiment to run for many months. However, if the mesocosm were any larger than that used, the resolution of the images would not be high enough to discern any changes in soil structure. As such, preliminary work showed that the two months the experiment would have run for would be enough for any structural changes to develop. This was seen when the soil was dried and gently crushed. The soil was still amorphous with no lines of weakness and nothing that could be described as a 'stable aggregate'. While there may have been some changes in the microporous structure of the soil, the resolution of the XRCT scans (45 μm) was too course to visualise or quantify these changes.

Another issue was the inability to conduct the experiment in an a-septic environment. This led to the autoclaved soils becoming visually colonised with air-borne fungi. These colonised the gap between the mesh and the soil all through the mesocosm. This occurred in both the autoclaved and non-autoclaved soils. As no analysis of microbial activity was done post-autoclaving to verify the sterilisation of the soil, it is impossible to know how effective it was. Trevors (1996) suggest that holding at heat for 10 minutes may not be sufficient to sterilise the soil, and that it may have needed up to an hour. While this would have been impractical for this purpose, it could have improved the sterilisation of the soil. Autoclaving is also known

to release ammonium-N and change soil structure (Alef and Nannipieri, 1995; Jenneman et al., 1986) so sterilisation by gamma radiation may have been a preferred method.

3.3 The role of tillage and molasses addition on the persistence of glyphosate in a UK soil. A field and lab trial.

3.3.1 Introduction

Increasingly, farmers and land managers are looking to reduce the environmental impact of their activities (Kassam et al., 2019). Conservation agriculture (CA) is a sustainable farming approach that aims to preserve and improve the health of agricultural ecosystems while enhancing crop productivity (Giller et al., 2015). CA comprises three key principles: minimise soil disturbance, maintain permanent soil cover, and use diverse crop rotations. These practices aim to reduce soil erosion, enhance soil fertility, and optimize water usage, all of which contribute to long-term environmental and economic benefits (Dumanski et al., 2006; Hobbs et al., 2007; Palm et al., 2014). A key part of CA involves the adoption of ZT, where soil is left undisturbed to protect its structure and the carbon within it. Within the context of ZT, glyphosate, a widely used herbicide, is a crucial tool for CA farmers due to its perceived low-toxicity and its ability to effectively control weeds and unwanted vegetation without the need for soil disturbance (Romano-Armada et al., 2017). However, the use of glyphosate is a topic of much debate due to mounting evidence suggesting it may not be as innocuous in the environment as previously thought and there may be legitimate human and environmental health concerns surrounding its use (Myers et al., 2016). There are calls for stricter regulations and controls on glyphosate use ahead of its UK license review in 2025 (Reynolds et al., 2016), however there is no economically viable alternative currently available to arable farmers wanting not to till their fields (Kehlenbeck et al., 2016). If restricted, many farmers who currently manage their fields using ZT to protect soil carbon may revert to tilling their land (Böcker et al., 2018). One study suggested that increased fuel use from tillage activities alone

could increase EU greenhouse gas emissions by 1.4–3.8 Mt CO_2 equivalents per year if glyphosate were banned (Wynn and Webb, 2022). Reducing the environmental impact and persistence of glyphosate in soils is of interest to environmentally minded farmers, and as glyphosate in the soil is decomposed principally by microorganisms (Martins et al., 2023), understanding the role of the soil microbial community and how to adapt or support it will play a key role in this.

The adoption of ZT has been shown in many cases to increase the microbial biomass in topsoil (Helgason et al., 2009; Sapkota et al., 2012) and alter microbial communities (Srour et al., 2020). The changes to the microbial community composition differs depending on various factors such as climate, soil texture and organic matter content but there are some general trends. Probably due to the increase crop residue in the topsoil, ZT soils tend to have higher abundances of actinomycetes and cellulose decomposers in the top 5-10 cm than tilled soils and a shift toward more stress tolerant organisms with wider metabolic capacity with depth. (Schmidt et al., 2018b)

It is well established that glyphosate is broken down by soil microorganisms (Martins et al., 2023; Ratcliff et al., 2006; Zabaloy et al., 2012; Zablotowicz et al., 2009). Glyphosate is a ready source of N, P for soil microbes and has been shown to temporarily alter soil microbial community composition and increase biomass and respiration as it is respired (Nguyen et al., 2016; Schmidt et al., 2018b; Zabaloy et al., 2012). The addition of simple carbohydrates, typically molasses or sucrose, to the GBH when applied is a technique used by some CA practitioners to increase the efficacy and reduce the persistence of GBHs. Aside from one mention by Story in 1939, there is no mention of this technique, let alone evidence for the efficacy of this practice in the scientific literature. However, the general principal is sound:

increasing the numbers of microorganisms with the addition of a source of readily available C would speed the degradation of glyphosate that encounters the soil. However, the amount of carbohydrate typically used is around 3 L ha^{-1} and there is scepticism as to whether there would be any impact of the nutrient addition. Furthermore, as glyphosate is a source of all three major microbial nutrients (C, N and P), the addition of more C may not result in the removal of a microbial growth limiting factor and the subsequent glyphosate degradation that practitioners hope for. Although an agronomically relevant effect seems unlikely, it is still considered worthy of study as if it were found to be effective it would offer a cheap method of reducing the half-life of a widely used and potentially harmful herbicide, and could reduce the time between the application of GBH and planting a crop; and if it is found to be ineffective, this will enable CA practitioners to save money on inputs.

This study uses XRCT images from intact cores and glyphosate degradation data from a field trial, alongside an incubation experiment to explore the effects of the addition of a simple sugar to glyphosate on the persistence of glyphosate and the interaction of different tillage treatments on the fate of glyphosate. It is hypothesised that i) glyphosate will be degraded more rapidly with the addition of a labile carbon source (molasses) and ii) glyphosate will degrade more rapidly and completely in ZT soils.

3.3.2 Methods

3.3.2.1 Laboratory incubation experiment

Soil was collected from a long-term field experiment at the University of Nottingham, Sutton Bonnington experimental farm (grid ref. 52°50'30.5"N 1°15'17.3"W). The experiment has been running for ten years with a complete randomised block design comparing ZT, CT and

MT all with and without residue removed. Soils for this experiment were taken from plots which had been under ZT without reside removal, and from plots with had been under CT with residue removed (see Table 3.2 for soil physical properties).

Table 3.2 Soil characteristics. From Furcloth and O'Sullivan (2023, personal communication)

	Conventional tillage	Zero Tillage
pH	6.43	6.72
Texture (sand:silt:clay)	Sandy clay (54:4:42)	Sandy clay (54:5:41)
SOM (%)	4.89	5.91
Bulk density (g cm^3)	1.077	1.078

Soil was dried and sieved to 2 mm before being spread thinly in trays (diameter 24cm, surface area 452.4 cm^2). Two levels of glyphosate, with and without molasses were applied to both soils in a full factorial design. The glyphosate used was Motif (Monsanto UK Ltd) containing 360 g L^{-1} of active ingredient as a phosphate salt and etheralkylamine ethoxylate as a non-ionic surfactant. This was applied at two rates equivalent to 1) 2.88 kg glyphosate ha^{-1}, a commonly applied rate for the control of annual weeds in an arable setting, and 2) 4.32 at kg glyphosate ha^{-1}, the current maximum annual glyphosate application rate in the EU (EFSA, 2015; Silva et al., 2018). Molasses was applied at 3 L ha^{-1} in line with amounts applied by farmers using the practice (Parton, 2023). A control for each soil was established with deionised water. Pots were wrapped in clingfilm to prevent drying, and holes were made to allow gas exchange. Pots were incubated in the dark at 21 ^oC for six days which has been shown to be sufficient time to see significant reductions in glyphosate concentrations (Al-Rajab and Schiavon, 2010). Following incubation three subsamples were taken from each pot, combined and dried at 105 ^oC, a temperature shown not to thermally degrade glyphosate or its metabolites (Narimani and da Silva, 2020). Samples were then ball milled (Retsch PM400, Retsch GmBH, Germany) in agate pots for five minutes at 200 rpm. Glyphosate was

extracted with 20 millimolar ammonium acetate and quantified with liquid chromatography tandem mass spectrometry as described in section 3.3.2.2.2.

