HARPER ADAMS UNIVERSITY

The effect of traffic and tillage management systems on soil organic carbon dynamics and crop performance

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Declaration

I, Ana Prada Barrio, hereby declare that this research work with the title "The effect of traffic and tillage management systems on soil organic carbon dynamics and crop performance” is my own and solely composed by myself and that no help was provided from other sources as those allowed. Except where stated otherwise by reference or acknowledgement, the work presented is entirely my own.

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List of abbreviations

SOM – soil organic matter

SOC – soil organic carbon

SMC – soil microbial community

POM – particulate organic matter

POM-C – carbon content of the POM fraction

MAOM – mineral associated organic matter

MAOM-C – carbon content of the MAOM fraction

MBC – microbial biomass carbon

p – p-value (statistical analysis)

cm – centimetre

Mg – Megagram (syn. Metric tonne for crop yield and carbon stocks)

CV – coefficient of variation

Treatment abbreviations:

CTF - refers to the non-trafficked crop area of controlled traffic farming systems, for all soil analyses

CTF (30% trafficked) – refers to the controlled traffic system with a 30% trafficked area, for the crop productivity analysis

STP – refers to random traffic with standard tyre inflation pressure

LTP – refers to random traffic with lower ground tyre pressure

Deep – deep tillage (25 cm)

Shallow – shallow tillage (10 cm)

Zero – zero tillage

Abstract

There is an increased interest in implementing soil compaction mitigation strategies in sustainable agricultural practices to promote soil health, crop productivity and resilience. However, knowledge gaps still exist on the long-term effects of alternative traffic systems, and their interaction with different tillage systems, on soil organic matter (SOM) dynamics and crop yield. This thesis aimed to determine the effects of three traffic systems—Standard Tyre Pressure (STP), Low ground Tyre Pressure (LTP), and Controlled Traffic Farming with 30% trafficked area (CTF)—interacting with three tillage systems (Deep 25 cm, Shallow 10 cm and Zero tillage) on SOM dynamics and crop performance, in a long-term 3×3 factorial field experiment with four replicates on sandy loam soil. After 12 years, the non-trafficked crop area of CTF with Zero tillage had significantly higher SOM concentration (0-30 cm), storing 5 Mg/ha more SOC stocks on equivalent soil mass than other treatments. This combination stored ~26% more particulate organic matter carbon (POM-C) and ~6% more mineral-associated organic matter carbon (MAOM-C). After introducing a C4 millet crop, the POM δ^{13}C was 4.5% higher and MAOM δ^{13}C was 0.4% higher than under the previous C3 crop, indicating that carbon storage was driven by the POM fraction.

Crop yield was significantly higher only for Spring oats, which yielded ~ 14% higher than STP Deep and ~ 10% higher than STP Shallow. CTF and LTP systems produced significantly higher yields than STP systems (~ 9% more for Winter wheat and ~ 7% more for Spring oats). Tillage effects on yield were not significant, indicating that long-term Zero tillage maintained equivalent yields. However, calculating for a more realistic CTF with 15% trafficked area provided ~4% additional grain yield increase.

CHAPTER 1 INTRODUCTION

1.1. BACKGROUND

Evidence suggests early humans developed agriculture approximately 10,000 years ago, thanks to a warmer climate following the last Ice Age (Tauger, 2010). This spark of early agriculture led to population growth, the development of cities and trade some 5,000 years later (Tauger, 2010). From those early years, there has been a broad evolution of techniques used in agriculture. More recently, there has been a shift in agricultural practices around the world towards the use of often large and specialised agricultural machines (such as tractors, combine harvesters, track loaders and trailers). As a result, most farms in the developed world have increased in size compared to historic averages and are powered by significantly heavier machines (Chamen, 2011). This has improved productivity and efficiency (Arvidsson, *et al*., 2001). However, this industrialised, high input, high yield type of agriculture has led to high levels of soil degradation, with soil compaction being one of the biggest contributors (Hamza *et al*., 2005) along with the depletion of soil organic matter (Reicosky *et al*., 1995). Soil compaction hampers plant growth and crop performance (Lamande and Schjonning, 2011). On top of that, the high levels of agrochemical inputs used, such as fertilizers and pesticides (Geiger *et al*., 2010), have an associated environmental damage such as polluting nearby water systems through run-off and leaching.

Globally, approximately 38% of the Earth's terrestrial environment is covered in agricultural land; this includes cropland (12%) and meadows for grazing livestock (25%) (FAO, 2020; Ramankutty *et al.,* 2000). In addition, the global population is predicted to increase to almost 11 billion people by 2100 (UN, 2019). The associated increase in demand for food, combined with increasing competition for settlement and other land resources such as timber production, will put increasing pressure on agricultural land. Currently, land conversion to agriculture continues to be a major driver of biodiversity decline and land degradation globally (Zabel *et al*., 2019), as well as the largest cause of greenhouse gas emissions (FAO, 2020).

To achieve the goals of the Paris Agreement (United Nations, 2015) and prevent the worst climate change damages, global net human-caused emissions of carbon dioxide (CO_2) must be reduced by approximately 45% from 2010 levels by 2030, reaching net zero by around 2050 (Net Zero Climate, 2022). Net Zero agriculture is a new approach that aims to reduce emissions, for example by improving the efficiency of production, producing seasonally, reducing harvesting wastes, but also to increase the removal of atmospheric CO_2 by storing carbon in soil and biomass. Therefore, in the 21st century, we are facing a very important challenge, to reach Net-zero agriculture by 2050, while at the same time increasing crop productivity to meet global food demands (Hemathilake *et al*., 2022) and safeguarding the remaining ecosystems and biodiversity (Seppelt *et al*., 2016).

Intensive and industrialized crop production have depleted soil organic carbon (Sanderman *et al.,* 2017), with some soils losing 40-60% of the organic carbon (UK Parliament POST 2022). Therefore, arable soils have a great potential for sequestering and storing atmospheric C. However, this potential varies in response to soil type, climate, vegetation cover, management practices and the interaction between them.

Soil organic carbon (SOC) is a component of soil organic matter (SOM), comprising about half of its mass (Pribyl, 2010) and plays a very important role in the physical, chemical and biological processes of the soil, affecting the fertility and productivity of the soil (Chen *et al*., 2004). SOC is also a critical component of soil health as it provides fuel for the soil biota, allowing it to provide ecosystem services that are vital for agriculture (Bünemann *et al.,* 2018). It helps soils retain water and nutrients (Esmaeilzadeh and Ahangar, 2014; Cobo *et al*., 2002), decreases soil bulk density and improves the soil structure (Strawn and Sparks *et al*., 2000).

An understanding of SOC dynamics and how they are affected by different management practices, in the context of different biotic and abiotic factors, is an essential pre-requisite for developing enhanced SOC storage in annual croplands via sustainable soil management. SOC dynamics refers to the processes and factors that influence the transformation, sequestration, storage, and loss of SOC in soils over time. To better understand SOC dynamics, Lavallee *et al*., (2020) proposed a physical separation of SOM fractions into particulate organic matter (POM) with a fraction size of 2000 mm – 53 µm and mineral associated organic matter (MAOM) with a fraction size of <53 µm. These two forms are fundamentally different in terms of their formation, persistence, and functioning. By exploring SOC dynamics within these fractions, advice could be drawn towards management practices aimed at increasing or protecting one or both fractions to help maintain or increase SOC stocks within soils.

SOC is made up of both of these fractions (POM and MAOM), which have different degrees of sensitivity to changes in management practices (both short-term and long-term). POM-C (Cambardella and Elliot, 1992) and microbial biomass carbon (MBC) (Jenkinson and Powlson,1976) are quickly mineralised and restored, responding rapidly to changes in C supply. Therefore, these SOC pools can be used as sensitive indicators to study the effects of different management practices on the overall SOC stock change (Ramesh *et al*., 2019). While MAOM-C, which helps long-term sequestration, have longer turnover times (the C is better protected and highly resistant to microbial activity) (Six *et al.,* 2002).

Traffic and tillage management systems used in crop production have a big influence on soil organic carbon and crop yield (Berner *et al*., 2008; Hussein, *et al*., 2021; Mouazen and Palmqvist 2015; Lal, 2004). Without the use of appropriate agricultural traffic management system, conventional traffic systems can cover up to 85% of the field area for each cropping season (Kroulik *et al.,* 2009). This leads to soil compaction, reducing soil porosity, water infiltration, water storage capacity and soil health (Soane and van Ouwerkerk, 2013). Soil compaction has a significant economic impact on the farmer, by reducing the crop yield, increasing the risk of disease development and a need to de-compact (loosen) the soil using additional fuel and time (Michelin, 2024).

Controlled traffic farming (CTF) is a method aimed at reducing the impact of trafficking. In CTF, all wheelways are restricted to the minimum possible area of permanent traffic lanes, which limits compaction to only those specific lanes. This leaves the rest of the soil free from compaction, which improves water infiltration (Chamen, 2015), reduces run-off and erosion, conserves SOM, enhances soil biodiversity and fertiliser use efficiency and reduces greenhouse gas emission from the non-trafficked soil (Mouazen and Palmqvist 2015**;** Hussein, *et al*., 2021). It also improves trafficability and timeliness of field operations when compared with conventional traffic systems (Antille *et al*., 2019). Equipment and system changes are necessary to match equipment widths and wheel track spacing as well as GPS-based accurate driving systems. In a long-term UK study on a sandy loam, CTF systems with a 30% trafficked area, resulted in yield increases of 4%, for arable crops, when compared to “random” (non-controlled) traffic with standard tyre pressure systems (> than 100 kPa / 1 bar), increasing to 7% when the trafficked area was reduced to 15% (Godwin *et al*., 2022). The same study by Kaczorowska-Dolowy (2022) with a bean crop, in the growing season 2017-18, showed that agricultural traffic had a negative effect on root growth, with CTF systems having significantly higher root biomass leading to significantly higher crop yield. CTF treatments also improved soil biology (soil fauna feeding activity and total springtail density). Root biomass and soil biology both play a major role in SOC sequestration. Some plants can move up to 30 – 50% of the carbon fixed in photosynthesis below ground and some of this carbon is lost to the soil through rhizodeposition (Baker *et al*., 2007). Despite this, CTF practices have been poorly adopted in the UK and Europe in contrast with other locations. For example, in Australia, 30 – 40 % of cereal production systems are managed under CTF (Antille *et al.,* 2019).

The use of low ground pressure tyres (< 80 kPa/ 0.8 bar) is an alternative traffic management system that has seen reductions in soil compaction (Antille *et al*., 2013; Chamen *et al.,* 2015). It provides a bigger contact patch, improves traction, reduces pressure on the soil and reduces fuel consumption (Michelin, 2013).

Tillage has also been shown to negatively affect soils (Briones and Schmidt, 2017). This has led to the development and increased adoption of reduced tillage and zero tillage conservation management practices. Reduced tillage has been more widely adopted in the UK, with 47.6% of arable land and 7% under zero tillage (Alskaf *et al*., 2020). In wet climates, reduced tillage and conventional ploughed systems deliver similar crop yields. Although after conversion from conventional to zero tillage, most cereal crops have a reduction of yield during the first 3–5 years of practice, after which yields typically return to levels comparable to conventional tillage (Chaman, 2011; Pittelkow *et al*., 2015; Kaczoroska-Dolowy *et al.,* 2019). However, in semi-arid regions, the benefits on crop yield are more pronounced, as water retention is promoted by reduced tillage. Nonetheless, both climates can benefit from better soil resilience to climate change adaptation (Busari *et al*., 2015).

Long-term studies worldwide show conflicting results on SOC from conversion from conventional to conservation / zero tillage. While some studies (Berner *et al.,* 2008; Lal, 2004) reported an increase of SOC in zero tillage systems in the top layers, others argue that the SOC is just differently distributed throughout the soil profile, with higher concentrations in deeper layers (deeper than 30 cm) under conventional tillage (Baker *et al.,* 2007; Deiss *et al*., 2021; Powlson *et al.,* 2014; Sun *et al*., 2011). While other authors such as Bai *et al*., (2019) have seen SOC increases in the whole profile when using biochar application as a conservation practice. These disagreements in SOC sequestration results could be attributed to the different management practices used (such as SOM input, fertiliser, use of cover crops, rotation), the duration of the study period, the soil sampling frequency and depth, methodological inconsistencies in the measurements used, as well as soil characteristics (soil type, compaction, initial C content), climate and vegetation cover (Bai *et al*., 2019; Li *et al*., 2020; Ugarte *et al*., 2014; Virto *et al.,* 2012; Wan *et al*., 2022). Consequently, this is a knowledge gap that needs further studies to improve the understanding of the processes involved and to assess the nature of SOC under all these variables.

By increasing soil carbon concentrations in arable land, soil health can be promoted, which in turn will support crop productivity, food security and environmental quality (Lal, 2016) as well as constituting a mitigation strategy for Climate Change (Jansson *et al*., 2021).

Global research has typically focused on the effects of tillage on soil carbon (Lipiec *et al.*, 2006; Schuller *et al.,* 2007; Six *et al.,* 1999), while others have focused on CTF (Mouazen and Palmqvist, 2015; Hussein *et al*., 2021; Antille *et al*., 2015). However, the effect of both management practices and their interaction on SOC and fractions is not yet clearly understood. There is also a knowledge gap on the effects that both management practices and their interaction have on crop yield over time. Thus, to achieve sustainable land management in the UK, there is a need to evaluate the quantitative effect of traffic and tillage and their interaction on SOC dynamics and crop yield.

The ability of soil to store SOC depends on the environmental-soil-management interactions, therefore identification of location-specific, suitable management practices is of vital importance to be able to provide robust information to farmers and land managers to maintain and improve soil health.

1.2. RESEARCH AIM, OBJECTIVES AND HYPOTHESIS

As discussed previously in this chapter, both traffic and tillage management practices are critical factors affecting SOC dynamics and crop yield. The separate impacts of these two factors on SOC dynamics and crop yield are important, but more so their potential interaction effects, which are less well understood.

It is crucial to better understand how cropland soils convert the plant-derived C into more persistent C forms, and how these processes are affected by the different management practices imposed. Assessing how the bulk of SOM is distributed among the different SOC pools can provide insights into SOM behaviour and how it is affected by the different management practices. Soil $^{13}C/^{12}C$ natural abundance measurements are another useful tool to investigate changes in SOC dynamics (Smith and Chalk, 2021) and yield insights into the dominant processes guiding SOC sequestration within the different systems.

The aim of the study was to determine if three traffic systems: Standard tyre inflation pressure (STP), Low ground tyre pressure (LTP) and Controlled traffic farming (CTF) interacting with three tillage systems: Deep, Shallow and Zero tillage for a sandy loam soil in annual temperate cropland have an impact on SOC dynamics and crop yield over time.

The specific objectives were:

1) To determine the individual and interacting effects of three traffic and three tillage management systems on SOM, SOM fractions and SOC stocks within 0-30 depth, and over time through the quantification of:

    a) SOM concentration, and microbial biomass carbon (MBC),
    b) SOC concentration, SOC stocks, and soil bulk density (BD),
    c) SOM fractions (POM and MAOM) and soil carbon-to-nitrogen ratio (C/N ratio), and
    d) ^{13}C and ^{12}C isotope analyses to determine the pathway of photosynthetic C moving into the different SOM fractions

2) To determine the individual and interacting effects of three traffic and three tillage systems on crop performance for the following crops:

    a) Winter barley cv. Belfry (*Hordeum vulgare* L.)
    b) Millet cv. White (*Panicum miliaceum L.)*
    c) Spring oats cv. Isabel (*Avena sativa L.*)
    d) Winter wheat cv. Extase (*Triticum aestivum* L.)

The hypotheses are:

1. Controlled traffic farming, Low ground tyre pressure systems and reduced tillage will lead to depth-specific increases in C storage and changes in C distribution among the SOM pools (e.g. POM and MAOM fractions, MBC).
2. Controlled traffic farming, Low ground tyre pressure systems and reduced tillage will lead to increased crop yields.
3. The interaction between traffic and tillage systems will impact SOC dynamics at different soil depths.
4. The interaction between traffic and tillage systems will impact crop yield.

1.3. STRUCTURE OF THE THESIS

The individual chapters addressing these objectives and hypotheses are detailed below:

Chapter 1. Introduction. Reasons are presented as to why agricultural traffic systems interacting with different tillage management systems are important on annual cropland in the UK and their likely impacts on SOC dynamics, showing the current gaps in knowledge. After this, the aim, objectives, and hypotheses to resolve those gaps are described, together with an outline of the structure of this thesis.

Chapter 2. Literature review. Following the introduction, this chapter collates and expands on the central topics and current knowledge on the effects and interactions of traffic and tillage management practices on SOC dynamics, SOC stock, and crop yield in annual croplands, identifying knowledge gaps in the literature.

Chapter 3. Methodology. Details the general methodology for the long-term field experiment at Harper Adams University, UK, over the three years of research.

Chapter 4. Results. Addresses Objective 1 a) and details the effects and interactions of traffic and tillage systems on the SOM (%) over 3 years.

Chapter 5. Results. Addresses Objective 1 b) and details the study carried out to assess dry bulk density, SOC concentrations and SOC stocks.

Chapter 6. Results. Addresses Objective 1 c) outlines the effects and interactions of traffic and tillage systems on the SOM fractions (POM and MAOM) and their C/N ratio over the 3 years.

Chapter 7. Results. Addresses Objective 1 d) and details the study carried out to assess $^{13}C/^{12}C$ isotope analysis and how is affected by the different traffic and tillage systems.

Chapter 8. Results. Addresses Objective 2, a), b), c) and d) and details the study carried out to assess the effects and interactions of traffic and tillage systems on crop growth and yield.

Chapter 9. Discussion. This chapter integrates the discussion on the processes involved in SOC storage and putting together all the findings, relating them, and examining the impact of the different types of traffic and tillage management practices on SOC storage and crop yield.

Chapter 10. Conclusion. Summarises the key findings of this study and draws some practical and scientific recommendations.

Chapter 11. References.

Chapter 12. Appendices.

CHAPTER 2 LITERATURE REVIEW

2.1. INTRODUCTION

In a period marked by rising global population and food demands, with increasing competition for settlement and resources and where the remaining ecosystems and biodiversity are under constant decline, the topics of crop yield and soil organic carbon have gained critical importance. Sustainable agricultural practices aim to enhance crop production while simultaneously improving soil health and resilience and minimising environmental damage. A key aspect of this approach involves the preservation and augmentation of SOC concentrations. However, the ability of a soil to store SOC depends on many factors such as soil type, climate, vegetation cover and the management practices used in crop production. Different traffic and tillage management systems have a big influence on soil organic carbon and crop yield (Berner *et al*., 2008; Hussein, *et al*., 2021; Mouazen and Palmqvist 2015; Lal, 2004), therefore particular emphasis will be placed on the impact that these different management systems and its interaction have on carbon dynamics and crop yield. To increase soil carbon concentrations in annual temperate cropland, it is necessary to understand the factors which contribute to soil carbon dynamics. To explore this issue, this literature review aims to investigate SOM and SOC dynamics in cropland to provide insights into its formation, persistence and function, as well as its relationship with crop yield and climate change mitigation potential.

2.2. WHAT IS SOIL?

Soils are highly diverse and complex systems that deliver numerous ecosystem services (Kibblewhite *et al.,* 2008; Tahat *et al*., 2020). They consist of a mixture of minerals (usually ~50%), organic matter (~2 – 6%), pore space consisting of water or gases, dependent on soil moisture content, and highly numerous and diverse organisms (Orgiazzi *et al.,* 2016). A spoonful of soil may contain up to 1 billion bacteria and myriad other organisms (UK Centre for Ecology and Hydrology, 2022). These organisms are of vital importance to ecosystem function because they decompose SOM and cycle nutrients, thereby supporting the entire food web by providing nutrients to plants (Lavallee *et al*., 2020).

The surface layer of soil, generally referred to as the A horizon or “topsoil” or cultivated depth, is where most of the soil’s biological activity occurs (Brady and Weil, 2008). It is variably defined as a layer that is usually somewhere between 0 – 10 cm or 0 – 30 cm in depth, depending on soil type and paedogenic processes. It is also where the highest concentration of nutrients is found (Brady and Weil, 2008).

The B horizon, or “subsoil”, is the layer located under the “topsoil” but before the bedrock. This layer has a much lower percentage of organic matter and nutrient content (Raper, 2005).

2.3. SOIL ORGANIC MATTER (SOM)

SOM consist of a complex mixture of compounds from animals, plants, and microorganisms in various stages of decomposition, combined with compounds excreted into the soils and their residues (e.g., root exudates such as enzymes or hormones) (Dungait *et al*., 2012; Polyakov and Lal 2004; Shepherd, 2022).
SOM forms only a small fraction of the soil between 2 - 10% by mass in most soils; however, some soils such as peat soils can have SOM >12% (Shepherd, 2022). Most agricultural soils have between 3 – 6 % organic matter (Fento *et al*., 2008).

SOM decomposition and transformation is regulated by the soil microbial community, whose activity and structure regulates the turnover and delivery of nutrients. There are complex feedback loops that take place between the soil microbial community and the soil environment (Jing *et al*., 2017). This will be discussed in more detail in Section 2.6. Soil Microbial Biomass.

SOM promotes healthy crops by improving a wide range of soil physical, chemical and biological properties:

- **Physical**: as SOM increases, soil bulk density decreases, with increases in aggregate stability, porosity, water infiltration, water holding capacity and soil aeration (Esmaeilzadeh and Ahangar, 2014). Surface crusting is also reduced. Therefore, increased SOM leads to reduced water run-off, leading to less flooding and soil erosion. These structural effects also improve the habitat provided by the soil to microorganisms, soil fauna and roots (Strawn and Sparks *et al*., 2000).
- **Chemical**: SOM is associated with better nutrient provision through its decomposition (Cobo *et al*., 2002). It also improves the pH buffering capacity of a soil and accelerates bioweathering of soil minerals (Esmaeilzadeh and Ahangar, 2014; Murphy, 2015).
- **Biological**: SOM sustains the living organisms in the soil by providing food, and enhancing soil microbial biodiversity and activity, which can aid at the suppression of diseases and pests (Magdoff and Weil, 2004). By providing energy to the soil biota, it

also assists with structural genesis within the soil (Esmaeilzadeh and Ahangar, 2014).

2.3.1. SOM CLASSIFICATION IN THE LITERATURE

Historically, SOM has been classified into different components by different studies, which can hinder cross-study comparison. In general, SOM research initially focused on modelling it as a single entity (Jenkinson 1990; Parton *et al*., 1988). However, subsequent studies have fractionated SOM into components that are chemically and physically distinct (Cookson *et al*., 2005; Magid *et al*., 1996), such as light fraction (partly decayed plant and animal products of low density <1.7 g cm^{-3}) and heavy fractions (also called humic substances which are generally mineral associated and have a higher density >1.7 g cm^{-3}) (Janzen *et al*., 1992; Song *et al*., 2012). Other authors, such as Esmaeilzadeh and Ahangar (2014), categorised SOM into three different pools: Active SOM (with a rapid turnover time from weeks to years), Slow SOM (with a turnover time from years to decades) and Stable SOM (sometimes also called passive pool or humus with a turnover time from hundreds to thousands of years).

Another approach separated SOM chemically through alkaline extractions to obtain humic substances. The International Humic Substance Society (IHSS) (2022) claimed that these substances naturally occur in soils "by biochemical and chemical reactions during decay and transformation of plant and microbial remains (in a process called humification)" and these complex molecules will remain in the soil. However, other authors argue that these are artefacts of the extraction process and do not naturally occur in soils (Baveye and Wander 2019; Lehmann and Kleber, 2015; Schmidt *et al*., 2011).

Lehmann and Kleber (2015) proposed the "soil continuum model" explaining that SOM is a continuum of progressively decomposing organic compounds. They claim that large fragments of plant material get decomposed by microbes into smaller and smaller molecules, each time releasing CO_2 as they decompose, rather than decomposing into larger-molecular-size and persistent "humic substances".

Other physical methods of separating SOM compounds have also been developed over the years based on size and/ or density. Cambardella and Elliott (1992) and other authors, such as Christensen (2001), separated SOM into two or three SOM forms: such as humus, particulate organic matter (POM) and mineral-associated organic matter (MAOM), which had different properties and rates of turnover. Subsequently, all the different SOM components helped to answer different research questions, leading to new knowledge, but leaving also disorganised and confused SOM classification scheme without a consensus (Poeplau *et al*., 2018).

To unify and simplify the scientific understanding, Lavallee *et al*. (2020) proposed separating SOM into POM and MAOM; two forms that are fundamentally different in terms of their formation, persistence, and functioning (Fig. 2.1). Current research has been focusing on these two pools and there is an increasing consensus that successful SOC management relies on a comprehensive understanding of how these two fractions respond to various management practices (Angst *et al*., 2023; King *et al*., 2024).

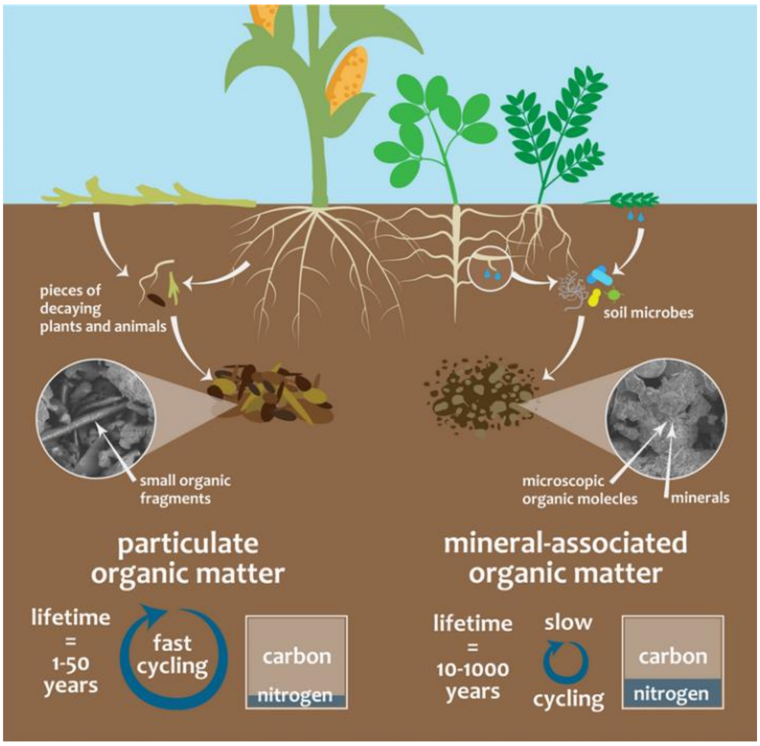


Figure 2. 1. - Particulate (POM) and mineral associated organic matter (MAOM) formation and function (Cotrufo and Lavallee, 2022).

POM is made up of organic materials, largely consisting of partly decomposed plant residues and fungal hyphae. It enters the soil mainly through fragmentation due to the action of soil fauna such as earthworms and microarthropods, which incorporate it into the soil. Because it is freely available to microorganisms, it turns over relatively quickly, with mean residence times <10 years (Fig 2.1). It might become protected in soil aggregates. POM is very vulnerable to environmental changes that promote microbial activity, such as the warming of cold and frozen soils and drying of waterlogged soils (Lavallee *et al*., 2020). It is also susceptible to management practices like tillage that disturb the soil, causing increased decomposition. POM is limited by the amount of C inputs that enter the soil (Six *et al*., 2002) and it contains less nitrogen per unit of carbon than the other SOM pool (MAOM – described below), however, it is more readily available for microbial decomposition (Table 2.1).

Table 2. 1. -Soil organic matter (SOM) components proposed by Lavallee (2020) and their characteristics as Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM) (table adapted from Lavallee et al., 2020).

	POM	MAOM
Protection mechanisms	None or occlusion in large aggregates	Mineral associations (occlusion in fine aggregates, organo-mineral clusters, and micropores; sorption to mineral surfaces)
Lifetime expectancy	<10 years—decades	Decades—centuries- millennia
Dominant formation pathway	Fragmentation, depolymerisation	In vivo transformation or ex vivo modification of low molecular weight compounds
Subject to saturation	No	Yes
Dominant chemical constituents	Plant-derived (e.g., phenols, celluloses, hemicelluloses), fungal-derived (e.g., chitin, xylanase)	Low molecular weight compounds of microbial (e.g., microbial polysaccharides, amino sugars, muramic acid) and plant origin
C:N ratio	10-40	8-13
Nutritional role	Not assimilable by plants, few or no assimilable compounds for microbes	More assimilable compounds for microbes and plants
Compounds	More complex compounds with high activation energies	More simple compounds with low activation energies
Size (diameter)	> 50 – 60 µm	< 50 – 60 µm
Density	< 1.6 – 1.85 g/cm^3	> 1.6 – 1.85 g/cm^3

In contrast, **MAOM** consists mainly of molecular compounds that have either leached directly from plant material (such as soluble extracts leaching from plant litter or root exudates) or been chemically transformed by the soil biota (Lavallee *et al*, 2020), including residues of dead microbes (necromass).

MAOM forms associations with mineral surfaces, and it can also form micro-aggregates (< 50 – 60 µm), which protect it from decomposition (Table 2.1). This mineral protection results in mean residence times in the order of decades to centuries or millennia, dependent on ecosystem properties (Schmidt *et al.*, 2011) (Fig. 1). It also has a higher density (when including the minerals, it is associated with) and is less vulnerable to disturbance and environmental change than POM (Lavalle *et al.*, 2020). Therefore, MAOM represents the most persistent and resistant SOM stock (Cotrufo *et al*., 2019). MAOM has a lower C:N ratio, (i.e., it contains more nitrogen per unit of carbon than POM), but because of the mineral association of this organic matter pool, N is less available and cycles much more slowly than from POM (Cotrufo and Lavallee, 2022).

2.3.2. SOM FRACTIONS AND THEIR IMPLICATIONS FOR LAND MANAGEMENT

Separating soil carbon into POM and MAOM aids in the understanding of its vulnerability to the different management practices and identification of the best effective carbon sequestration strategies.

POM is the SOM fraction considered to be the most sensitive and vulnerable to changes in the environment, such as due to tillage practices (Lavallee *et al.,* 2020). However, much like tillage, drying- rewetting cycles may also increase SOM decomposition by breaking soil aggregates and exposing SOC that was otherwise protected (Lal *et al*., 2015). Cropland soils generally exhibit relatively low levels of POM due to the harvesting and removal of plant biomass, low levels of root input due to growing annual crops with shallow root systems, and frequent tillage destroying soil aggregates and aerating soils, which exposes SOC to decomposition, promoting a fast turnover of any crop input into the soil (Cambardella and Elliott, 1992). POM can be a precursor to MAOM, because increased POM supports higher levels of microbial biomass, much of which eventually becomes microbial necromass, a main component of MAOM (Lavallee *et al*., 2020). However, MAOM formation is not always linked to POM, soluble extracts of plant litter have long been known to sorb to minerals (Kramer *et al*., 2012) as well as living root inputs (exudates), which through rhizodeposition can lead to MAOM formation, which in fact have a preferential retention compared to shoot inputs (Sokol *et al*., 2018).

One way to investigate the formation pathway of POM-C and MAOM-C is to study their C/N ratio. If MAOM has a C/N ratio of 8-13, it suggests a predominance of microbial contributions to this fraction, while a POM with a C/N ratio of 10-40 reflects major contributions from plant litter (Lavalllee *et al*., 2020). This is in line with Angst *et al*. (2021), who observed that a MAOM C/N ratio (>15) suggests a larger contribution of plant-derived C to MAOM, for example in wet forest soils, while a MAOM C/N ratio (<15) was attributed to a fast microbial turnover and thus a major proportion of microbial-derived C to MAOM. Furthermore, Yu *et al*. (2022) also reported that in annual cropland soils, POM is mainly plant-derived (except when manure is applied) and MAOM is mostly microbial-derived. However, if perennial crops are used, this will no longer be the case, because they have bigger and more complex root structures, increasing the plant-derived C, similar to forest soils.

Arable and permanent cropland soils hold more of their TOC in MAOM fraction Lugato *et al.* (2021). This agrees with different authors such as Begill *et al*. (2023) who using the German soil database found that in intensively managed temperate cropland soils, on average 86% of the TOC was stored in the MAOM fraction, while 14% was stored in the POM fraction. And Matus (2021) who in a recent meta-analysis looking at a wide range of cropland soils also observed that the MAOM fraction contained on average 83% of the TOC of the soil. This indicates, that in croplands most of the C is stored in the stable carbon stocks with potentially long mean residence times. However, although the MAOM fraction cycles much slower than the POM fraction, it is also under constant turnover and decomposition, therefore, to maintain SOC levels in croplands, it is recognized that constant carbon inputs are essential (Luo *et al*., 2017).

MAOM formation is also likely sensitive to environmental factors. Increasing MAOM relies mostly on favourable soil conditions for the biotic transformations (such as pH, oxygen availability, soil humidity and temperature, soil texture) and type and quantity of OM inputs (low C/N ratio) (Angst *et al*., 2023).