3.3.2.2 Field experiment site and soil collection

A field trial was established on a field at a mixed arable farm near Coven, Wolverhampton (grid ref. 52°39'34.2"N 2°09'51.4"W) in Spring 2023. The field had been under ZT and had not been ploughed for 8 years. Soil texture was a sandy loam. The field was split into four plots, each 8 m by 50 m. Four glyphosate and cultivation treatments were established, as per table 3.3. Glyphosate and/or molasses was applied on 27.03.23 and tillage treatments were established on 05.04.23.

Table 3.3 Glyphosate and tillage treatments established

	F1	F2	F3	F4
Glyphosate (L ha^{-1})	4	2.5	2.5	0
Molasses (L ha^{-1})	0	3	3	0
Cultivation	8 year ZT	8 year ZT	10 cm disc	10 cm disk

Intact topsoil soil cores (8 cm wide by 10 cm deep, 503 cm^3) were collected manually by driving

Table 3.4 Spraying, cultivation and sampling dates

Activity	Date	Day after spraying
TP0 Bulk soil sampling and intact soil core collection	26.03.23	-1
Glyphosate application	27.03.23	0
TP1 Bulk soil sampling	28.03.23	1
TP2 Bulk soil sampling	02.04.23	6
Cultivation of F3 and F4	05.04.23	9
TP3 Bulk soil sampling	09.04.23	13
Intact soil core collection	12.04.23	16
TP4 Bulk soil sampling	15.04.23	19
TP5 Bulk soil sampling	23.04.23	27
TP6 Bulk soil sampling	01.05.23	35
Intact soil core collection	24.05.23	58

the cores into the ground and carefully extracting with a spade (see Table 3.4 for sampling dates). Cores were collected at three timepoints (before cultivation, one week post cultivation

(12/04/23) and seven weeks post cultivation (24/05/23)) and stored at 4°C for non-destructive soil structural analysis using X-ray CT (within two days).

Bulk soil samples from 0-10 cm were collected in duplicate weekly for 6 weeks from the start of the experiment and stored at -20 for glyphosate quantification with liquid chromatography with tandem mass spectrometry (LC-MS/MS).

3.3.2.2.1 X-ray Computed Tomography

X-ray Computed Tomography (XRCT) offers non-destructive visualisation and quantification of soil structure at a range of spatial scales. This is particularly useful for soil research where examining the soil pore geometry at millimetre and centimetre scales well as micrometre aggregate scale (Helliwell et al., 2013). For more information see section 2.3.3.

Soil cores were scanned at the Hounsfield Facility, University of Nottingham, Sutton Bonnington, using a Phoneix v|tome|x M scanner (GE Measurement and Control Solution, Wunstorf, Germany). Voxel resolution was set to 45 µm, with a potential energy of 172 kV and a current of 200 µA. Soil cores were scanned in two sections (i.e., depths) to allow greater resolution, with a total scan time of 18 minutes per core. A total of 2,845 image projections were captured for each core. The resolution at which the cores were scanned, and the subsequent image processing, enables only measurement of pores typically defined as macropores (> 50µm diameter).

Scanned images were reconstructed at 16-bit using Phoneix Datos x2 reconstruction software. Images were optimized to correct for any movement of the sample during the scan and noise was reduced using the beam hardening algorithm in Datos × 2, set at level 8. Datos x2 Multi|Scan feature was used to automatically merge the two sections. The topmost slice was

determined visually as the first slice with more than 60% soil cover and 2,310 slices below this were used for analysis.

An image processing and analysis pipeline was established in ImageJ v.1.54f (illustrated in fig. 3.11) and a macro was created to complete the processing and analysis. Image stacks were filtered using the 'enhance contrast' function to set saturated pixels at 0.4%. Noise was removed using the 'Remove Outliers' function using a deviation threshold of 50 and radius of two and. Thresholding is the technique of dividing the grayscale image into two classes of pixels, one which represents airspace and the other that represents solid matter (see section 2.3.3 for more detail). Thresholding was completed before cropping as this achieved more

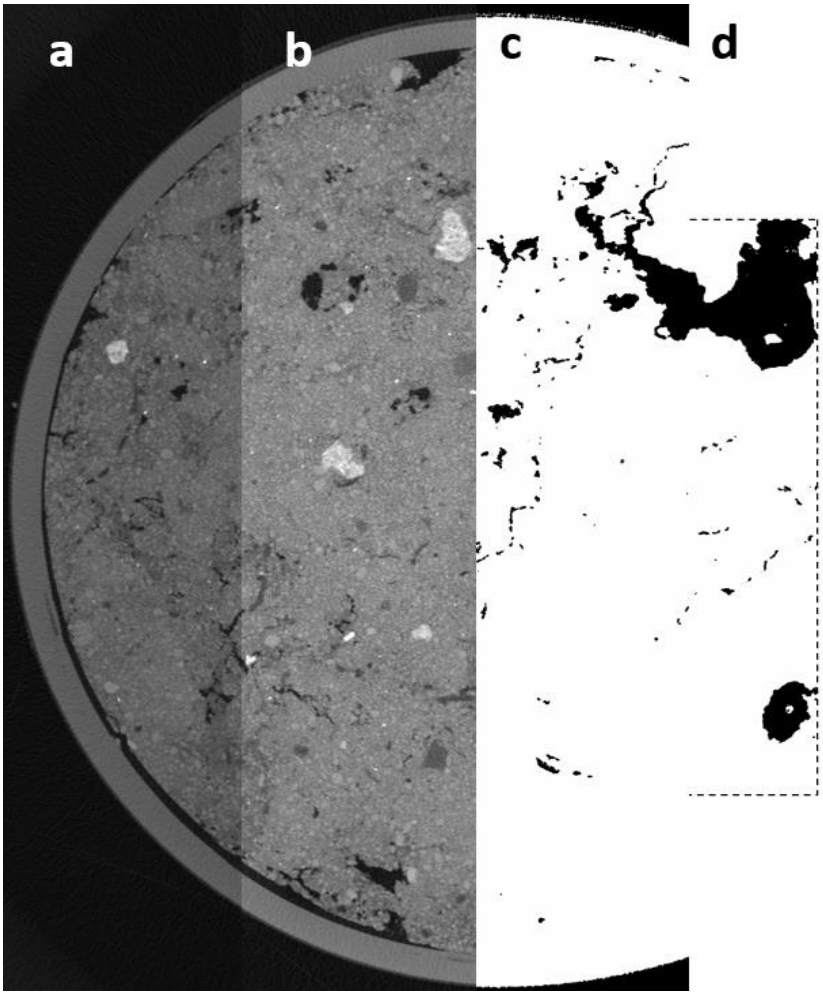

Fig 3.11 Image processing pipeline showing (a) the reconstructed radiograph, and (b) filtered image, which is then thresholded and binarised (c) and cropped (d) before analysis .

consistent threshold value and was done using the histogram of the whole image stack to ensure consistency between slices. The entropy-based thresholding Li algorithm was used (Li and Tam, 1998) as this offered the best estimation of airspace and minimal erroneous inclusion of organic matter.

A 900 x 900-pixel region of interest (ROI) was cropped from the centre of the resulting binary image to avoid any cracking around the edge of the column. The particle analyser within the BoneJ plugin (Doube et al., 2010) was used to analyse 3D porosity characteristics and pore size distribution and to create thickness maps. The particle analyser plugin performs connected components labelling to map unique (unconnected) particles and then analyse each particle separately. When used for soil pore structure analysis, the pore spaces are the particles which are analysed. Parameters measured in this research were as follows: The number of pores (sum of unique particles/pores), average pore volume (mean volume of pores), percentage porosity (sum of all pore volumes, expressed as a percentage of the total volume), largest pore (volume of the largest unique pore), porosity accounted for by largest pore (percentage of the total porosity that is part of the largest connected pore). BoneJ defines thickness as the diameter of the largest sphere that fits within the pore structure, average thickness of all the pores as well as the maximum pore thickness was used to assess these cores. Thickness maps were also created to enable visual comparisons of the pore structure. Connectivity of the pore structure is quantified using the Euler characteristic which describes the topography of the pores in terms of the holes and cavities within a pore (Odgaard and Gundersen, 1993; Toriwaki and Yonekura, 2002).