The **soil carbon saturation concept** is based in the assumption that soils have a finite capacity to store C, due to MAOM associates with soil mineral surfaces (silt and clay minerals) and these vary between soils but are ultimately finite (Six, *et al*., 2002; Stewart *et al*., 2007). Hassink (1997) was the first one to use this term to explain the relationship between silt and clay particles and MAOM on a wide range of soils from temperate to tropical climates. Other studies have demonstrated that clay-size particles are rich with C compared to sand and silt particles, at any soil type and depth (Rumpel *et al*., 2004). Since then, there has been a widespread assumption that MAOM-C behaviour is determined by saturation dynamics (Angst *et al*., 2023; Just *et al*., 2023), and many models use clay content as a parameter to predict SOC turnover (Jenkinson and Rayner, 1977; Müller and Höper, 2004: Prout *et al.,* 2022).

A study conducted by Cotrufo *et al.* (2019) using the European database of grassland and forest soils (LUCAS) reported an upper limit for MAOM-C of 45 g C kg^{-1} bulk soil, while POM-C continued to increase with increased SOC. However, Begill *et al*. (2023) challenged this concept, noting that the dataset analysed by Cotrufo *et al.* (2019) lacked representation of soils with SOC content higher than 45 g kg^{-1}. As a result, Begill *et al*. (2023) used the German Agricultural Soil Inventory to test for an upper threshold of MAOM-C storage by selecting a wide range of agricultural soils with SOC (5–118 g kg^{-1}) and clay contents (30-

770 g kg^{-1}). And in contrast to Cotrufo *et al*. (2019), no upper limit of MAOM-C storage was detected in any texture class. The proportion of MAOM-C increased with the fine fraction content, but its accumulation was not constrained by texture. Furthermore, they also observed a pilling of SOC in the MAOM fraction when the biding sites were limited. These findings are in line with other research that found that MAOM-C can form organo-mineral clusters (Schweizer *et al*., 2021). Additionally, Vogel *et al*. (2014) also observed a patchy accumulation of MAOC in the soil, happening in hotspots preferentially forming in rough surfaces. And when new carbon was added, it ended up pilling in areas with pre-existing high concentrations of carbon, instead of the free surfaces. The formation of MAOM-C clusters might depend on the abiotic soil properties such as mineralogy, pH or soil compaction, limiting oxygen and water supply. Schweizer (2022) also proposed that within each pile, there might be different gradients of decomposability. Begill *et al*. (2023) also noticed that the proportion of MAOC-C was found to be surprisingly stable across the whole range of investigated soils and up to a very high bulk SOC content. These observations suggest that the concept of carbon saturation in cropland soils may be influenced by additional factors beyond the traditionally considered.

In any case, there are a number of factors indicating that to achieve a theoretical saturation level of MAOC in cropland soils may not be a realistic prospect. Firstly, cropland soils have been shown to be strongly depleted of SOC (Sanderman *et al*., 2017). Furthermore, there is always a limit to the amount of C inputs from crop biomass (Janzen *et al*., 2022) and organic amendments that a farmer can put into the soil, as well as important socio-economic constraints.

On annual cropland, over long periods, MAOM-C is assumed to evolve around equilibrium dynamics, so-called steady-state assumptions (Castellano *et al.,* 2015). This steady-state level of MAOM-C is derived from the constant SOC input and output from soil systems. In most cases, the C inputs are not high enough and/or the rate of C mineralization is too fast to reach very high levels of MAOM-C.

However, the rate of SOC sequestration is not constant, it is usually highest in the years immediately after the management practices change is introduced, and it slows down over time as the soil reaches a new equilibrium (Fig. 2.3) (BSSS, 2023).

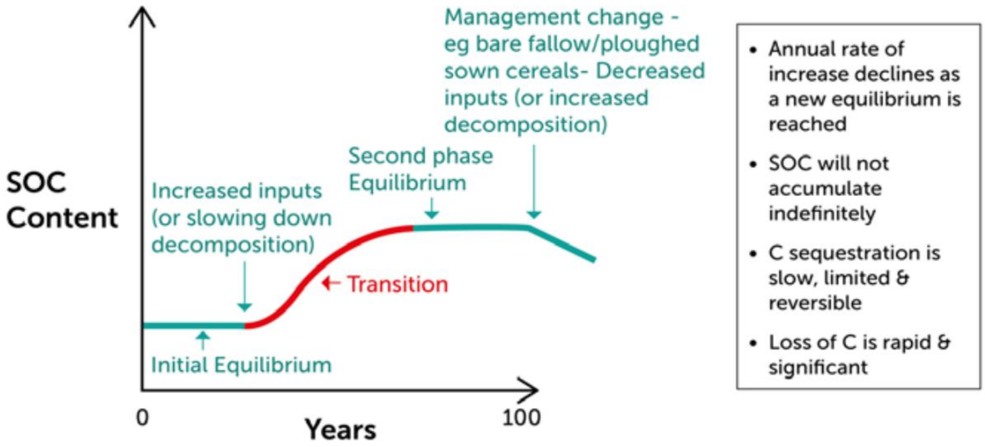


Figure 2. 2. – SOC accumulation rates change over time (Diagram from: BSSS, 2023).

Recent studies conducted in long-term field experiments on temperate arable soils suggest that MAOM-C is reaching this steady state or equilibrium even across different soil types, management practices and fertilizer inputs. When this happens, the additional SOC seems to accumulate on the POM-C instead. For instance, a long-term (36 years) field experiment conducted by Mayer *et al*. (2022) in Switzerland, investigating the impacts of different fertilizer types and rates on the temporal dynamics of SOM fractions, revealed that no additional C was sequestered in the clay-sized MAOM fraction (<6.3 um) under any of the fertilised systems. In fact, the amount of C in this pool remained unchanged over the 36-year period, (while in the unfertilized control treatment, it decreased by 20%). TOC increases were stored only within the labile POM fractions. They also observed strong annual POM-C fluctuations depending on the timing of soil sampling after harvest. This illustrates the need for careful management to protect and increase the POM fraction. And raises concerns about the potential of arable soils to act as a long-term C sink as a climate change mitigation strategy. It also emphasizes the need for continuous SOM inputs to maintain elevated levels of labile POM fractions, thereby maintaining soil fertility, crop performance and food security.

Another long-term (29 years) field study by Rui *et al*. (2022) conducted in mollisols soils in the North Central United States investigated the effects of different regenerative management practices on TOC accrual and distribution among SOM fractions in comparison to conventional continuous maize monocropping with annual tillage. Their results suggested for the systems incorporating reduced tillage, crop rotation, cover crops with legumes and manure addition that MAOM-C did not increase. However, incorporating legumes and manure into annual cropping systems enhanced POM-C, microbial biomass, and microbial carbon use efficiency, but did not significantly increase microbial necromass accumulation, MAOM-C or TOC storage. While these management practices improved soil health metrics which are crucial for maintaining soil nutrient and water conservation, they were unlikely to increase the more persistent form MAOM-C.

However, there are long-term studies showing an increase in both POM-C and MAOM-C, such as the study by Kauer *et al.* (2021) in Estonia, who in a long-term (10 years) study compared conventional rotational cropping (with mineral fertilization) and organic cover cropping (with additional manure) with a control treatment (with no additional mineral fertilizers or manure). These results are to be expected because the control treatment will be losing both their POM-C and MAOM-C as the system needs a continuous supply of C inputs and fertilizer rates to maintain itself.

Soil abiotic factors (such as soil mineralogy, pH and climatic factors) can also play an important role on MAOM-C formation (Beare *et al*., 2014). Other important factors are the initial C content of the soil (Haddix *et al*., 2020) and vegetation cover (Wiesmeier *et al.,* 2019). King *et al.* (2023) showed that across a different climatic and soil texture gradient, MAOM was promoted by a higher precipitation and lower sand content, while higher initial soil C content promoted the formation of POM.

Other studies have also shown that substrate quality (Ridgeway *et al*., 2022) and quantity (Janzen *et al*., 2022) plus root quality (Poeplau *et al*., 2023), might also play an important role in MAOM-C formation and stabilisation by affecting microbial carbon use efficiency (CUE) and priming or mineralisation of native SOC (POM-C an MAOM-C) (Guenet *et al*., 2018; Ridgway *et al.,* 2022).

Lower substrate quality inputs (high C:N ratio) should favour POM formation, increasing C sequestration in the short-term. However, if the field is ploughed afterwards, this will increase the decomposition rate of SOM and the benefit will be lost (Cotrufo *et al*., 2013). While higher quality SOM inputs (low C:N ratio) will favour MAOM formation, thanks to the microbes decomposing and realising more N to the soil (Lavallee *et al*., 2020).

In cropland soils, incorporation of high concentrations of artificial nitrogen may support a highly efficient microbial transformation of plant residues, increasing POM and promoting MAOM formation (Cortufo *et al*., 2013). Although too many N additions could also promote soil acidification, in which microbial activity will be limited, causing an accumulation of undecomposed POM *(*Averill and Waring*,* 2018). However, when N inputs are limiting, additions of fresh residues might stimulate the microbial community to mine the more recalcitrant forms of C for N, resulting in losses of MAOM (Diochon *et al*., 2016).

Understanding POM and MAOM behaviour under different management practices can give us important information for managing our soils for nitrogen-efficient carbon sequestration.

To maximise the productivity of both POM and MAOM, best practices should focus on:

i) diversifying cropping systems (e.g. including legumes and perennials with deep roots in rotation),

ii) maintaining residue inputs into the soil (especially with high-quality litter -high N-),

iii) maintaining plant cover all year round and

iv) minimising soil disturbance from tillage practices. This will provide a variety of recalcitrant and labile plant components and will support the biota and their diversity, leading to the formation of new SOM (Cotrufo *et al*., 2019).

2.4. CARBON TO NITROGEN RATIO (C/N) OF SOM INPUTS

The C/N ratio refers to the amount of C relative to the amount of N present in a substance. The C/N ratio has an important effect on organic matter decomposition and crop nutrient cycling (predominantly nitrogen) (Wang *et al*., 2018). Soil C/N ratio has been recognised as a good indicator of soil fertility (Zhang *et al*., 2016).

Microbes must acquire enough carbon and nitrogen from the environment to meet their energy and nutritional requirements. They need an ideal diet with a C/N of about 24 (Brust, 2019). An explanation of the different C/N ratios of SOM inputs and its effects on N are shown in Table 2. 2.

Therefore, SOM holds N that is not available to the crop or plants until is mineralised by soil microorganisms. Depending on the C/N ratio of the SOM inputs, it might favour N immobilisation or mineralisation, however, this is always temporary. For example, when low quality SOM inputs are added to the soil, to be able to consume all the C, microbes will also consume the available N in the soil, but when they start to die and decompose, they will release back the N contained in their bodies to the soil (mineralization). Arable soils have a C/N ratio that ranges from 9 to 14 (Johnston *et al*., 2009), however this ratio will be variable when organic matter has been added to the soil. This ratio is lower in arid regions than humid regions. And the C/N ratio is usually smaller in subsoils (Kramer *et al*., 2017).

Table 2. 2. – C/N ratio of SOM inputs and its effects on soil N (table adapted from Brust, 2019; USDA, 2011; Kicklighter *et al*., 2019; Watson *et al*., 2022).

SOM input	C/N ratio	Example	Effects on N
Low quality SOM	C/N ratio (> 30) It favours POM formation	Example: Wheat straw (C/N ratio 80:1) To be able to consume all the excess C, microbes will need to find additional N in the soil.	Immobilization (temporary N deficit in the soil, due to soil microbes use it to be able to consume the excess C).
Medium quality SOM	C/N ratio (20 – 30)	Example: Alfalfa Hay (C/N ratio 25:1) Microbes will consume it quickly with no excess C or N left over.	Equilibrium state between immobilisation and mineralization (no excess C or N left over).
High quality SOM	C/N ratio (1 -20) It favours MAOM formation	Example: cover crop of leguminous plants (C/N ratio 11:1) Microbes will consume the C and leave the excess N in the soil.	Mineralisation (temporary N surplus, N is released into the soil for immediate crop use).

2.5. SOIL CARBON

Soil C can exist in two forms: soil inorganic carbon (SIC) and soil organic carbon (SOC).

SIC, the predominant forms are carbonate minerals either derived from weathering of parental material, from reaction of soil minerals with atmospheric CO_2, or material applied as part of soil management to increased soil pH (i.e., "lime") (Lal *et al.,* 2015). SIC forms in alkaline soils with a pH typically around 7.5 – 8.5. This type of soil is very common in semiarid and arid climates (Dwevedi *et al*., 2017). SIC, particularly calcium (and magnesium) carbonates are formed through the following reactions:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (1)$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + H_2O + CO_2 \quad (2)$$

The pH, carbon dioxide (CO_2), Ca^{2+}/Mg^{2+} and water (H_2O) content of a soil will determine its formation. For example, under high soil pH, reaction (1) will be driven to the right, producing HCO_3^- and available Ca^{2+}/Mg^{2+} will precipitate forming calcium carbonate ($CaCO_3$). Under low soil pH or an increase in CO_2, reaction (2) will be driven to the left. Therefore, acidic soil conditions would lead to the dissolution of carbonates, causing a decrease in SIC stock, while an alkaline soil environment will promote the formation of carbonates (Guo *et al*., 2016).

SOC is the measurable carbon concentration of SOM; usually about 50% (Pribyl, 2010). It is derived from the decomposition of dead animals, plants and microorganisms and their activities (Bronick and Lal, 2005). Soils contain more C than the atmosphere and biosphere combined (Cho, 2018).

Soil carbon sequestration (SCS) refers to the storage of atmospheric CO_2 that has pass through plants and/ or animals to the soil C pools through leaching or decomposition processes (Bhattacharyya *et al*., 2022). Carbon enters the soil via decomposition of organic matter or roots and as root exudates. Labile C pools refer to the C that exists in roots or microbial biomass and is bioavailable (POM). And stabilised or recalcitrant C pool is the organic material resistant to decomposition (MAOM) (Fig. 2.1). Many plants' roots form symbiotic associations with mycorrhizae, providing the fungi with energy in the form of C, while the fungi provide the plant with nutrients such as phosphorus. Soil microbes can also decompose SOM and retain a small portion of the original carbon in the soil (forming POM and MAOM), as well as producing some carbon loss (in the form of CO_2) through respiration (Ontl and Schulte, 2012) (Fig. 2.3).

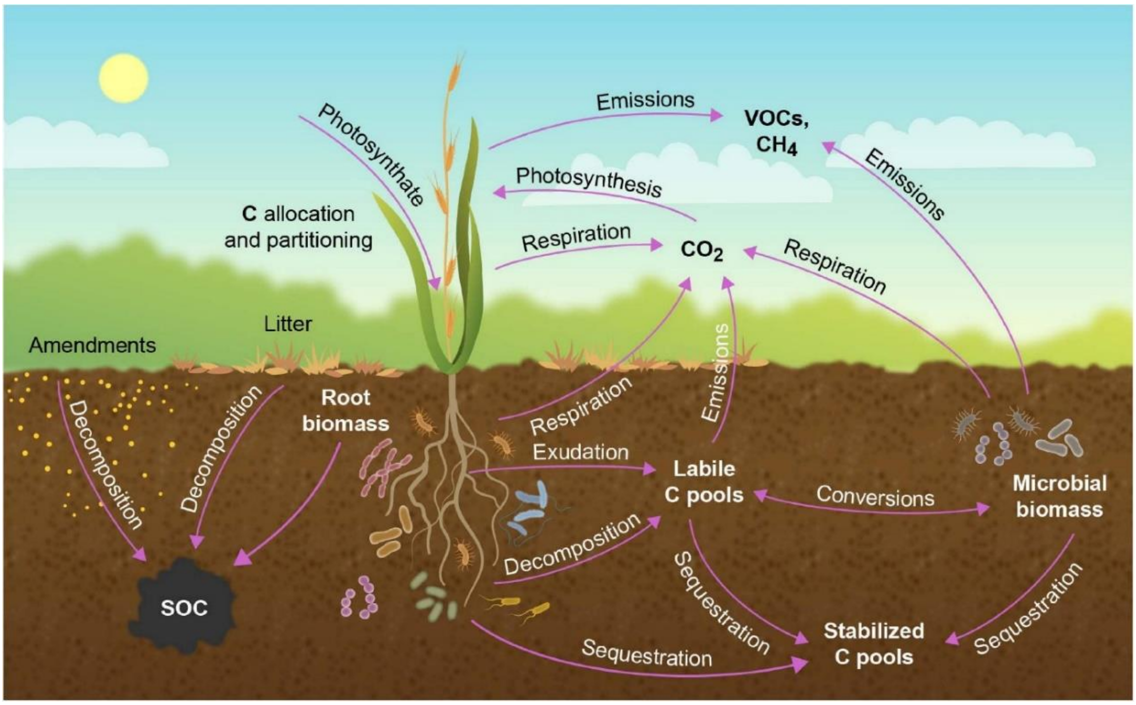


Figure 2. 3. – Carbon cycle and its storage in different C pools. (From Jansson *et al.,* 2021).