3.3.2.2.2 Liquid chromatography tandem mass spectrometry

3.3.2.2.2.1 Extraction Protocol

Soil was air dried and sieved to 2 mm before 1 g of soil was weighed and transferred into a 15 mL falcon tube. The extraction solvent used for the extraction of the glyphosate in soil samples was 20 mM ammonium acetate and 5 mL of extraction solvent was added to the falcon tubes containing the soil samples. The samples were vortexed for two minutes and stored at 4°C for overnight. The samples were vortexed again next day for two minutes and centrifuged for 20 minutes at 5000 RPM. The supernatant layer of the samples was subjected for solid phase extraction for sample clean up using Oasis HLB extraction cartridges. Samples from the incubation experiment were diluted 10-fold and samples from the field trial were submitted without dilution for LC-MS/MS quantification analysis. Standards, blanks and quality control samples were also extracted using this optimized method to minimize the matrix effect (Jha, 2023, personal communication).

3.3.2.2.2.2 Instrumentation:

Glyphosate was analysed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) from SCIEX. The column used for the elution of glyphosate was ZIC-pHILIC column (4.6 × 150 mm, 5µm particle size, Merck Sequant, Watford, U.K.). 20 mM ammonium carbonate (A) and acetonitrile (B) were used as mobile phase at the flow rate of 0.5 mL min^{-1} in isocratic mode and the percentage of mobile phase-A was 95 % while percentage of mobile phase-B was 5 % with 20 µL as injection volume of the sample. The total run time of the method was 5 min where retention time for glyphosate was 2.47 minute. Qtrap-6500 was the mass spectrometer used for the quantitation of glyphosate and the mass of glyphosate was optimised in negative ionization mode. Ion spray voltage (IS) = -4500,

curtain gas (CUR) = 35.0, Collison gas (CAD) = high, temperature (TEM) = 400, Ion source gas 1 (GS1) = 40 and Ion source gas 2 (GS2) = 40 were the condition for source parameters while declustering potential (DP) = -40, entrance potential (EP) = -10, collision energy (CE) = -14 and cell exit potential (CXP) = -15 were the condition for compound parameters for the optimized method. The scan type used for the quantitation was multiple reaction monitoring (MRM) and the mass transition of glyphosate was 167.786→149.700 at dwell time for 800 msec. The data generated from the mass spectrometer were analysed using MultiQuant v3.0.2 (AB Sciex Pte. Ltd). A signal to noise ratio of 10 was set as the cut off for determining the glyphosate peak. A method to quantify AMPA concentrations was attempted but was ultimately unsuccessful. (Jha, 2023, personal communication).

3.3.2.2.3 Statistical analysis

Normal data distribution was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variance was tested using the Brown-Forsythe test (Brown and Forsythe, 1974). For the incubation experiment, two-factor analysis of variance (ANOVA) with molasses addition and glyphosate level as factors. Pairwise post-hoc analysis was completed using the Holm-Šídák method (Holm, 1979). All statistical tests were completed using SigmaPlot 15.0 software (Systat Software Inc., San Jose, CA). Insufficient replicants were returned from the LC-MS/MS analysis to statistically analyse data from the field trial as a full factorial design.

3.3.3 Results

3.3.3.1 Effects of cultivation on soil pore architecture

There were no differences in any soil porosity characteristics as measured by XRCT in the soil that remained uncultivated over the course of the trial, as such all the ZT soil from all time

points were considered as one treatment. There was a significant ($p < 0.001$) increase in XRCT-measured total porosity from 7.99% before cultivation to 16.91% one week after cultivation and no significant change between week one and week seven (Fig 3.12c).

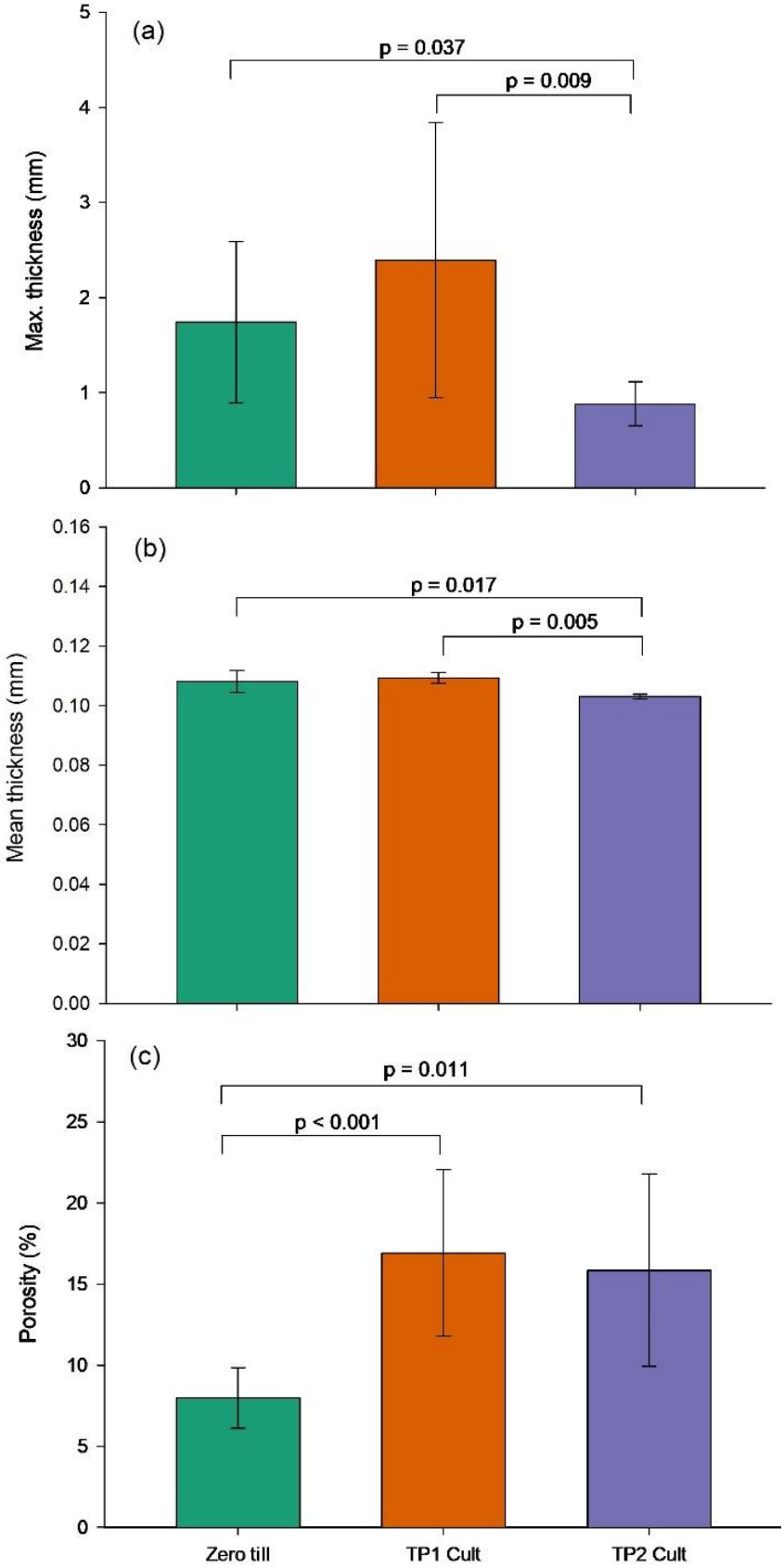

Fig. 3.12 a, b, c Maximum pore thickness (a), average pore thickness (b), percentage porosity (c) for the top 10 cm of soil in ZT soil, one week after cultivation (TP1 cult) and seven weeks after cultivation (TP2 cult).

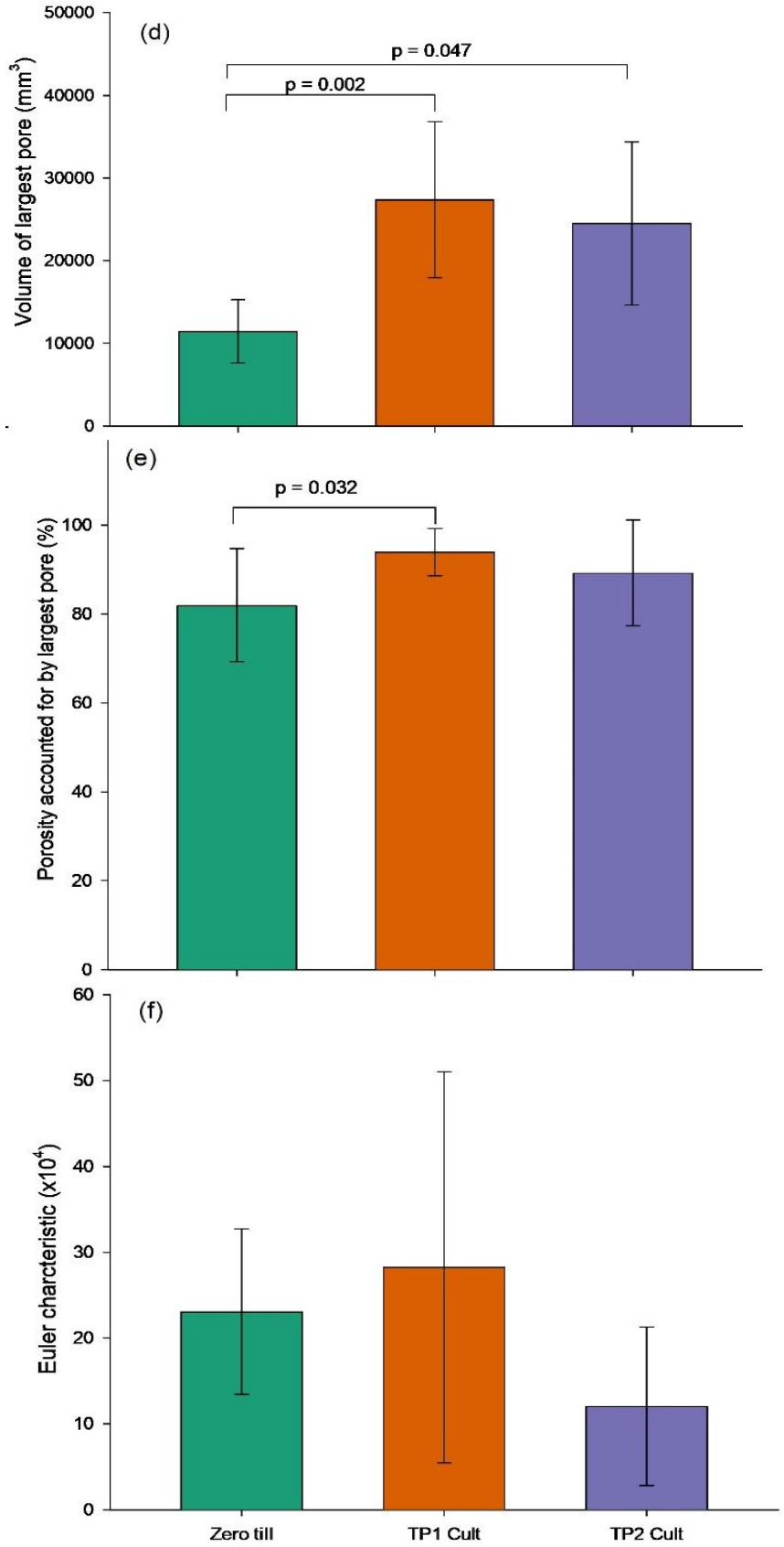

Fig. 3.12 d, e, f Volume of the largest pore (d), the percentage of total porosity accounted for by the largest pore (e) and the Euler characteristic (f) for the top 10 cm of soil in ZT soil, one week after cultivation (TP1 cult) and seven weeks after cultivation (TP2 cult).

Most of the porosity (>80%) was accounted for by one large, connected pore (Fig. 3.12d and illustrated in Fig. 3.13). The size of the largest pore shows the same pattern as the total porosity data (Fig. 3.12c) with an increase from 11452.28 mm^3 in the ZT soil to 27395.25 mm^3 one week after cultivation and then no significant shift in the seven weeks post cultivation. The proportion of the total porosity accounted for by the largest pore also increased seven days after cultivation from 81.99 % to 93.94 % (p=0.032) before decreasing to 89.26 %, which

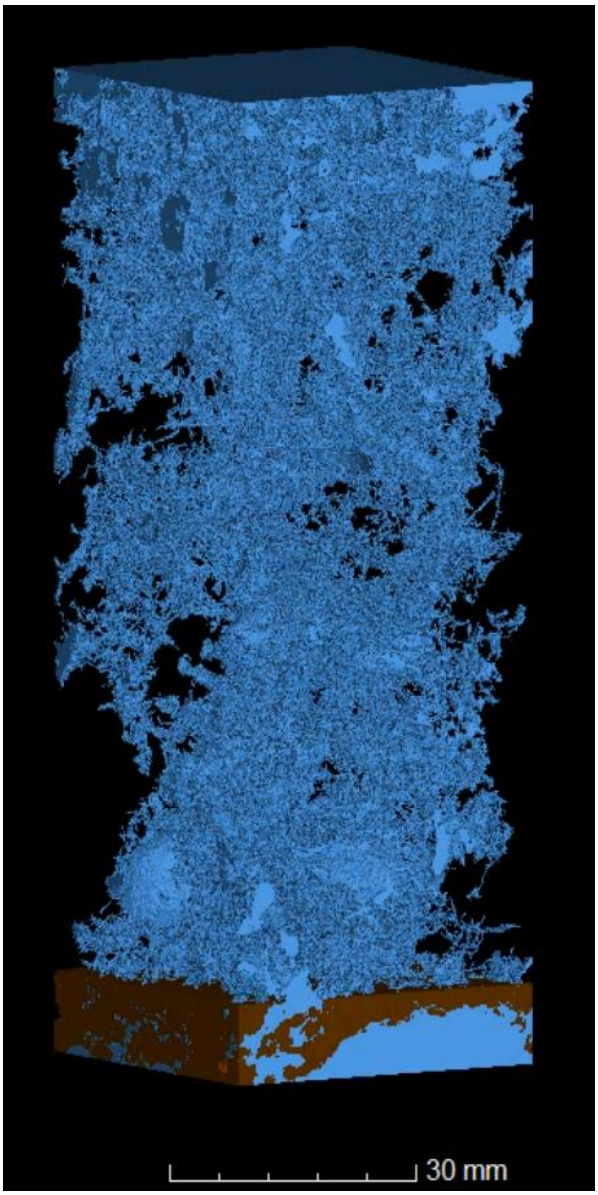

Fig. 3.13 3D render of the single large, connected pore that contains around 90% of the porosity in both the ZT and CT soil. Video available here

is not significantly different to the ZT soil. The remainder of the pore space in the soil comprised pores between 0.000182 mm^3 (smallest detectable pore at this scan resolution) and 316 mm^3 (Fig. 3.14). In the ZT soil, the volume of pores was similar in each pore size class. Following cultivation, pores smaller than 3.16 mm^3 appeared to be stable with no change in their cumulative volume after cultivation. There was a decrease in volume of pore space in pores between 3.16 and 316 mm^3 one week after cultivation and a further decrease between week one and seven. For all treatments and time points there were no pores in the 1000 ≤ 3162 or 3162 ≤ 10000 mm^3 pore size classes.