Some plants can move up to 30 – 50% of the carbon fixed in photosynthesis below-ground. This carbon is then used for autotrophic respiration or root growth, and some is lost to the soil in organic forms, through rhizodeposition (Baker *et al*., 2007).

Rhizodeposition is a process in which living plant roots release carbon compounds in their surroundings (which leads to a proliferation of microorganisms around them) but they also release other materials such as dead fine roots, water-soluble exudates, secretions of insoluble materials and gases, such as CO_2 and ethylene (Cheng and Gershenson, 2007). This complex mixture of substrates is responsible for the rhizosphere priming effect (RPE) (el Zahar Haichar *et al*., 2014; Kuzyakov, 2010). This effect can slow SOM decomposition by 50% or stimulate it by 380%, when compared to laboratory soil incubations without plants (Chen *et al*., 2014). In a recent meta-analysis by Huo *et al*. (2017), they showed that RPE varied significantly among plant types and soil texture, but on average, it enhanced the SOC mineralisation rate by 50% across all studies. Additionally, crop plant types had the lowest RPE when compared to grasses and woody species and soils with coarser texture had lower RPEs than soils with finer texture. More surprisingly, they found that RPE was positively correlated with aboveground plant biomass, but not with root biomass, as was commonly believed.

Soil carbon storage is controlled by the balanced between inputs; from plants (e.g., litter and root exudation) and amendments (e.g., manure and compost), and outputs or biological losses (e.g., respiration from microbes and roots) (Jansson *et al*., 2021) (Fig. 2. 3) (Table 2. 3).

Table 2. 2. - Soil carbon sequestration (SCS) and storage are controlled by the balance between plant inputs and outputs (Jansson *et al.,* 2021).

Input – Output = Storage	**Inputs**: Photosynthesis and soil amendments. **Outputs**: Plant and microbial respiration **Storage**: Plant and microbial biomass, soil carbon as SOC and SIC pools

In terrestrial ecosystems, soils are the largest carbon store (2,344 PgC) (Jobbágy and Jackson, 2000). (PgC = Pentagrams of carbon, where 1 PgC = 1 billion metric tonnes carbon). Agricultural soils have the lowest carbon density of all land covers, but because of the large area they occupy, they hold the largest topsoil carbon stocks (6.7 ± 1.2 PgC)" (Lugato *et al.,* 2021). Nevertheless, SOC stocks are very slow to change, generally taking over 6 years to change after an agricultural system change (Blanco-Canqui *et al*., 2015;

Cooper *et al*., 2021; Poeplau and Don 2015). And as previously discussed, soil C concentrations are also affected differently by soil type, climate and vegetation cover.

It is also worth noting that SCS rates will reduce as the soil carbon stock reaches an equilibrium where the soil carbon sink is saturated (i.e., in which soil carbon inputs are similar to soil carbon outputs) (Paustian *et al.,* 1997; West and Six, 2007). It is thought that this process in agricultural soils could take from 10 to 100 years, depending on soil type and climate (Sauerbeck, 2001; West and Six, 2007).

Additionally, measurements of SOC stocks have proven problematic over the years. Poeplau, Vos and Don (2017) found that most studies had an overestimation of SOC stocks, as they did not include in the bulk density measurements the rock and root fragment content and/ or depth in the calculations. They showed that more than 5 vol.% rock/root fragment content will inevitably overestimate SOC stocks by an average of 144 % (i.e., more than doubling the real SOC stocks).

Von Haden *et al*. (2020) also proposed that to be able to assess changes in SOC stocks in soils, the equivalent soil mass (ESM) method should be used. They reported that comparisons of SOC stocks at fixed depth intervals (as the product of soil carbon concentration, soil bulk density and depth) are subject to errors when changes in dry bulk density occur due to different management practices (such as tillage, drilling, etc), and it can also vary through time.

Another potential problem when estimating soil carbon stocks is the depth of sampling. Many studies have shown that carbon sequestered in subsoils can contribute to 30 – 75 % of the total stocks within a soil profile and this carbon is also characterised by higher mean residence times (Chaopricha and Marin-Spiotta, 2013; Harrison, Footer and Strahm, 2011; Rumpel *et al*., 2012) indicating that it is more resistant to decomposition probably because it is protected from physical disturbance such as tillage. But while some studies have shown differences in SOC stocks in deeper layers (> 30 cm) between conventional and conservation tillage (Baker *et al*., 2007), others found that it stored the same amount of C in deeper layers (Blanco-Canqui and Lal, 2008; Haddaway *et al*., 2017).

In this context, Mary *et al*. (2020) proposed 5 methodological recommendations for a rigorous assessment of SOC stocks and SOC changes: "1) direct measurement of bulk density, 2) deep sampling, so that the sampling depth exceeds the maximum tilled depth, 3) calculation of stocks on an equivalent soil mass (ESM) and not on a depth basis 4) pre-treatment baseline measurement in the plots before treatments are applied and 5) use of diachronic (e.g. a time series analysis) rather than a synchronic approach".

2.6. SOIL MICROBIAL COMMUNITY

Soil microbial community (SMC) refers to the microbial populations living in the soil, their diversity and interactions. SMC and their activity play a major role in soil C dynamics and soil carbon sequestration. Therefore, understanding the interactions between SMC, SOM and the different arable management activities will be crucial to understand their full contribution to soil carbon sequestration and improve the sustainability of soil management (Bhattacharyya *et al.,* 2022).

SMC have two very important and contrasting jobs: reducing SOC stocks (through decomposition of SOM) and increasing SOC stocks (through their own biomass and its residues (Kallenbach *et al.*, 2015; Kästner and Miltner, 2018). Soil microbial biomass and its diversity affect rhizosphere processes and alter carbon dynamics (Hartmann *et al*., 2015). Soil microorganisms (such as bacteria and fungi) contribute substantially to the recalcitrant SOC or MAOM through processes of population growth, cell generation, death, decay and necromass formation (Ma *et al.*, 2018; Buckeridge *et al.*, 2020). In fact, for temperate agricultural soils, 55.6 % of total SOC is considered microbial necromass (dead microbial cells and their degradates) (Fig. 2.4). From these necromass, fungal necromass carbon makes higher contributions (>70% of total necromass) to SOC than bacterial necromass carbon (26%-28%) (Liang *et al*., 2019).

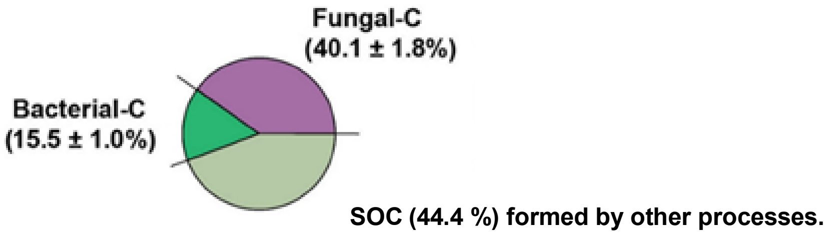


Figure 2. 4. – Diagram showing SOC composition in temperate agricultural soils, with 55.6% considered microbial necromass (and from this, >70% is considered fungal necromass). (Adapted from Liang et al., 2019).

Conventional agricultural practices (such as tillage, chemical inputs, annual mono-cropping) have a direct impact on the structure and function of the soil community and typically result in a reduction of soil microbial biomass carbon (MBC) (Postma-Blaauw et al., 2010). In agricultural soils, MBC is estimated to be less than 2.5% and strongly correlated with the quantity and quality of C inputs as well as SOM concentrations (Fierer *et al*., 2009). It has a fast turnover time and therefore is highly sensitive to changes induced by management practices as well as environmental conditions or disturbances (Alvarez and Alvarez, 2016).

2.6.1. FACTORS AFFECTING SMC

The quality (C/N ratio) and availability of SOM are factors that will affect the ability of soil microorganisms to survive and the size of their communities (Nguyen and Marschner, 2016). If the supply is cut, SMC might die or transform into spore forms and enter a dormant state, therefore their biomass will be reduced (Shahbaz *et al*., 2017). However, if the supply is increased, it will increase soil microbial biomass improving the available nutrient content of the soil, which is also an important pool for soil nitrogen (Naorem *et al*., 2021).

Soil microorganisms must acquire enough carbon and some nitrogen from the environment to stay alive. They need an ideal diet with a C/N of 24:1 (USDA, 2011).

As previously discussed, soil microorganisms need to consume carbon and nitrogen from the environment to stay alive. Therefore, organic nitrogen availability is an important factor affecting the contribution of microbial necromass to SOC. Its limitation might slow down the decomposition of plant litter, turnover to microbial biomass and therefore microbial necromass, affecting the carbon movement and resulting in less MAOM where it gets stabilised (Averill and Waring, 2018; Cotrufo *et al*, 2013).

However, there are other factors such as soil management practices, soil type, climate, vegetation and living soil organisms, that can all affect the abundance, activity and composition of SMC (Gałązka and Furtak, 2019). For example, the amount of traffic will affect soil compaction which will affect soil porosity and therefore the spatial distribution of SMC as well as soil C stocks (Kravchenko *et al*., 2019).

2.7. TRAFFIC AND TILLAGE FOR SOIL MANAGEMENT IN ARABLE LAND

Soil management is a fundamental part of crop production. Tillage has been used for seedbed preparation for thousands of years (Warkentin, 2001). Contemporary agricultural machinery has increased in size and mass due to the use of more powerful and larger vehicles aimed at increasing productivity and efficiency. This has increased the risk of soil damage due to compaction (Keller *et al.,* 2022; Lamande and Schjonning, 2011).

2.7.1. TILLAGE SYSTEMS

Conventional tillage (CT) systems are widely practiced by farmers across the world. They are based on turning over the topsoil with different implements and to different depths >25 cm, mainly for seedbed preparation (Morris *et al.,* 2010). Other uses include weed control, alleviating soil compaction, incorporating manure and fertilizer and turning over cover crops. The mixing of the topsoil develops changes in soil structure as well as decreasing soil aggregation (Lipiec *et al.*, 2006). This promotes changes in the microbial activity and its community composition, increasing SOM oxidation (by breaking soil aggregates and exposing protected carbon and by redistributing SOM across the whole profile), reduced water storage capacity and increased risk of erosion (Schuller *et al.*, 2007; Six *et al.*, 1999). Therefore, conventional agriculture has created many challenges over the years, such as soil compaction, pollution of water bodies and the need to use fertiliser and pesticides. It also has high fuel and labour needs. This is how and why conservation agriculture started (Kassam *et al.*, 2009).

Conservation tillage or zero tillage systems cover a wide range of tillage operations where the soil is either direct drilled (drilling the seed directly into the soil with no previous tillage practice to bury or partially bury crop residues) or shallow tillage (to a depth <10 cm using discs or tines) (Morris *et al.*, 2010). These systems aim to preserve the soil structure and SOC content by maintaining the maximum amount of cover on the soil surface and promoting a good environment for plant growth (Czyz and Dexter, 2008; Derpsch *et al.*, 2014). It also minimises soil erosion, increases biological activity and promotes aggregate stability (Green *et al.*, 2007; Song *et al.*, 2016). A more detailed review of its effects on soil properties is explained in section 2.8.1.

Other benefits of replacing conventional tillage with conservation tillage is that they improve the cropping systems' resilience (Lal, 2015) as well as a decrease in fuel consumption (reducing GHG emissions) and working hours (Dyer and Desjardins, 2007; Mileusnić *et al.*, 2010).

However, zero tillage is not always beneficial and it can potentially lead to soil compaction in the surface, increased weeds, insect pests and higher slug populations, sub-optimal seedbed preparation, poor germination in wet, anaerobic conditions and pollution of nearby water systems due to the mobilised nutrients that can accumulate near the soil surface (Abdollahi *et al.*, 2014; Godwin, 2014; Holland, 2004; Shao *et al.,* 2016; Skaalsveen *et al.*, 2019; Van den Putte *et al.*, 2010). Despite this, conservation tillage practices have been quite widely adopted in the UK, data from 2010 indicate that zero tillage practices were adopted by 32% of farmers, and 46% adopted some form of conservation or reduced tillage (Townsend *et al.*, 2016).

2.7.2. TRAFFIC SYSTEMS

In conventional farming practices most of the soil gets trafficked by conventional field driving operations. A study by Kroulik *et al.* (2009) showed the extent of the trafficked area on conventional traffic farming practices for wheat production. Using GPS system tracking devices, they were able to show that at least one wheel pass covered 85% of the area for conventional tillage, 65% for minimum tillage and 42% for zero tillage practices. This random field traffic induces soil compaction, particularly when the soil is wet. The compaction reduces soil porosity, altering the soil structure and reducing soil hydraulic conductivity (Soane and van Ouwerkerk, 2013).

Controlled traffic farming (CTF) is a method aimed at reducing the impact of trafficking. In CTF, all wheel ways are restricted to the minimum possible area of permanent traffic lanes, which limits compaction to only those specific lanes. This leaves the rest of the soil free from compaction, which improves water infiltration (Chamen, 2015), reduces run-off and erosion, conserves organic matter, enhances soil biodiversity and fertiliser use efficiency and reduces greenhouse gas emission from the non-trafficked soil (Mouazen and Palmqvist 2015; Hussein *et al*., 2021). Equipment and system changes are necessary to match equipment widths and wheel track spacing as well as GPS systems, but farmers could adjust to this over time when equipment needs to be replaced without needing a large investment. In a long-term UK study on a sandy loam by Kaczorowska-Dolowy *et al*. (2019), CTF systems resulted in yield increases of 4% from the beginning when compared to standard tyre pressure systems, as well as improved root development and soil health (Kaczorowska-Dolowy, 2022).

Another important factor affecting soil compaction, is the selection of tyre construction, load, inflation pressure, and resulting contact area. By reducing the tyre load and inflation pressure, the pressure that penetrates the soil profile is also reduced. This also improves tractive efficiency of field operations (Chamen *et al*., 2003).

Agricultural vehicles drive with different loads for a range of field operations, on different surfaces and at different speeds, including higher speed highway conditions. Therefore, to reduce the effects of soil compaction it is important to be able to change the tyre pressure depending on these different variables, but also to increase the tyre longevity (preventing unnecessary wear and tear) and reduce fuel costs (Treadfirst, 2020). To deal with this problem, agricultural tyre manufacturers have created different data sheets with the recommended tyre size, loads and inflation pressures for each specific need. However, improved types of tyres are reaching the market, for example Michellin ultraflex technology allows the tyre pressure to be adjusted over a wider range and thanks to its wider wheel footprint, which allows for better soil protection and increased longevity and fuel efficiency (Brookes, 2022). Therefore, farmers should consider all aspects (such as soil type and its vulnerability to soil compaction, crop rotation, size of the farm and field, vehicle load) when choosing the appropriate tyres for their needs.

There is a most recent line of research studying the feasibility of intelligent machines and smaller automated tractors for agricultural operations to increase efficiency and reduce soil compaction and environmental impacts. In the UK, the Hands Free Farm project (a 35-hectare farm) at Harper Adams University has just completed its third-year crop, using a fleet of light weight autonomous vehicles and drones to plant, monitor and harvest a crop without operators in the driving seat (Spencer 2017; Lowenber-Deboer *et al*., 2020). However, these technologies are in their infancy and have some reliability issues to be resolved (Virk *et al*., 2020).

2.8. SUSTAINABLE MANAGEMENT PRACTICES TO ENHANCE SOIL C SEQUESTRATION AND YIELD PRODUCTIVITY

2.8.1. USE OF CONSERVATION TILLAGE PRACTICES

Soil physical, biochemical and biological properties: By planting into the residues of the previous crop, conservation tillage enriches the soil with SOM and nutrients, it prevents erosion and reduces the risk of compaction, as well as enhanced water availability. It is also linked with the increased quantity and persistence of soil aggregates (Doran, Elliott and Paustian, 1998; Kraut-Cohen *et al*., 2020; Sun *et al.,* 2011). Furthermore, these minimal soil disturbance systems, promote an increase in earthworm biomass (Pelosi *et al*., 2014). Which in turn improves surface connected porosity and macroporosity, encouraging deeper rooting growth (Fischer *et al*., 2014), which is likely to assist with water infiltration and storage capacity (Soane *et al*., 2012). Zero tillage systems tend to have a larger proportion of deep borrowing earthworms (that create vertical burrows connected to the soil surface), as they would normally be killed during conventional tillage operations (Peigné *et al*., 2009). These earthworms primarily feed on surface residues, which are often pulled down into their burrows, increasing SOM movement down the profile but also improving water drainage and availability.

Zero tillage systems are also beneficial for arbuscular mycorrhizal fungi, which form mutualistic symbiotic relationships with approx. 80% of terrestrial plant species (Wilkes *et al*., 2021).