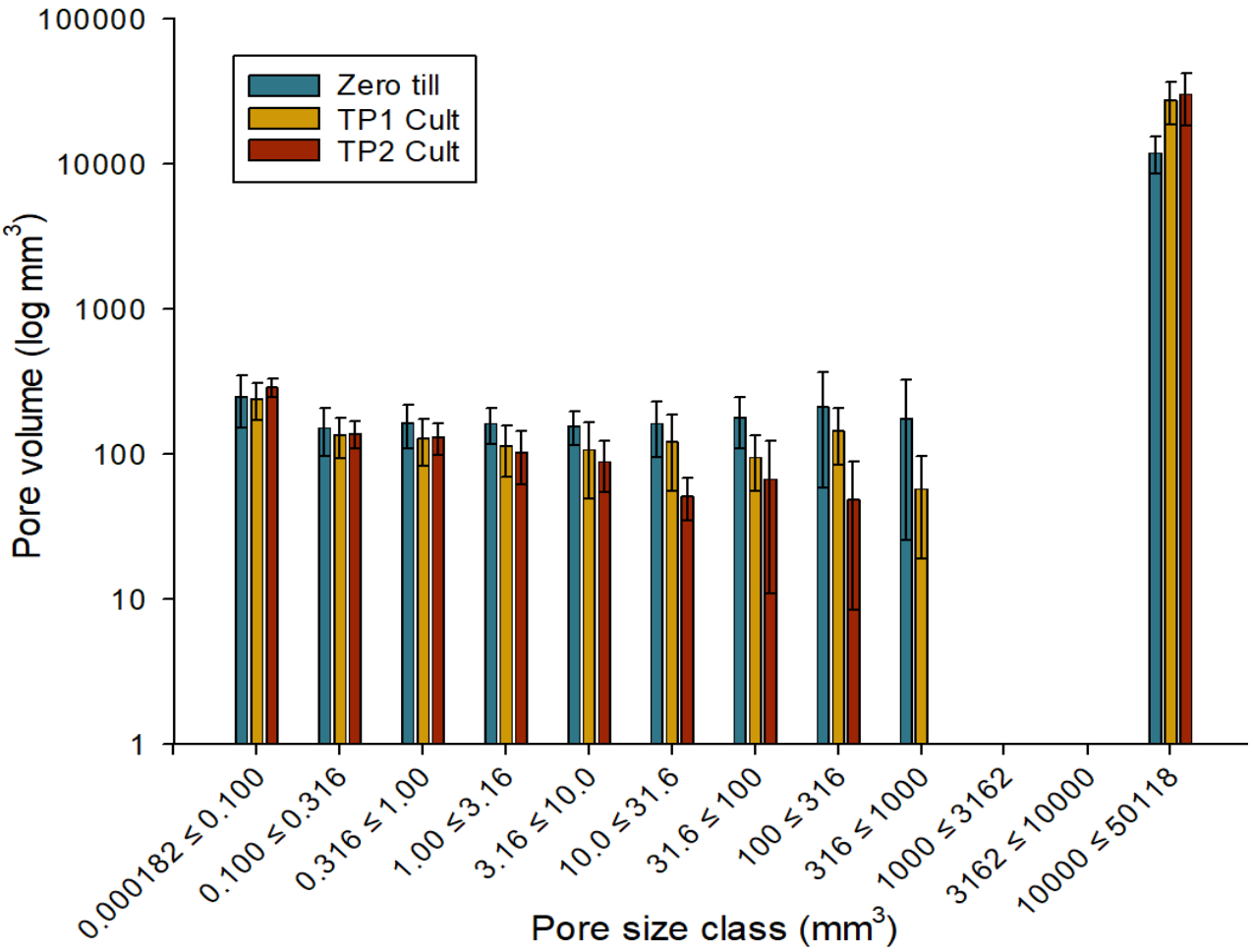

Fig. 3.14 Pore space distribution of pores between 0.000182 mm^3 (smallest pore detectable at scan resolution) to 50118 mm^3 before (Zero till), one week after (TP1 Cult) and seven weeks after (TP2 Cult) cultivation. Error bars are standard error. Vertical axis is log^{10} transformed.

No significant difference was seen between any treatment on any of the XRCT measures of connectivity (Euler characteristic (Fig 3.12f), connectivity, connectivity density) although they followed the same pattern as the proportion of total porosity accounted for by the largest pore. Maximum and average pore thickness did not change between the ZT soil and one week after cultivation (Fig 3.12a and b). However, the average and maximum thickness both

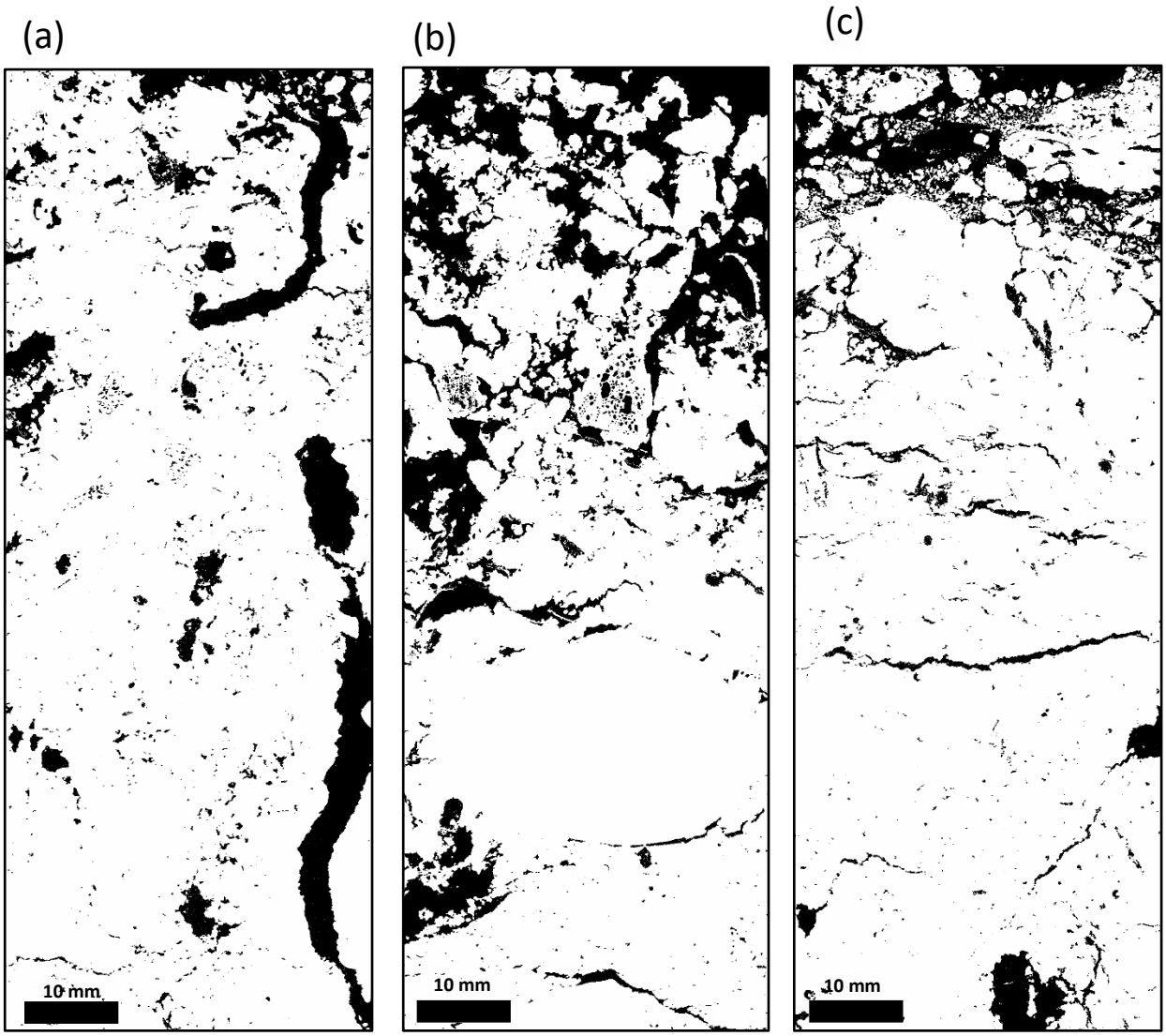

Fig. 3.15 Representative segmented and binarised images from the before tillage (a), one week post tillage (b) and seven weeks post tillage (c). Large, vertical biopores are clearly visible in the untilled soil.

decreased between one and seven weeks after cultivation with the average decreasing a small but significant from 0.109 mm to 0.103 mm (p = 0.005) and the maximum thickness decreasing from 2.39 mm to 0.882 mm (p = 0.009) by seven weeks after cultivation.

Both the average and maximum pore thickness were lower (p = 0.017 and p = 0.037 respectively) at seven weeks post cultivation than in the ZT soil which had an average pore thickness of 0.108 mm and maximum thickness of 1.74 mm. The same patterns were seen when the large, connected pore (10,000 ≤ 50118 mm^3) was removed from the analysis. Fig. 3.15 shows representative images from a ZT and tilled plot. The image of the ZT soil (Fig. 3.15a) shows large, vertical, cylindrical bio-pores created by anecic earthworms, other burrowing invertebrates and plant roots. These bio-pores were disrupted by the action of cultivation (Fig. 3.15b) which increases total porosity. Seven weeks after cultivation (Fig. 3.15c) the soil had slumped and many of the medium sized pores (10 ≤ 1000 mm^3) have collapsed and accounted for a smaller proportion of the pore volume (Fig. 3.14).

3.3.3.2 Field trial

The theoretical limit of quantification (LOQ) for the method used to quantify glyphosate is 0.295 mg/kg^{-1}, and the theoretical limit was estimated to be around 1 mg/kg^{-1} (Jha, personal communication, 2023). No residual glyphosate was detectable in any of the plots before glyphosate was applied. After application, glyphosate was detectable on all plots to which it was applied. The plot which received 4 L ha^{-1} had slightly more (1.854 mg kg^{-1}) than the 2.5 L ha^{-1} plots (1.802 mg kg^{-1}; Fig. 3.16). Detectable glyphosate levels did not change significantly between one and six days after application (1.802 and 1.823 mg kg^{-1} respectively, p = 0.837). Cultivation of F3 and F4 was undertaken 9 days after glyphosate application. Following cultivation, the glyphosate level in the cultivated plot which had glyphosate added fell below

the level of detection by day 13. The detectable glyphosate in both ZT plots (F1 and F2) remained similar, decreasing slightly, over the next 10 days. Between 19 and 27 after glyphosate application glyphosate levels in F1 and F2 fell below the detection limit. F4, the cultivated plot which did not receive any glyphosate had undetectable levels of glyphosate at all sampling point aside from 6 days after glyphosate was applied to the other plots where it had 1.729 mg kg^{-1}. This number was deemed to be erroneous and was excluded from figures and analysis.

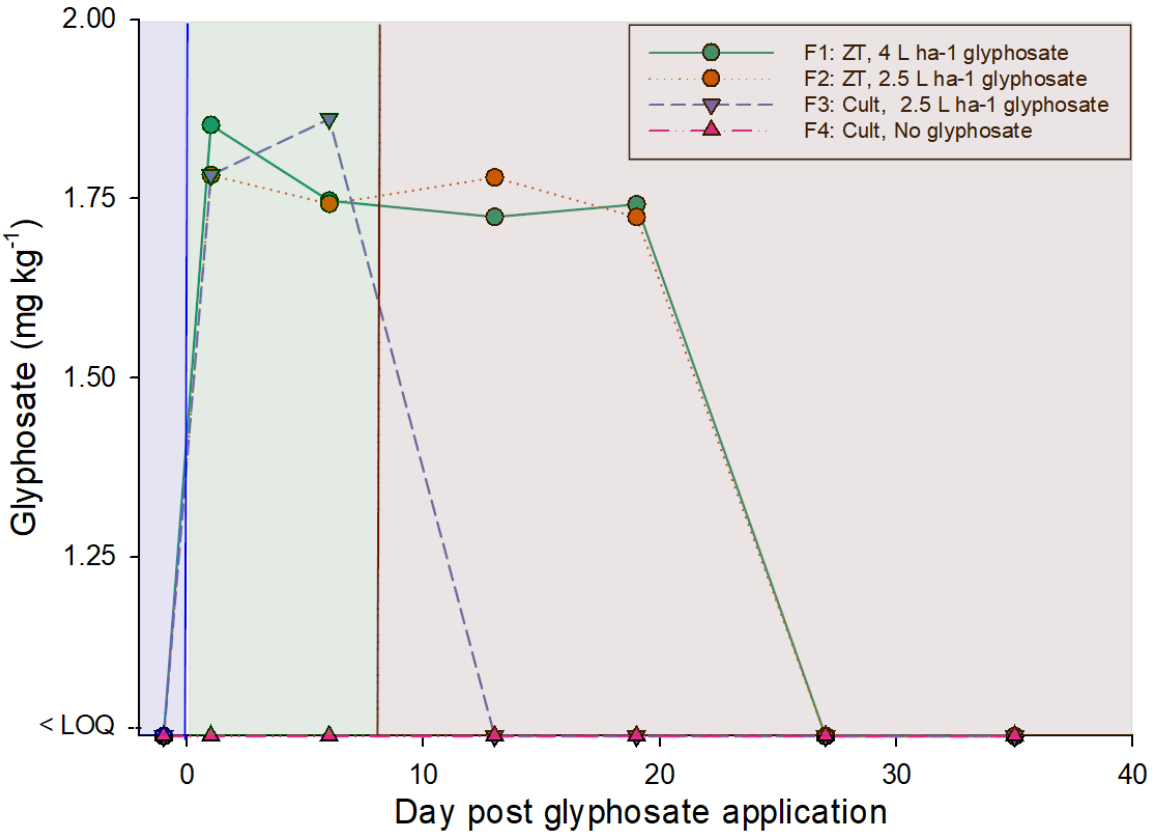

Fig. 3.16 Glyphosate levels before glyphosate application (blue shaded area), after application and while all plots remained uncultivated (green shaded area), and after cultivation was carried out on plot F3 (purple dashed line and triangles) on day 9 (brown shaded area). Plots F1 and F2 (green solid line and circles, orange dotted line and circles, respectively) remained untilled for the duration of the experiment. Readings below the limit of quantification (<LOQ) are represented as zero values.

3.3.3.3 Glyphosate degradation incubation experiment

Two levels of glyphosate (low (LG) and high (HG)) were added to soil from either ZT or CT managed plot with or without molasses in a full factorial design and glyphosate levels were measured after incubating for 6 days. There is a small but statistically significant difference between the CT and ZT control soils (16.048 and 14.215 mg kg^{-1} respectively, p = 0.002).

Glyphosate levels in all treatments were similar by the end of the trial with all treatments falling to just over 100 mg kg^{-1} (Fig. 3.17a). The control CT soil has 2 mg kg^{-1} more glyphosate than the control ZT soil at the end of the incubation despite the experimental site they were collected from receiving the same herbicide treatments.

The HG treatments had significantly more glyphosate remaining than LG treatments (123.21 and 83.47 mg kg^{-1} respectively, p = 0.004). When expressed as a percentage of the applied glyphosate, both LG and HG were not significantly different (4.34 % and 4.95 % remaining respectively; Fig. 3.17b). There is a main effect of cultivation with significantly more glyphosate remaining in the ZT soil that the CT soil in percentage terms (5.13% and 3.95 % respectively, p = 0.039); the same pattern is present in the concentration data however, the difference is not significant (113.88 and 90.69 mg kg^{-1} respectively, p = 0.121).

The addition of molasses has opposing effects in the ZT and CT soil (Fig. 3.17a and b). In the soil from the plots managed with CT, the addition of molasses with the glyphosate significantly decreases the degradation of glyphosate, doubling the percentage of glyphosate remaining in LG and HG treatments. In the soil from the ZT plots, the opposite is the case: addition of molasses increases the degradation of glyphosate and decreases the glyphosate remaining in

the soil from 103.09 to 64.40 in the LG treatment and from 132.61 to 98.59 in the HG treatment.