Soil microbial community (SMC): Zero tillage systems create a better and more stratified habitat for soil microorganisms to live, as well as other micro-and macro-fauna, improving their biodiversity (Doran *et al*., 1998; Matthew *et al*., 2012; Tsiafouli *et al*., 2015). This agrees with other authors who also claimed that no-till systems increased the activity, diversity and the quantity of soil microorganisms in the upper layer (Blanco-Canqui and Lal, 2007;

Cookson, Murphy and Roper, 2008). Other authors have also seen an increase in total nitrogen and microbial biomass (Alvarez, 2005; Balota *et al.,* 2003). Since SMC regulates SOM decomposition rates and plant nutrients turnover and supply, as well as having a high concentration of stabilised C in the microbial necromass (section 1.5.), it is considered as the main driver for soil conservation and sustainable management practices.

SOC: in zero tillage systems the only source of C input below surface is the movement of SOM by soil organisms and root exudes. Six *et al*. (1999) suggested that this might decrease SOM decomposition rate. This agrees with Balesdent *et al*. (2000), who reported that the SOM mean residence time (inverse of mineralisation rate) could be higher under zero tillage, which might be due to the increase of micro and macro-aggregates protecting SOM from decomposition. This also agrees with Du *et al*. (2015), who reported that long-term zero tillage systems promoted macro-aggregates in the surface and increased soil carbon sequestration. However, other studies (Haile-Mariam *et al*., 2008; Murage *et al.,* 2007) found no difference in mean residence time between no-till and conventional tillage systems.
Lal (2015) claims that following a “holistic conservation agriculture” approach, and given favourable soil moisture content and temperature, there are examples around the world of increasing gains of SOC sequestration in the whole soil profile. His approach includes: (i) increase SOM inputs (above and below ground such as high-quality plant litter, living roots and root exudates) by including cover crops with legumes in the rotation and leaving their residues as surface mulch, (ii) reduce tillage depth or move to zero tillage practices, as these decreases the rate of decomposition of SOM and increases the rate of protection within aggregates. They also improve the habitat for soil micro and macro-fauna such as earthworms, and iii) improve soil fertility by integrated nutrient management. Following this approach, Gan *et al*., (2014) reported a lower carbon footprint of wheat, taking more CO_2 from the atmosphere than emitted during its production (each kg of wheat grain produced a net soil sequestration of 0.027-0.377 kg CO_2).
Higher SOC stocks in the topsoil not only develop a more productive soil, improving the biological activity and water holding capacity (Lal 2016) but also provides resilience to extreme weather conditions (Haddaway *et al*., 2017). This agrees with Droste *et al*., (2020) who showed that increasing soil carbon provided farmers with more yield stability and more resilient production.

2.8.2. USE OF COVER CROPS AND ROTATION SYSTEMS

Cover crops have been recommended as a sustainable management strategy (especially including them in conservation/ zero tillage systems) to increase SOC sequestration (Bai *et* *al*., 2019; Mazzoncini *et al.,* 2011). Other benefits have also been reported, such as a reduction of N losses via leaching (Abdalla *et al*., 2019) and improving overall soil quality (Chahal and Van Eerd, 2019). However, the evidence relating to yield in the literature is inconclusive, while some authors report an increase in yield on the following crop (Balkcom and Reeves, 2005; Chahal and Van Eerd, 2018), other authors report decreased yields (Nielsen and Vigil *et al*., 2005). In fact, this seems to be dependent of the type of crop, with vegetable crops profit margins increasing, but not for grain and oilseed crops (Chahal *et al*., 2020). Despite the mentioned benefits above, cover crop effects are also dependent on the management practices such as plant species used and quality of their residues, time of planting and termination, duration of the experiment, cropping system and climatic conditions (Tonitto *et al*., 2006).

2.9. KNOWLEDGE GAPS

The importance of understanding soil carbon dynamics and carbon sequestration has gained interest to improve soil health and mitigate climate change (Wiese *et al*., 2021).

Conventional or intensive agriculture has led to depleted SOC due to erosion (e.g., Olsen *et al*., 2016), decomposition (e.g., Haddaway *et al*., 2017), and leaching (e.g., Nakavahali *et al.,* 2020). Practices that minimise soil disruption such as reduced and zero tillage, generally lead to reduced SOM mineralisation. When combined with traffic management systems such as CTF (Vermeulen *et al*., 2010) and low inflation tyre pressure systems, this has the potential to increase crop yields, which absorb more CO_2 through photosynthesis, driving increased biomass production. This can then mean more residues are returned to the soil post-harvest, which may then lead to increased SOM development and long-term C sequestration (Antille *et al*., 2015). There can also be beneficial interactions between tillage and traffic management. For example, where soil compaction is avoided, there is a reduced need for tillage, which improves soil structure and the protection of SOM into stable aggregates, which may otherwise be mineralised (Kraut-Cohen *et al*., 2020; Keller et al., 2022).

As mentioned earlier, the effects of conversion from conventional agriculture practices to conservation/ zero tillage on SOC are not clear from experimental studies worldwide. While some studies showed higher SOC concentrations under zero tillage (Berner *et al*., 2008; Lal, 2004) others show no differences (Baker *et al*., 2007; Haile-Mariam et al., 2008; Powlson *et al*., 2014). There also remains a disparity among global studies on SOC distribution down deeper layers (30-60 cm) of the soil profile, with some authors reporting higher SOC storage in conventional than conservation agriculture (Baker *et al*., 2007), while others found no significant differences (Beare *et al*., 2010; Haddaway *et al*., 2017; Six *et al*., 2002). All these disagreements in SOC sequestration could be attributed to the different management practices used (such as SOM input, fertiliser type and rates, use of cover crops, rotation), the duration of the experiment, the soil sampling frequency and depth, methodological inconsistencies in the measurements used, as well as soil characteristics (such as soil type, compaction, initial C content), climate and vegetation cover (Bai *et al*., 2019; Li *et al*., 2020; Ugarte *et al*., 2014; Virto *et al.,* 2012; Wan *et al*., 2022).

Therefore, there is a need for studies that look at the effect of different tillage systems based on thorough assessments of the soil profile, to be able to have a better understanding of the processes involved in SOC sequestration and how it behaves under all those variables. And because SOC and crop yield can behave differently under all those variables, there is also a need for location-specific long-term studies to be able to provide robust information to farmers and land managers on how ultimately increase crop yield and soil health.

Heavy agricultural machinery has been shown to cause soil compaction (Venkatesh and Shearer, 2021) and research indicates that soil compaction reduces the physical, chemical and biological indicators of soil health (Shaheb, Venkatesh and Shearer, 2021; Frene, Pandley and Castrillo, 2024). These effects can significantly reduce crop yield (Lamande and Schjonning, 2011; Zhang, *et al.,* 2024). However, limited research exists on the long-term effects of different traffic management systems and their interaction with different tillage systems on SOC dynamics and crop yield. Some studies have looked at the effects of CTF on soil carbon and crop yield (Antille *et al*., 2015; Hussein *et al*., 2021: Mouazen and Palmqvist 2015). But there is only one study by Lee *et al*., (1996) in Alabama, that looked at the effects of different wheel traffic and tillage treatments on SOC content. They reported no significant effects on SOC content after 6 years. However, research on this area is limited and therefore significant knowledge gaps still exist.

Carbon sequestration in soils depends to a large degree on the microbial community and its structure. Zero tillage soils on arable land have a greater abundance of fungi, bacteria, arbuscular mycorrhizal fungi and actinobacteria than conventional tillage (Mathew *et al*., 2012). However not many studies have examined the distribution of microbial communities comparing different tillage and/ or traffic systems at depth, therefore more studies are needed to assess the nature of subsoil organic C.

This long-term project on the effect of traffic and tillage has already demonstrated the beneficial effects of reduced traffic and tillage on soil health, including both physical and biological properties, crop yield and farm economics (Godwin et al., 2017; Millington, 2019;

Kaczorowska-Dolowy, 2022). However, data on the long-term effects of the complex interplay between traffic and tillage management systems on SOC dynamics and crop performance is lacking. Therefore, by examining various SOC-related parameters such as SOM and SOC concentrations, bulk densities, POM-C and MAOM-C, $^{13}C/^{12}C$ isotope analysis and other biological carbon indicators, the research sought to provide a comprehensive understanding of how the different management systems affect soil organic carbon cycling.

Numerous studies on SOC rely on single-point measurements throughout the experiment, which might be influenced by various factors (such as seasonal variations, spatial heterogeneity, sampling and analytical errors, etc). To account for temporal fluctuations, this study measured SOC post-harvest for four crop cycles, incorporating time as a factor. The increased sampling enhances the statistical power and data resolution.

This study also extended the long-term agronomic assessment of the project by evaluating not only the management system impacts on winter barley, millet, and spring oat growth and yield, but also provided further insights into yield responses to inter-annual weather variability, crop rotation effects, soil condition, etc.

The findings of this comprehensive investigation will offer valuable insights into SOC dynamics and crop performance under different traffic systems and tillage intensities, potentially leading to informing farmers and policymakers on how to enhance carbon sequestration while maintaining or improving crop yield.

CHAPTER 3 METHODOLOGY

3.1. INTRODUCTION

A long-term traffic and tillage experiment was established in 2011 on the Large Marsh field at Harper Adams University, Shropshire, UK, (*52°46'58.0"N 2°25'43.9"W*) (Fig. 3.1- a) and b)) to understand the different effects that traffic and tillage systems have on soil properties and crop performance. For the last 10 years, it has already demonstrated the beneficial effects of reduced traffic and tillage on soil health, crop yield and economics (Godwin *et al*., 2017; Kaczorowska-Dolowy, 2022).

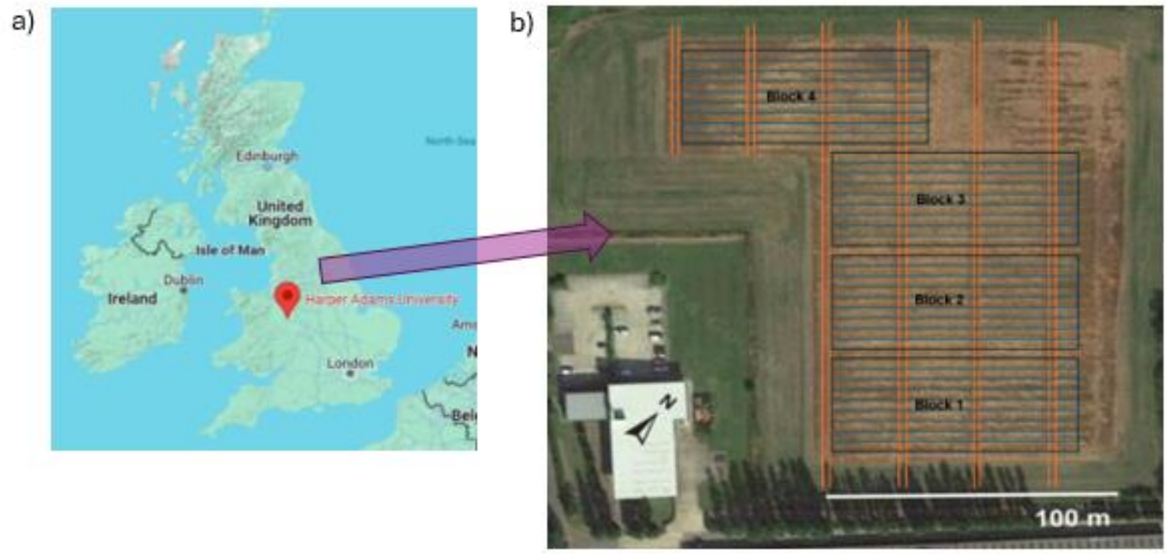


Figure 3. 1 – **a)** Map of the United Kingdom showing the location of Harper Adams University (*source adapted from: Google Maps, 2024*). **b)** Aerial photo of Large Marsh field, at Harper Adams University (source adapted from: Google Earth, 2024).

Large Marsh field was selected for its relative uniformity. The soil is a Claverley (Cl), with small areas of Ollerton (O) and Salwick (Sal) (Bread, 1988) (Fig. 3.2). They are characterised by being slowly permeable soils and seasonally waterlogged (LandIS, 2024).

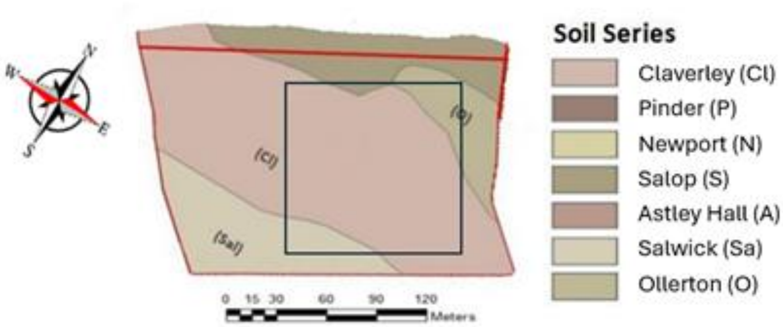


Figure 3. 2 - Soil series distribution for Large Marsh, showing the experimental area (black box) at Harper Adams University (source: adapted from Kristof *et al.,* 2012).

The soil texture is a dark brown slightly stony sandy loam (65% sand; 15% silt; 19% clay) (Soil Survey of England and Wales, 1984) (Fig. 3.3).

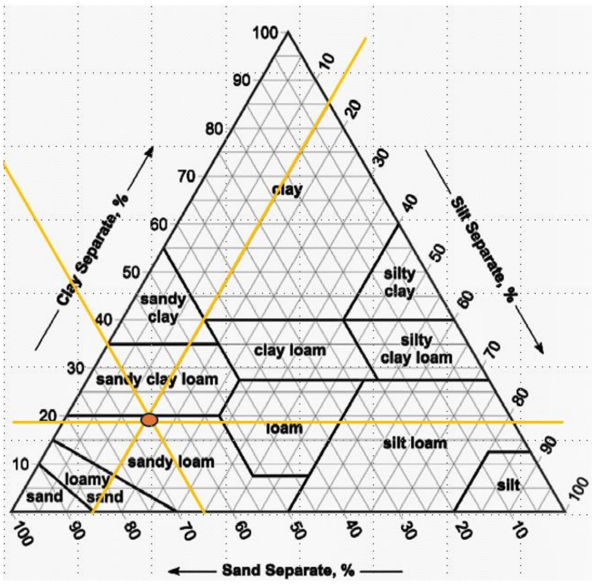


Figure 3. 3 - Soil texture triangle based on the class intervals of the Soil Survey of England and Wales. The orange circle represents the identified texture. (Source adapted from: https://www.nrcs.usda.gov/resources/education-and-teaching-materials/soil-texture-calculator)

The total area of the field experiment is 3.12 ha, which includes the experimental plots and surrounding headlands (Fig. 3.1.b) and Fig. 3.4).

Before initiating the experimental field trial, the field was subjected to conventional agricultural practices with a barley crop during 2008 and 2009, followed by a grass vegetation cover in 2010. To prepare the site for the field experiment, the field was under-drained at 1 m depth with 13 m drain spacing, using gravel backfill, followed by subsoiling operations to a depth of 0.5 m to remove any deep compaction (Smith, *et al*., 2014). Underdrains and subsoiling operations were conducted perpendicular to the treatment plots. After this, the whole field was mouldboard ploughed to allow normalisation, and a winter wheat (*Triticum aestivum var. Duxford*) crop was drilled in 2011. The crop yields were uniform across the experimental area. The site had a topsoil depth of approximately 35 cm, corresponding to the cultivation depth, with a pH of 6.6 and a subsoil pH of 6.1 (Godwin *et al*., 2022).

3.2. EXPERIMENTAL DESIGN

The experiment consists of four replicated blocks, each containing nine plots, for a total of 36 plots set up in a randomised block design. Each plot is 4 m wide x 85 m long, apart from Block 4, which is 4 m wide x 82 m long. To avoid a surface drain inlet, Block 4 was shifted southwest by 24 m (Fig. 3.4). A spare plot separates each block, and spare plots 1-3 have typically been used to set up the tillage and drilling depths on the agricultural machinery. The width of the plots was chosen to be 4 m to keep the experimental field within the uniform soil zone and to match the available machinery. Permanent tramlines were created perpendicular to the plots and at 24 m spacing for the use of all fertiliser and spraying operations to ensure consistency across plots.

The experimental design aimed to simulate the trafficked areas for different tillage farming practices according to the findings of Kroulik *et al*. (2009), who quantified the percentage of total wheeled area during a cropping season for different tillage practices in big cereal farms in the Czech Republic. His findings showed that 85% of the field was covered with at least one wheel pass for traditional mouldboard plough (deep) tillage practices, while for shallow and zero tillage practices, the traffic area was reduced to 65% and 45%, respectively.

To achieve these values, traffic was precisely applied using a Trimble RTK system, following the established protocol designed by Smith (2017) and amended by Millington (2019) (Appendix A3.1)

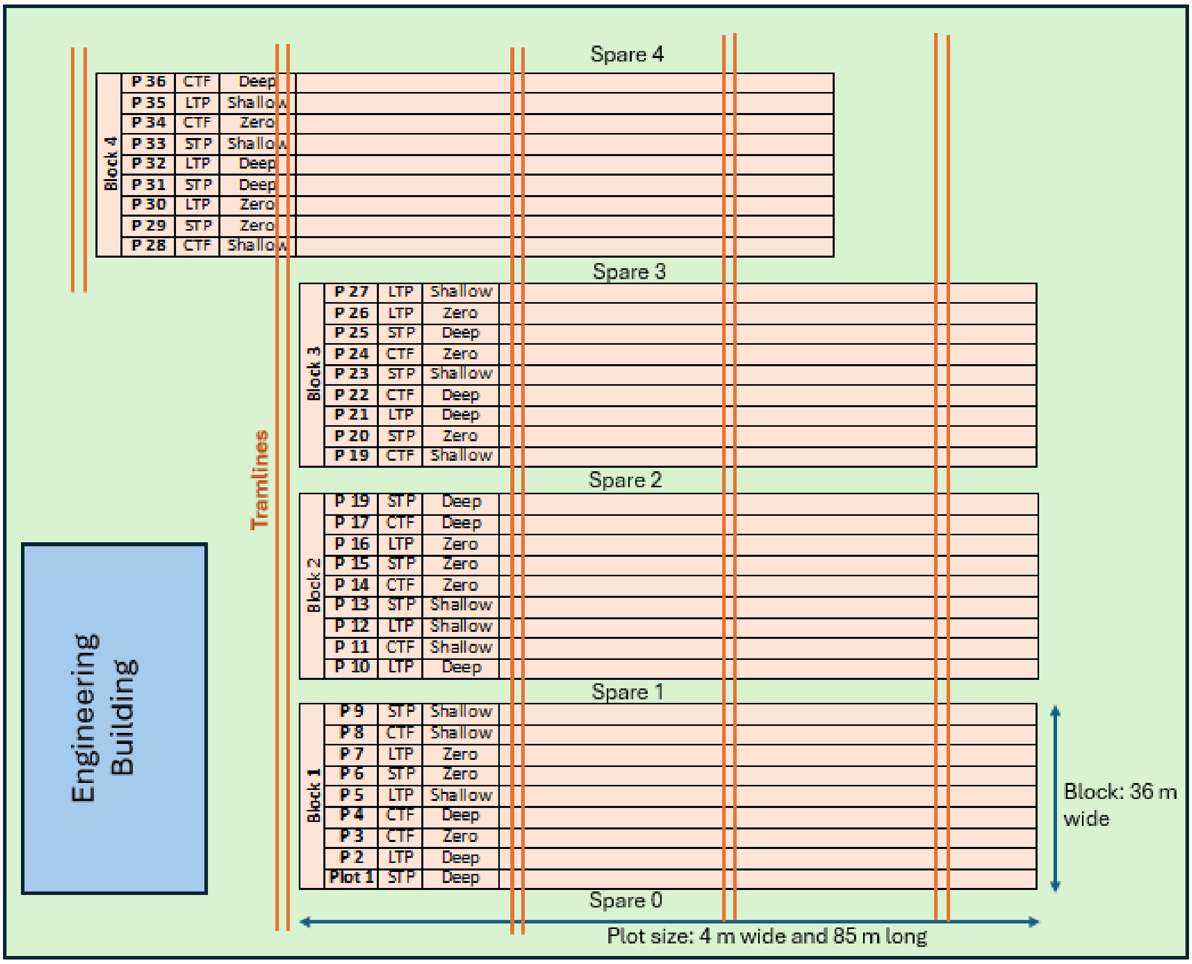


Figure 3. 4 - Experimental design map showing the distribution of the blocks, plots, tramlines and the different traffic and tillage treatments.

To investigate the relationship between three traffic management systems and three tillage depths, a 3 x 3 factorial design was selected for the study. The three traffic management systems were:

i. **STP**: standard inflation pressure tyres (front 1.2 bar, rear 1.5 bar)

ii. **LTP**: low inflation pressure (high flexion) tyres (front and rear 0.7 bar)

iii. **CTF**: controlled traffic farming (front and rear 0.7 bar)

Three tillage treatments were:

i. **Deep** (25 cm)

ii. **Shallow** (10 cm)

iii. **Zero**

In all traffic and tillage systems, the crop residues, such as cereal straw were retained on the soil surface. In Zero tillage systems, discs were used on the surface to break the straw. In Shallow or Deep tillage systems, crop residues were incorporated into the soil down to 10 or 25 cm, respectively.

Further details of the experiment design can be found in Smith *et al*. (2012), Kristof *et al.* (2012), and Smith *et al*. (2014), including further soil characteristics.

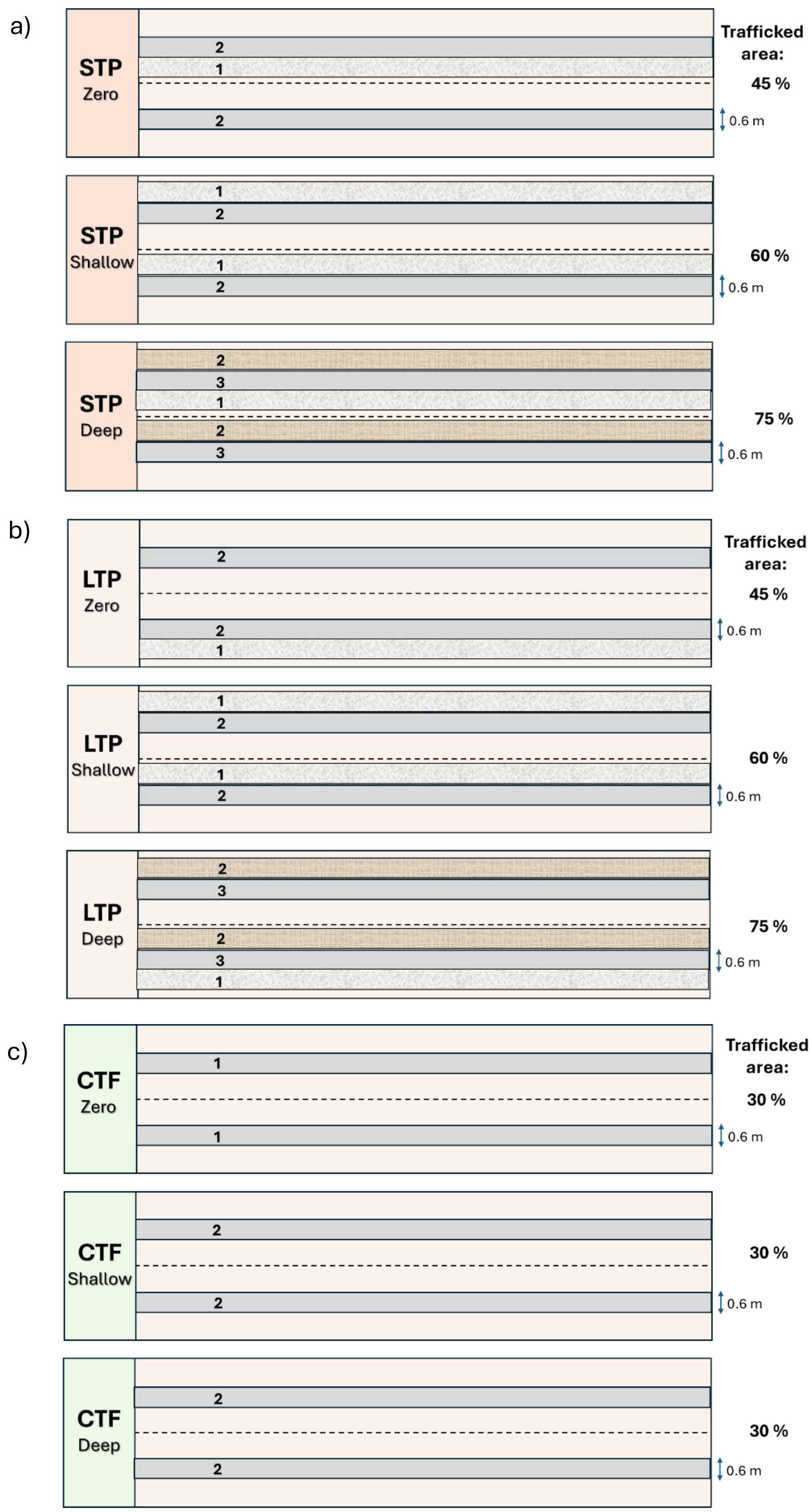
3.3. TRAFFIC (COMPACTION) OPERATIONS

To mimic the random traffic on an arable farm during a cropping season, additional wheel passes were applied at the end of each cropping season using an in-tractor Trimble RTK system. The traffic area for each treatment is based on the findings of Kroulik *et al*. (2009). The traffic area and tractor details are shown in Table 3.1 and Fig. 3.5. The constraints of the 4 m wide plot sizes led to the CTF plots having 30% traffic area (from the tillage and drilling operations, no extra traffic was applied). In comparison, an average farm with a CTF system of 12 m spacing can restrict the traffic area to 13% (Chamen, 2015).

Table 3. 1. Trafficked area after the traffic treatments and tractor characteristics.

Traffic	Tillage	Traffic area
STP and LTP	Deep	75% of the plot
STP and LTP	Shallow	60% of the plot
STP and LTP	Zero	45% of the plot
CTF	Deep, Shallow, Zero	30% of the plot
Tractor	**Additional load**	**Tractor wheels**
290 hp Massey Ferguson 8480 (vehicle mass: 12.55 tonnes, track width: 2.05 m)	1400 kg on rear linkage and 540 kg on the front linkage	Michelin AxioBib tyres (IF 650/85 R38 TL 179D, rear and IF 600/70 R30TL 159D)

More details on the total trafficked area and position and number of wheel passes per plot can be seen in Fig. 3.5.



1Figure 3.5 - Plot layout of the traffic area, position and number of wheel passes of each plot for the different traffic treatments: **a)** STP, **b)** LTP and **c)** CTF with their different tillage treatment interactions and showing the trafficked area for each plot. Permanent traffic wheels in each plot are marked grey, additional traffic wheels are marked with a different texture and colour. Numbers represent the nominal tractor passes. Dash lines represent the middle of the plot (Adapted from: Abell, 2016; Smith, 2012).

3.4. CULTIVATION OPERATIONS

Cultivation treatments were performed by the Massey Ferguson 8480 with an extra load of 1400 kg in the front, coupled with a 4 m Väderstad TopDown cultivator that can be adjusted for Shallow (10 cm) and Deep (25 cm) tillage depth. The cultivator has 14 standard tines at 27 cm spacing and the front discs were set to 5 cm depth (Fig. 3.6 a) and b)).

All tillage and drilling operations were applied using the primary wheel ways. The protocol used can be seen in Appendix A3.2 and A3.3.

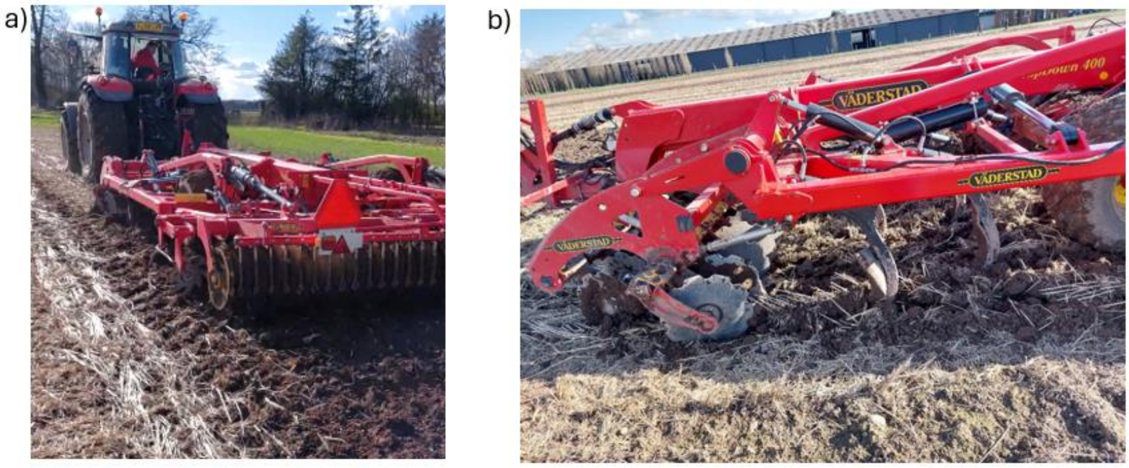


Figure 3. 6 – **a)** Väderstad 4 metre wide Topdown cultivator pulled by the Massey Ferguson 8480 tractor. **b)** closer look of the Väderstad 4 metre wide Topdown cultivator showing the tines and discs. (Source: Author's own)

Protocol modification (cultivation on 07 March 2023, pre-spring oat drilling): all tyre pressures were reduced to 0.7 bar (front and rear) to minimise soil compaction due to previous traffic treatments conducted under high soil moisture conditions.

3.5. DRILLING OPERATIONS

Drilling operations were performed using a Massey Ferguson 8480 with an extra front load of 1400 kg, coupled with a 4 m Väderstad Spirit pneumatic seed drill (Fig. 3.7). The cultivating element was raised for drilling the Zero tillage plots to avoid additional soil disturbance. The Spirit drill has 24 seed coulters, however, to avoid overlapping plots and provide a gap between plots, the outside coulters (1 and 14) were blocked. Wheel track eradicators were lifted out on Zero tillage plots, except for the Spring Oats crop. Dates and details of drilling crops are in Table 3.

Figure 3. 7 – Drilling operations performed with a 4 m Väderstad Spirit pneumatic seed drill. (Source: Author's own)

Before 2015, the cultivation and drilling operations were carried out with a Caterpillar Challenger MT765C tractor, while the compaction treatments were done with the Massey 8480 (290 hp tractor). The Caterpillar Challenger tractor applied lower ground pressures consistently to all traffic treatments (STP, LTP and CTF). In 2015 the Caterpillar was replaced by a Massey Ferguson 8480, with increased flexion tyres (Michelin AxioBib IF 600/70 R30 159D TL on the front and IF 650/85 R38 179D TL on the rear axle), which allowed adjustment of the tyres pressure, having higher tyre pressure on STP treatments and lower on LTP and CTF treatments. In 2021 the tyre pressures were slightly adjusted to better represent random traffic farming operations and the benefit of using low tyre pressures (Table 3.2).

Table 3. 2. Tyre pressures (bar) for the compaction, cultivation and tillage operations from 2015-2024.

	Compaction, cultivation and drilling 2015		**Compaction, cultivation and drilling 2021-2024**	
	Front	Rear	Front	Rear
STP	1.1	0.9	1.2	1.5
LTP	0.8	0.8	0.7	0.7
CTF	0.8	0.8	0.7	0.7

Protocol modification (drilling spring oats on 08 March 2023): the protocol was modified to drill in both directions and save time, however, the wheel eradicators (10 cm) were left by mistake in zero tillage plots.

Apart from the mentioned changes, the compaction, cultivation and drilling protocols have remained consistent since 2011 (Smith, 2017 and Millington, 2019). Further details of the experiment design can be found in (Smith *et al*., 2012; Kristof *et al,* 2012), including further soil characteristics.

Compaction treatments, cultivation and drilling operations were carried out using a Trimble FmX integrated display unit 2018 (GPS technology), which allowed us to find the same AB line (at the centre of each plot).

3.6. CROPS AND VARIETIES

The crop rotation was chosen to follow a typical arable rotation in the UK, with cereals as the main crops and cover crops when possible. A table with all the crops since the beginning of the experiment can be found in “Annexe A3.4”.

This study (2021-2024) focuses on the 9th to 12th cropping cereal seasons with the crop types, dates, fertiliser and spraying applications represented in Table 3.3.

Table 3. 3. Table of crops, dates, fertiliser and spraying applications.