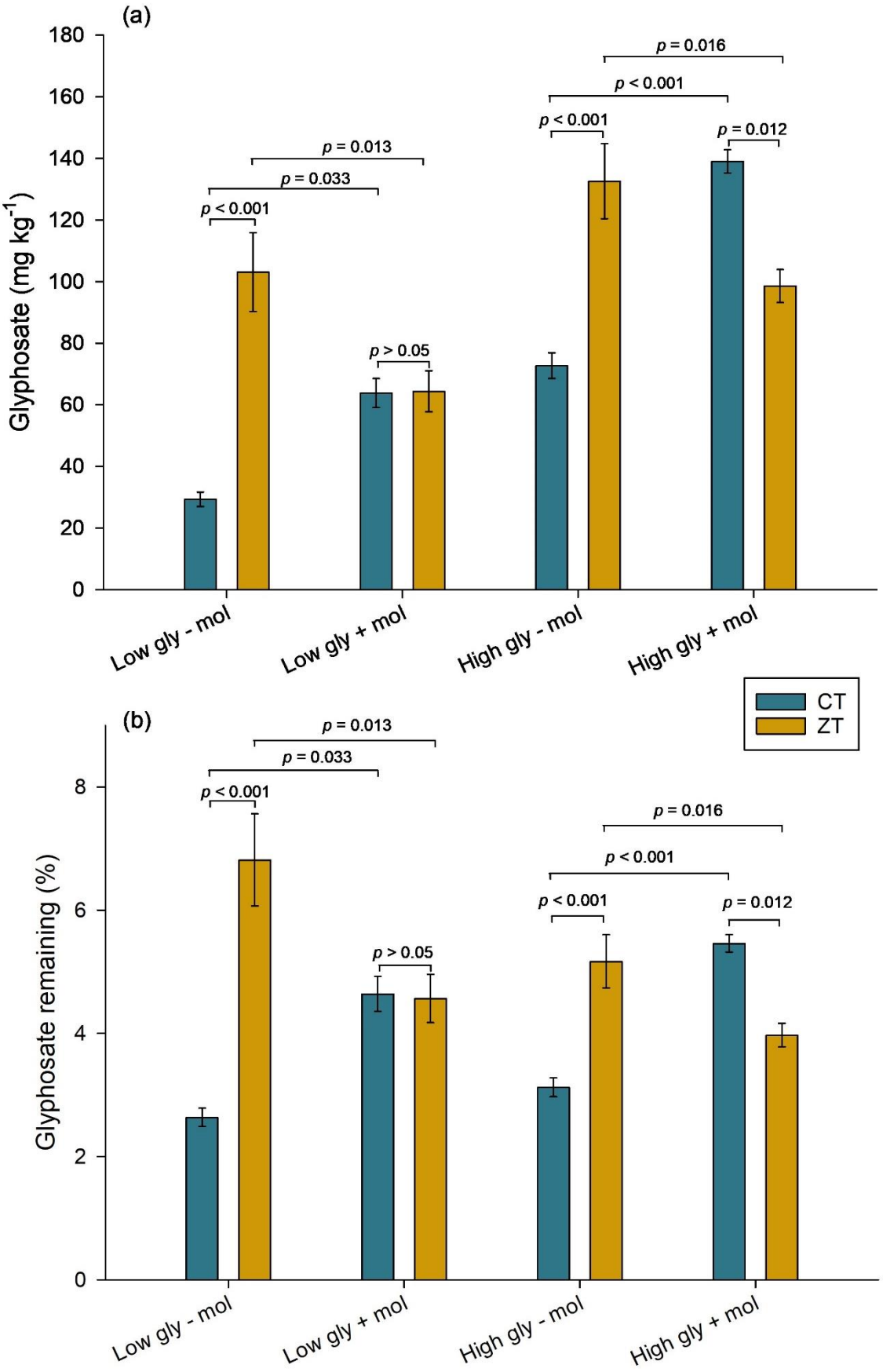

Fig. 3.17 a) Glyphosate level in each treatment after 6 day incubation; b) Percentage of glyphosate remaining in soil after 6 day incubation

3.3.4 Discussion

3.3.4.1 XRCT

The increase in total porosity within the upper 10 cm following cultivation aligns with expectations, and with the literature (Głąb and Kulig, 2008; Lipiec et al., 2006), considering that the primary aim of cultivation is to enhance crop germination by loosening the soil and consequently reducing mechanical impedance. This increase in total porosity appeared to be stable over the seven weeks following cultivation only decreasing a small, insignificant amount. This work categorises pore sizes into 4 groups as follows: very small, ≤ 1mm^3; small 1 ≤ 10 mm^3; medium, 10 ≤ 1000 mm^3 and large, > 1000 mm^3. Contrary to the commonly reported idea that cultivation homogenises pore sizes (Kravchenko et al., 2011), the PSD presented here shows the opposite trend with pore volume in very small pores (≤ 1 mm^3) increasing and pore volume in medium sized pores (10 ≤ 1000 mm^3) decreasing in the weeks following cultivation. According to Bacq-Labreuil et al. (2021), a decrease in the homogeneity of a soil's PSD is indicative of a less complex network of pores. This decrease in complexity could lead to fewer storage and transmission pores, resulting in impeded flow of water and nutrients.

The volume of pore space in the large connected pore (> 1000 mm^3) increases following cultivation and accounts for upwards of 90% the pore space in the soil. This suggests that connectivity might increase following cultivation. However, no significant changes were found in connectivity as measured by the Euler characteristic. A concern for farmers adopting ZT is that the reduction in porosity may negatively influence crop establishment and soil functioning (Alskaf et al., 2020). However, as our understanding of soil functioning develops, importance of connectivity rather than total porosity is becoming clearer (Rabot et al., 2018).

Pore connectivity is one of the most important parameters, affecting air permeability, hydraulic functioning and nutrient fluxes (Lucas et al., 2021). Farmers should therefore be reassured that, despite of ZT lowering total porosity, this study shows the connectedness of these pores is comparable with a cultivated soil after eight years and continues to increase the longer a soil is managed without cultivation (Galdos et al., 2019).

That said, the Euler characteristic is an algebraic method of describing the complexity of a shape which may be too simplistic to be meaningful when used to describe shapes as complex as the pore architecture of a soil, especially when it is employed to attempt to distinguish between shapes which are all very complex. The connectivity described by the large, connected pore is also not a particularly insightful metric if functional connectivity is of interest. Pore space is included in this pore no matter how small the point of connection, or 'throat', is; so, while it may be connected in the XRCT image, information on the functionality of the connection is lost and potential differences in hydraulic function, nutrient and pesticide flow, and microbial habitat diversity are obscured. Reanalysis of the XRCT images could break this massive pore down and offer insights into its functionality. Using the 'Region Grower' function of VGStudio, different thresholds could be set to quantify functionally relevant connections and pathways within the soil.

The maximum thickness data could offer some insight into the structural stability of the newly formed pore space. Or and Ghezzehei (2002) found that pore space established by cultivation operations tend to be structurally unstable and susceptible to collapse. They suggest that along with the action of rain drops, the capillary action of water causes aggregates to re-join. This action works on the micro- and macropore level but will also destabilise very large pore spaces created by cultivation causing them to collapse (Silva, 1995). This is possibly what is

observed in the maximum pore thickness seen in the XRCT data – large pores are established following cultivation and subsequent collapse by week seven. The maximum pore thickness decreases to below that seen before cultivation suggesting that cultivation may destabilise pre-existing pores as well.

Studies comparing the structure of soils which have been under ZT and CT for long period of time often find greater difference is structural parameters than were observed here (Or et al., 2021). Comparatively little structural change was observed following cultivation of the soils in this experiment as the soil had been uncultivated for eight years and regeneratively managed with a focus on increasing SOC and improving 'soil health'. Several studies show that increasing time under ZT increases the resilience of the soil structure (Mondal and Chakraborty, 2022; Qi et al., 2022). Increased SOC is reported to be the main driver for this (Qi et al., 2022). Long term ZT soils will also contain more earth worms (Johnson-Maynard et al., 2007; Reeleder et al., 2006) and while the population would be slightly reduced by the cultivation implemented in this trial, numbers would still be high and post- cultivation re-establishment of large elongate bio-pores by anecic earthworms would be rapid following cultivation in a long term ZT field. This has important implications for hydraulic functioning of the soil. Despite being a sandy loam, and as such more susceptible to structural damage than less sandy soils (Davies et al., 1992), this sporadic cultivation event does not appear to cause lasting structural damage.

3.3.4.2 Glyphosate degradation in the field trial

The baseline samples from all plots were below the detection limit for glyphosate (0.295 mg/kg^{-1}) which demonstrates that while glyphosate has been routinely applied to the field for many years, it does not accumulate in the soil. This is in line with findings from other authors

(Gandhi et al., 2021; Shushkova et al., 2010; Smith and Oehme, 1992). Bioavailability of glyphosate decreases over time (Okada et al., 2019) and could also become less extractable by the ammonium acetate used in the LC-MS/MS analysis which may explain the non-detectable levels in the baseline soils as well as all the plots after 35 days. Further testing of the method would be needed to confirm this. Without a quantification method able to detect lower levels glyphosate and a method to quantify glyphosate metabolites it is impossible to know where the adsorption and occlusion, or microbial degradation are the main drivers of the decrease in glyphosate levels.

It was observed that when the soil is cultivation, glyphosate levels drop below the LOQ while in the soils that remained uncultivated quantifiable concentrations of glyphosate remain for another 10 days. Due to its strong propensity for adsorption to mineral particles and SOM, glyphosate is not very mobile in soil and it will tend to stay where it makes contact with the soil (Okada et al., 2019). In uncultivated soil this means the soil will sit on the surface where it is not optimally available for microbial degradation. Incorporation of glyphosate through cultivation is reported to increase the degradation rate as it is brought into closer contact with the active microbial community of the topsoil (Okada et al., 2019). Soil respiration rate is known to increase directly after cultivation (Fiedler et al., 2015) and several studies report a positive correlation between pesticide degradation rate and the soil respiration rate (Franz et al., 1997; Thorstensen and Lode, 2001). Results from the field trial show porosity of the soil increases. The increased microbial activity from the increased aeration and available carbon released from the mechanical disturbance of aggregates could well explain the rapid decline of glyphosate to below quantifiable levels following cultivation. The soil was cultivated to a depth of 10 cm, and glyphosate samples were taken from the top 10 cm, so this is not the

effect of surface glyphosate being 'diluted' the cultivation. No comparisons were able to be drawn between molasses amendments made by the farmer on plots F2 and F3 as they were not controlled for, and any potential effect could not be separated from the effect of cultivation.

Glyphosate levels in the uncultivated plots remained above quantifiable levels for more than 19 days after application before dropping below the LOQ by sampling on day 27. After staying consistent for the first 3 weeks after application, levels seem to drop off quickly between these two time points. One possible explanation for this is that there may be a lag time between the application of glyphosate and the development of a microbial community able to degrade it. There is research showing that degradation follows the typical sigmoidal microbial growth curve with an initial lag before an exponential increase and subsequent saturation phase (Ibrahim et al., 2023). However, this saturation phase more normally happens in the first few days, rather than weeks following application. Furthermore, soils with a history of glyphosate application, as is the case with the soils in this trial, have a microbial community adapted to degrade glyphosate (Lane et al., 2012). The rapid decrease of glyphosate seen in the six day incubation experiment discussed below is more in line with what would be expected.

3.3.4.3 Lab experiment

The difference between the ZT and CT control soils at the end of the incubation suggests there may be differences in the way glyphosate interacts with soil when it's managed under either ZT or CT. Some reports in the literature suggest glyphosate will break down faster in disturbed soil (Cassigneul et al., 2016; Soracco et al., 2018) so we would expect the residual glyphosate levels in the CT control soil to be lower than the ZT if this were the case. Other research suggests that as the microbial communities of ZT soil are often more active and diverse,

glyphosate entering the soil will be degraded more rapidly and completely (Li et al., 2020; Schmidt et al., 2018b). It could also be due to crop residue left on the surface of the ZT plots which intercepts the glyphosate before it reaches the soil surface (Aslam et al., 2018; Soracco et al., 2018).

More residual glyphosate in the HG treatment compared to the LG treatment after incubation was to be expected. However, in both treatments around 95% of the added glyphosate was degraded meaning considerably more glyphosate was degraded in the HG treatment in absolute terms. This suggests that despite glyphosate being added at a relatively high rate, the limit of the microbial population to degrade glyphosate was not reached: more could potentially be added most of it would still be broken down.

The effect of the addition of molasses was inconsistent between both soil management treatments and glyphosate levels. The increase in glyphosate degradation in the ZT soil when applied with molasses may be due to the ZT soil having a more active and diverse microbial community better able to take advantage of additional labile carbon as an energy source to co-metabolise glyphosate; whereas the CT soil where we see the opposite pattern may not have a microbial community able to take advantage of the labile carbon source. While this suggested mechanism would need more work to corroborate it, there is literature to suggest differences in microbial community functioning between differently managed soils. Many studies have shown a switch to ZT shifts microbial communities to be more diverse with greater abundances of gram-positive bacteria, mycorrhizae and actinomycetes (Mbuthia et al., 2015b; M. Nunes et al., 2020; Saikia et al., 2019). Gimsing et al. (2004) showed correlation between *pseudomonas* species and glyphosate mineralisation, and Rathore et. al. (2023) found a greater abundance of *pseudomonas* species in soils under conservation tillage

compared to CT soils. However, it should be noted that other studies (Smith et al., 2016; Srour et al., 2020) find no difference in microbial community structure between ZT and CT, and it is not clear whether any shift that may occur would indeed be towards a community structure better suited to glyphosate degradation.

3.3.4.4 Challenges

Extraction of glyphosate from soil is widely agreed to be challenging, as adsorption and binding of glyphosate to organic matter, clay, and metals in soil leads to low and irreproducible recoveries (Bernal et al., 2012; Simonetti et al., 2015). The method developed showed good extraction of glyphosate from spiked soils and was able to quantify the levels of glyphosate in the incubation experiment (80 – 180 mg kg^{-1}) with confidence due to a very high signal-to-noise ratio. However, the much lower concentrations from the field samples (~1.7 mg kg^{-1}) was too low to easily distinguish the glyphosate peak from background noise and in many cases the signal-to-noise ratio was less than 10, the minimum required to be sure that the peak is a signal according to the standard operating procedures of the mas spectrometer.

3.3.5 Conclusion

With so many samples retuning glyphosate levels below the LOQ from the field trial, it was not possible to statistically test our original hypotheses, and the assertions above are observations and postulations, rather than statistically founded findings. However, there does seem to be a pattern worthy of further investigation – the residence time of glyphosate applied to a microbially active, 'healthy soil' is in the order of weeks, not months and this is decreased by cultivation after application. Furthermore, even a sandy loam when managed sensitively following the principles of CA will develop a structure robust enough to withstand

occasional light cultivation if need to manage weed burden or alleviate compaction from traffic.

4 Final Conclusions

This work started with a comprehensive review of the concept of soil health and how conservation agriculture, and alternative tillage practices in particular, may be able to increase the sustainability of agriculture, maintain or increase yields while ensuring soils are still able to perform their numerous ecosystem functions. This opened several avenues of research, namely strip tillage's impact on soil structure and the contribution of AMF to aggregation, where there were gaps in the literature that investigations with XRCT seemed well place to fill. Unfortunately, due to methodological constraints and the availability of suitable field sites neither of these experiments led to any of the original hypotheses being tested. However, they did highlight the key role soil texture plays in soil structural development, and how differing soil textures between sampling sites can mask changes occurring due to different soil management practices.

While developing these early experiments the topic of glyphosate use in ZT systems was frequently raised and led to the development of the experiments in section 3.3. With glyphosate being so widely used and relatively under studied, there was a lot of scope for new research in this area. Using XRCT alongside a post-spraying time series of in-field glyphosate degradation revealed interesting findings. It is highly likely that these can form the basis of further work. The XRCT analysis showed that while the total porosity of a long term ZT soil increased following cultivation, the pore connectivity was not greatly affected and began to return to pre-cultivation levels quickly. This suggests that time under ZT builds structural resilience, increasing a soil's ability to withstand, and recover from, mechanical disturbance.

The drop in detectable glyphosate following tillage, and the extended residence time without tillage would be of interest to farmers and policy makers making choices around balancing the benefits of ZT with the potential negative impact of glyphosate use.

A key limitation of this research was the experimental design of the field trial. Due to time budget constraints and farmer decision making, the trial was not randomised or blocked so the statistical power of the results was limited. Furthermore, the treatments applied did not include appropriate controls for all treatments of interest. In addition, only two samples were collected from each plot at each time point, of which only around a quarter had detectable levels of glyphosate. Further work would require a glyphosate assay able to quantify level below one mg per kg of soil. Also, a method for the quantification of the glyphosate metabolite, AMPA, would offer further insight into glyphosate dissipation pathways and the impact of this understudied degradation product. Further work to understand the dynamics of glyphosate degradation in relation to soil management should include measures of functional diversity of the soil microbiomes such as metagenomics. Using these techniques, shifts in microbial community structure and functioning following cultivation, as well as following glyphosate additions could be mapped to understand the implications of glyphosate use more thoroughly in ZT systems. Glyphosate plays a key role in enabling ZT farming in the UK; however, despite its ubiquity little is known about how its fate, the work described here forms the first steps towards deepening this understanding.

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