Crop	Variety	Date of Drilling	Seed Rate	Fertiliser	Spraying	Combine Harvest Date
Winter barley	var Belfry (hybrid)	17/10/2020	124 kg ha^{-1} (TGW 49.5) 250 plants m^{-2}	23/03/2021: Pyramid 27N37S. Quantity: 154 kg, area 1,900 ha. 09/04/2021: Pulan 34.5%N	04/08/21: Herbicide (Doxstar fluroxypyr + triclopyr) 2000 litres	27/07/2021
Cover crop	Wynnstay cover crop mix 1 cc1: 80% Black Oats, 15% Vetch, 5% Phacelia	10/08/2021	20 kg ha^{-1}		02/2022: Herbicide (Roundup Flex glyphosate, 781.2 l ha-1). 19/05/2022: Herbicide (Roundup Flex glyphosate, 781.2 l ha-1).	Cover crop was left to decompose
Millet	var White Millet	**1st time:** 24-25/05/22, **2nd time**: 08/07/2022.	**1st time**: 20 Kg ha^{-1} **2nd time**: 30 kg ha^{-1} (TGW 6) 500 plants m^{-2}	16/07/2022: My Premium 33.5, quantity: 435 kg. (30–40 kg ha^{-1})	09/06/2022: Herbicide (Roundup Flex glyphosate) 2.250 Litres, area 4800 ha. 8/10/22: Herbicide (Barclay Gallup Biograde). 3,000 litres. And Adjuvant (X-Clude) 0.250 litres.	31/10/22 crop was mowed and left to decompose
Spring Oats	var Isabel	08/03/2023	160 kg ha^{-1} (TGW 38) 420 plants m^{-2}	01/04/2023: LAT An 33.5%, rate: 119 kg ha^{-1}. 14/04/202: Origin 26-0-0-35, rate: 123 kg ha^{-1}	26/04/2023: Spray (Paramount Max. rate: 25 g ha-1) and (Starane Hi-Load rate: 0.400 lts ha^{-1}). 12/05/2023: Spray (Cyflamid rate: 0.200 lts ha^{-1}) and (Firefly 155 rate: 1000 lts ha^{-1}) and (Trinestar rate: 0.150 lts ha^{-1}. 19/05/2023: Spray (Firefly 155 rate: 1.246 lts ha^{-1}) and (Medax Max rate: 0.250 kgs ha^{-1})	07-08/09/2023
Winter Wheat	var Extase	17/10/23	220 kg ha^{-1} (TGW 56) 400 plants m^{-2}	18/10/2023: Origin 33N 30So3, rate: 183 kg ha^{-1}. 14/04/2024: LAT AN 33.5%, rate: 185 kg ha^{-1}. 16/05/2024: Ammonium Nitrate, rate: 185 kg ha^{-1}.	24/04/2024: Atlantis Star (20011), rate: 0.33 kg ha^{-1}. Biopower (ADJ0617), rate: 1 L ha^{-1}.Micromix Elevate (MBS970), rate: 3 L ha^{-1}. 17/05/2024: YaraTera KRISTA SOP (MBS1215), rate: 4.196 kg ha^{-1}. YaraVita Croplift (MBS095), rate: 4 kg ha^{-1}. 21/06/2024: Prosaro (16732), rate: 0.699 L ha^{-1}. Pixxaro EC (17545), rate: 0.279 L ha^{-1}.	18/09/2024

3.7. COMBINE HARVEST OPERATIONS

Crop harvesting operations were initially executed by a Claas Dominator 85 combine harvester with a 4-m header. However, due to mechanical failure in 2019, the combine was replaced by a New Holland Claydon 8080 combine (4-m header). To quantify the grain yield per plot, an external hopper lifted by a JCB telehandler was used (Fig. 3.8. a) and b). A sample was taken from each plot to measure hectolitre weight and grain moisture content. Grain weight was adjusted to 15% moisture content.

a) b)

Figure 3. 8 – a) Combine harvester working along a plot and b) after each plot, the grain was weighed using an external hopper on a load cell lifted by a JCB telehandler. (Source: Author's own)

For the combine harvest of winter barley 27/07/2021, the combine chopper broke down on plot 12. Therefore, a Massey Ferguson 8480 tractor equipped with the mower attachment (Fig. 3.9) was used to break the straw.

Figure 3. 9 – a) Tractor 290hp Massey Ferguson 8480 with a mower attached working behind the combine harvester along the plot. (Source: Author's own)

The millet crop did not reach physiological maturity suitable for mechanical harvest; consequently, on October 31, 2022, the vegetation was mowed using a Massey Ferguson 8480 tractor equipped with the mower attachment, as shown in Fig. 3.9.

For the combine harvest of spring oats on 08/09/2023, the combine chopper broke down on plot 29. Therefore, a John Deere 7480i self-propelled silage harvester was used to break and distribute the straw (started on plot 28, covering all Block 4), and distribution was okay. In 2024, a mechanical failure prompted the replacement of the combine harvester with a John Deere 1470 model.

Spraying operations were executed in the permanent tramlines (perpendicular to the plots and a 24 m spacing) by an Agrifac sprayer of 14.5 tonnes, with Michelin XEOBIB tyres 710/60R 38, tyre pressure 1 bar.

3.8. WEATHER DATA

The climate in the UK is classified as warm temperate, fully humid, with cool summers and cold winters (Kottek, *et al.,* 2006). The monthly average temperature (°C) and total monthly rainfall data from January 2021 to December 2024, were collected from a meteorological station situated within 500 m of the experimental site. The meteorological data shows that the temperatures of all four years were very similar in range, with maximum temperatures peaking during July for 2021 and 2022, June for 2023 and August for 2024. The average yearly temperature for the three years of this study (2020-2024) was 11°C, 0.6° degree higher than the yearly average for the last 20 years of 10.4°C. On average, the coolest month was January (4.2°C) and the warmest was July (17.5°C). The total annual rainfall for 2021 was 661 mm, followed by 626 mm in 2022, 779 mm in 2023, with the highest of 883 mm in 2024. The average annual rainfall for the years 2021-2024 was 737 mm, which was similar to the mean annual for the last 20 years of 712 mm (Fig. 3. 10 and 3.11).

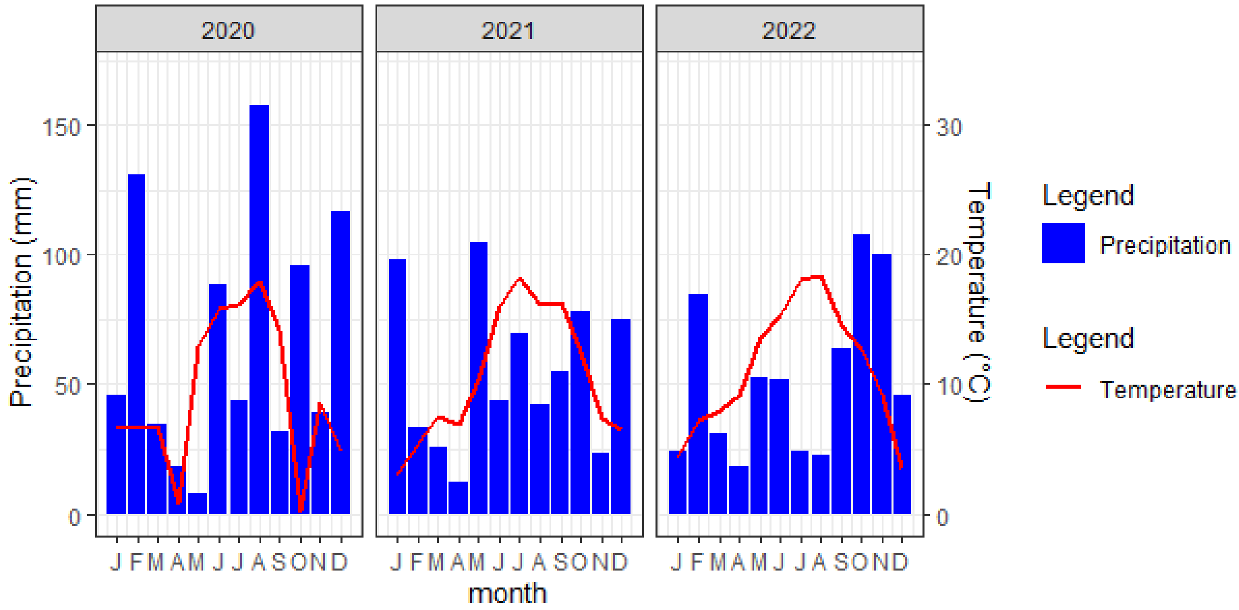


Figure 3. 10 –Total monthly Rainfall and average monthly temperature records for Large Marge at Harper Adams University during this study (2020-2022). Data was obtained from the meteorological station situated at Harper Adams University, UK.

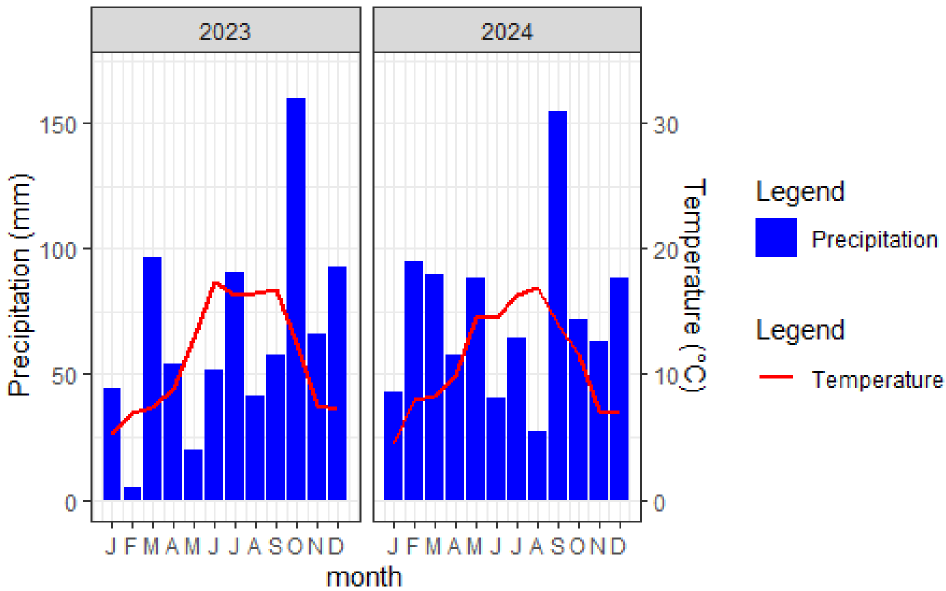


Figure 3. 11 –Total monthly Rainfall and average monthly temperature records for Large Marge at Harper Adams University during this study (2023-2024). Data was obtained from the meteorological station situated at Harper Adams University, UK.

October and November 2022 recorded total precipitation levels of 100 mm, rendering soil conditions unsuitable for drilling our winter crop. As a result, a spring crop was scheduled. October 2023 was one of the wettest months with a total rainfall of 160 mm (avg. rainfall period 2000-20: 66 mm). However, on 17th October, after a window of two days with no rain, the next crop of winter wheat was established.

The summer of 2022 was characterised by a two-week heat wave, marked by an absence of precipitation (Fig. 3.12). Unfortunately, this coincided with the second drilling of millet, which took place on 8th July, just before that two-week heatwave, which affected the emergence stage, producing a very patchy crop.

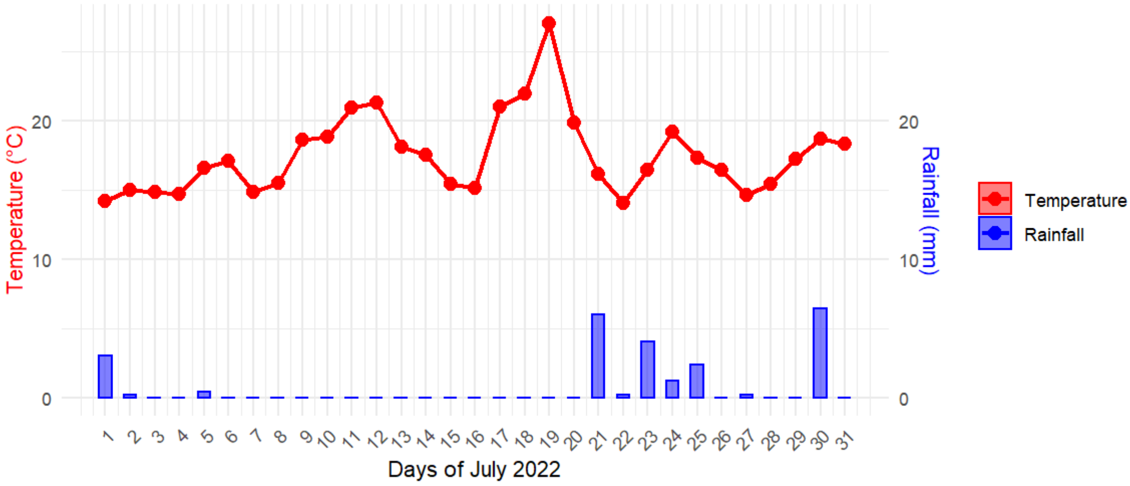


Figure 3. 12 – Average daily temperatures and total daily rainfall record for July 2022 at Large Marge Field (Harper Adams University).

The sum of precipitation for the winter barley crop season (October 2020 – August 2021) was 681 mm, for the Millet crop season (July 2022- Oct 2022) was 218.4 and for spring oats (March 2023- September 2023) was 411.6 mm and winter wheat crop season (October 2023- August 2024) was 824.6 mm.

Monthly total precipitation throughout the study period (2020-24) focused on the main vegetation season for cereal crops (March-July) is displayed in Fig. 3.13. Millet was excluded from this graph because it was planted in July and grew outside of this season.

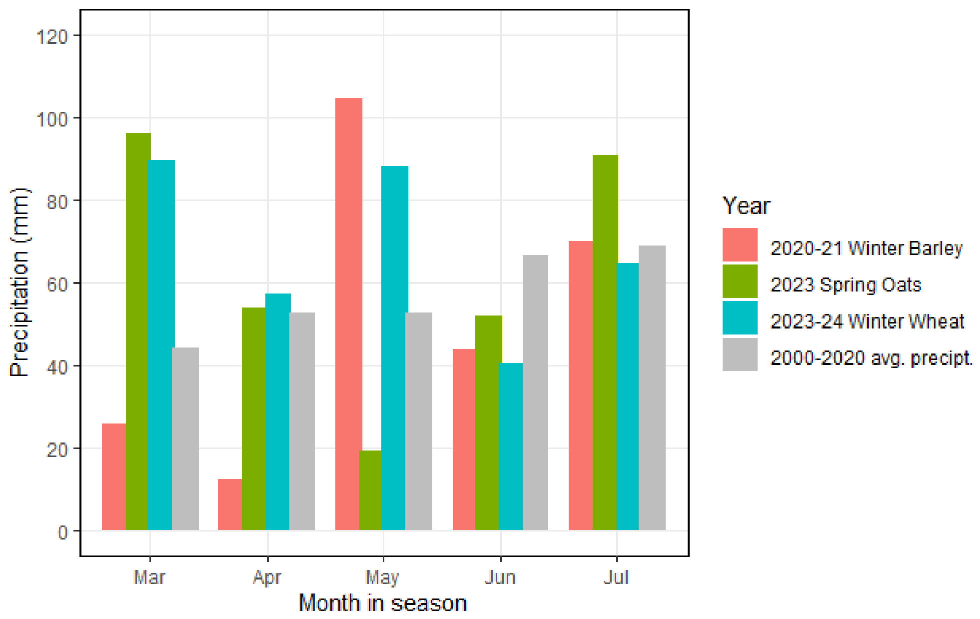


Figure 3. 13 – Total monthly precipitation throughout the assessed period (2020-2024) focused on the main vegetation season (March-July) for different cereal crops. Data was obtained from the meteorological station situated at Harper Adams University, UK.

3.9. SOIL SAMPLING AND PROCESSING

In order to quantify the effect of long-term traffic and tillage systems on SOM and SOC, soil samples were collected four times at the end of each cropping season (from August 2021 to Sept 2023), following the same sampling protocol as previously employed in this long-term field experiment (Kaczorowska-Dolowy, 2022). Briefly, CTF treatments were sampled in the middle of the plot (non-trafficked crop area, between crop rows 11 and 12) and STP and LTP treatments were sampled in the primary wheel way (between crop rows 4 and 5) (Fig. 3.14). This approach was adopted because CTF systems in a more realistic farming scenario maintain 85% non-trafficked field area, whereas the random traffic patterns of LTP and STP systems result in complete field trafficking over time. Soil compaction from trafficking persists long-term, making this distinction methodologically important. These areas enable the assessment of the different trafficked areas under different tyre inflation pressures across the different systems (Kroulik, *et al.,* 2009). Therefore, soil carbon analysis for CTF systems represented only the non-trafficked crop area (70% of the plot area), further referred to as CTF. In contrast, the C analyses for LTP and STP systems were performed in areas of maximum traffic intensity (Fig. 3.14). Future studies could examine both the trafficked and non-trafficked areas of CTF systems to have a more comprehensive view. However, the combine harvest did not allow separation of yield from trafficked versus non-trafficked areas.

Therefore, reported yields represent whole plots, with CTF plots containing 30% trafficked area.

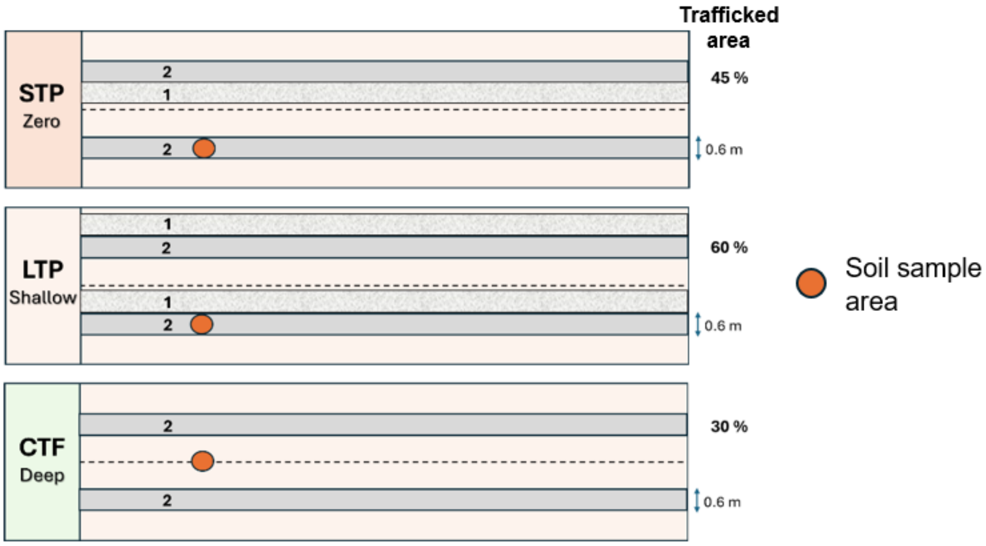


Figure 3. 14 – Plot layout of the three traffic treatments showing the location of soil sample points marked with an orange circle.

3.9.1. SOIL SAMPLING FOR SOIL CARBON ANALYSIS

Two soil cores per experimental plot were collected along the same sampling row using a Duch auger (30 cm depth and 4 cm diameter, Fig. 3.15. a), approximately 2-3 m left of the 2nd and 3rd tramlines. The soil cores were sectioned into 10 cm depth increments, with corresponding depth intervals from multiple cores, homogenised into composite samples for biological analysis. However, during the 4th soil sample collection, a larger soil quantity was needed; hence, three cores were collected per plot to the left of the 2nd, 3rd and 4th tramlines and composited, maintaining the same sample number but with increased volume. Soil samples were stored at 4°C in a press-grip plastic bag until processing. Stones and plant residues were removed from the soil before homogenising the samples by sieving the fresh soil. The first soil sample collection was sieved through a 4 mm mesh, while subsequent collections were sieved through a 2 mm mesh. Post-sieving, subsamples were kept refrigerated at 4°C and subjected to microbial assays within 10 days of collection (Wang, *et al.,* 2021). The remaining soil was air-dried at 30°C until fully desiccated and then stored for further SOC analysis/ quantification. Further information on the SOM and SOC methodology can be found in Chapters 4 and 5.

Deep tillage was implemented to a depth of 25 cm. Soil sampling depth was down to 30 cm to account for potential effects of the different traffic and tillage operations on SOC accumulation and bulk density parameters beyond the direct tillage zone. This study focused on the topsoil (0-30 cm) because the effects of the different management practices are most notable here (Thomas *et al*., 2020), despite acknowledged impacts on SOC dynamics in deeper horizons (Gregory *et al*., 2016).

3.9.2. SOIL SAMPLING FOR DRY BULK DENSITY (BD) ANALYSIS

The soil sampling for BD was also performed at the end of each cropping season, following the same sampling protocol as explained above. Except for the 1st soil sample collection, when the samples were collected after the combine harvest of winter barley, the compaction treatments and the drilling of the cover crop, which all happened in a very short time at the end of July and beginning of August 2021. This added some extra soil compaction compared to the rest of the soil sample collections.

Soil bulk densities were determined in all plots, over the whole depth profile using a 30 cm Dutch auger (Fig. 3.15 a)) for the 1st soil sample collection and a 30 cm Royal Eijkelkamp auger with a liner (Fig.3.15 b)) -that can take undisturbed soil samples-, for the rest of the soil sample collections. For each soil sample collection, one sample per plot was collected following the sample strategy already mentioned. Sampling was conducted at 2-3 meters away from the first tramline, moving a bit further in subsequent collections.

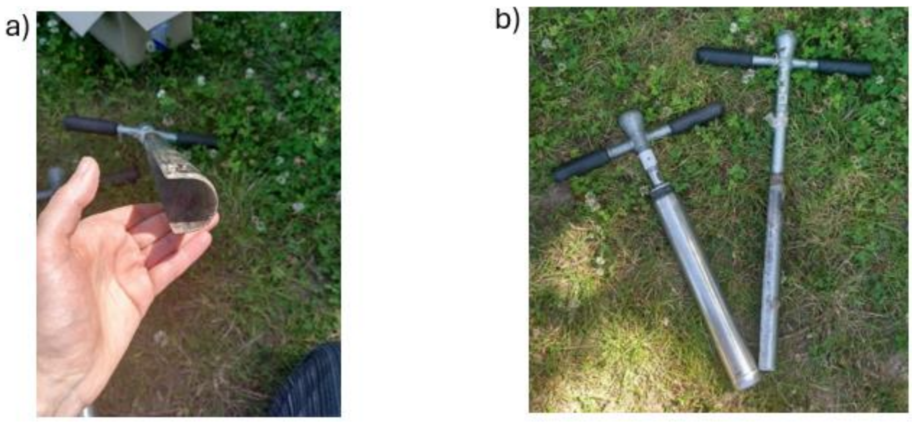


Figure 3. 15 – **a)** Duch auger with open core (30 cm) and **b)** on the left is the Royal Eijkelkamp auger with liners (30 cm) and on the right is the Duch.

Samples were stored at 4°C until further analysis. Soil samples were taken at three depth increments (0-10, 10-20 and 20-30 cm), resulting in 432 samples (36 points x 3 depths x 4 times) for bulk density and soil C analysis. Further information on bulk density methodology can be found in Chapter 5.

A timeline of field operations and soil sampling collections can be seen in Fig. 3.16.

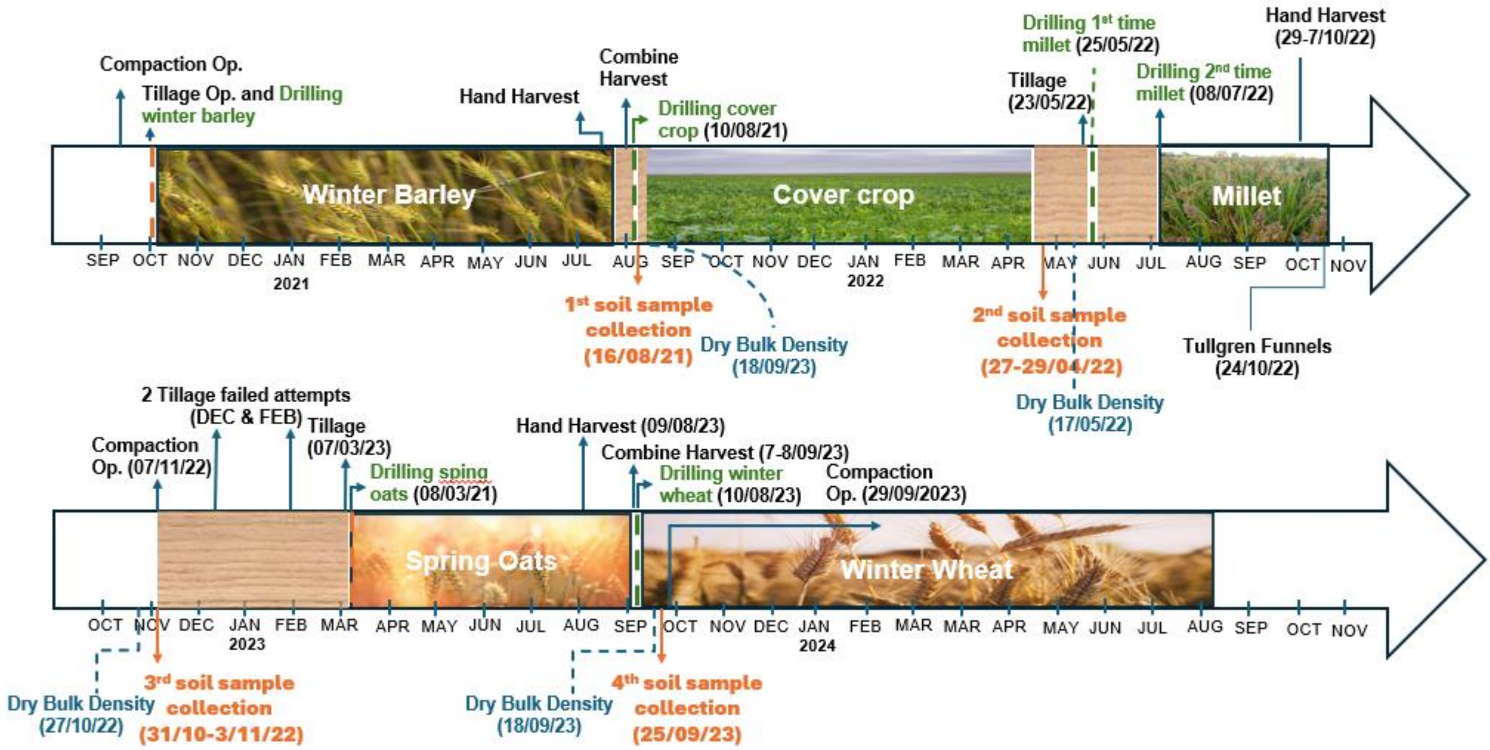


Figure 3.16 – Timeline of field operations and sampling.

3.10. STATISTICAL ANALYSIS

Statistical analyses were performed using the RStudio software (R version 4.0.5, 2021). A linear mixed-effect model was used with soil traffic and tillage treatments as fixed factors and block and collection as random factors (lmer package). When soil depth was included, it was used as a fixed factor. When looking at the effect of traffic, tillage or traffic-tillage interaction over time, collection was included as a fixed factor. Analysis of variance (ANOVA) and *Post-hoc* test for significant differences of means were carried out with Tukey's test with 95% confidence, using the *package nlme (*Pinheiro *et al.,* 2019).

CHAPTER 4

THE EFFECT OF TRAFFIC AND TILLAGE MANAGEMENT SYSTEMS ON SOIL ORGANIC MATTER

4.1. INTRODUCTION

SOM is a crucial indicator of soil health. SOM is responsive to external changes such as the environment and the management practices imposed, especially in agro-ecosystems (Janzen *et al*., 1997). It plays an important role in many physical, chemical and biological properties, such as improving soil structure, aggregation and compaction, allowing better aeration, water infiltration and water holding capacity (Esmaeilzadeh and Ahangar, 2014). It increases nutrient cycling and fertility, fuelling the entire soil food web system (Cobo *et al*., 2002; Strawn and Sparks *et al*., 2000). It also improves ecosystem functioning, such as improving water availability and quality and reducing the risk of soil erosion (Weil and Brady, 2017). Despite comprising a small percentage of most soils, SOM has a profound influence on soil function and health. SOM and soil health are integral to sustainable agriculture, defined as maintaining continuous food production without environmental degradation (Tahat *et al*., 2020).

Heavy agricultural machinery causes soil compaction (Hamza and Anderson, 2005; Shaheb, Venkatesh and Shearer, 2021) and soil organic matter depletion (Mikha *et al*., 2013). Numerous studies have shown that soil compaction reduces the physical, chemical and biological indicators of soil health (Frene, Pandley and Castrillo, 2024; Shaheb, Venkatesh and Shearer, 2021). It reduces the pore space, limiting oxygen and water infiltration and diffusion, as well as the living space for soil organisms and plant roots (Batey, 2009; Willatt, 1986). As a result, plant growth, yield and quality are also reduced (Batey, 2009; Lamande and Schjonning, 2011; Zhang *et al.,* 2024). In some cases, crop yields can be reduced as much as 50% (Shaheb, Venkatesh and Shearer *et al.,* 2021).

Soil compaction can also have negative effects, such as increasing fuel consumption, working hours, and abrasion of agricultural instruments, increasing mechanical operations costs (Hamza and Anderson, 2005; Horn *et al*., 1995; Soane and van Ouwerkerk, 1993). It can also exacerbate negative environmental effects, such as pollutants (nitrates and pesticides) runoff into water courses (Soane and Van Ouwerkerk, 1995). Soil compaction may also increase atmospheric greenhouse gas emissions (i.e. CO_2, CH_4 and N_2O), contributing to climate change (Horn *et al.,* 1995).

Together with other unsustainable cultivation practices such as high levels of tillage, extensive use of monocultures and removal of the crop biomass, soil compaction has led to the loss of SOM (Sanderman *et al.,* 2017) with some UK soils losing 40-60% of their organic carbon (Environment Agency, 2024; Bellamy *et al*., 2005).

SOC is a component of soil organic matter (SOM), usually 50 to 60% by mass (Pribyl, 2010). It has gained prominence in recent years owing to increasing interest in the use of soils for carbon sequestration to mitigate climate change. It has often been converted from SOM using a pedotransfer function to provide an estimate of SOC concentrations by using measured SOM concentrations (van Bemmelen, 1891). And although it has been suggested for decades, that care is needed when applying this pedotransfer function (e.g. Howard and Howard, 1990), it was confirmed in 2010 as being unreliable across a wide range of soils (Pribyl, 2010). Therefore, to gain insights into both SOM as an indicator of soil health (this chapter) and SOC as a metric of soil carbon storage (Chapter 5), it is necessary to measure both.

Soil compaction can exert contrasting effects on SOM. On the one hand, it decreases SOM mineralisation rate, potentially leading to higher organic matter accumulation (Neve and Hofman, 2000; Ziyaee and Roshan, 2012; Nawaz, *et al*., *2013*). But it also reduces the microbial transformations and microbial growth which leads to less stabilised SOM. On the other hand, reduced OM inputs into the soil due to decreased crop productivity, combined with increased erosion and run-off, reduce SOM concentrations with potential negative consequences for soil health.

Increasing SOM in cropland can be achieved in two main ways: (1) increasing organic matter inputs to soil, and (2) using management practices that slow the rate of SOM decomposition. Despite the existence of management strategies to implement these two principles, for example, by using cover crops and increasing crop diversity, Controlled Traffic Farming (CTF) systems, tyres with low inflation pressure and conservation agricultural practices such as reduced tillage (Zhang *et al.,* 2024), farmer adoption in the UK and Europe is still limited. A recent survey in the UK revealed that about 30% of UK farmers apply some of the principles of conservation agriculture (Jaworski *et al*., 2024).

The effects of traffic intensity and the interaction with different tillage systems on SOM dynamics remain poorly understood. In particular, significant knowledge gaps remain regarding the long-term effects of alternative traffic systems and their interactions with different tillage practices on SOM dynamics.

Microbial biomass carbon (MBC) refers to the carbon contained in the microbial populations living in the soil (typically comprises 1-4% of SOM) (Khoshru *et al.,* 2023). How MBC responds to the different traffic and tillage management practices may be important for understanding SOM dynamics, therefore, this will be further explored in this Chapter.

4.2. AIM AND HYPOTHESES

This chapter aims to quantify the effects of alternative traffic systems and their interaction with different tillage systems on SOM dynamics and MBC in a long-term field experiment over the last three years (2021-2023).

The hypotheses for this chapter are:

1. Soil compaction from agricultural vehicles has a negative effect on SOM. Therefore, reduced traffic and wheel pressure will lead to higher (depth-specific) SOM concentrations and MBC content.
2. Soil disturbance by tillage increases SOM decomposition. Therefore, reduced tillage will lead to higher (depth-specific) SOM concentrations and MBC content.
3. The interaction between traffic and tillage systems will impact SOM content and MBC at different depths.

4.3. METHODOLOGY

SOM concentration was quantified by loss-on-ignition (Sutherland, 1998) and SOC (%) was quantified using the dry combustion method (Bertsch and Ostinelli, 2019) (Chapter 5). These methods have been recognised as “best practice” (e.g. Hoogsteen *et al.*, 2015; Schumacher, 2002) when studying the SOM and SOC concentrations in soil.

For a detailed account of the sampling strategy, the number of samples taken, and the methods used for the soil extraction, please refer to Chapter 3. Methodology. 3.9. Soil sampling and processing.

4.3.1 LABORATORY ANALYSIS

4.3.1.1 SOIL ORGANIC MATTER CONCENTRATION

Soil organic matter content was determined by the loss on ignition method (Zhang and Wang, 2014), which measures the weight change associated with high-temperature oxidation of organic matter. Fresh samples were sieved to 4 mm for the first soil sample collection (16/08/2021). However, for subsequent soil sample collections, they were sieved to 2 mm. After sieving, the samples were oven-dried overnight at 105ºC, and the dry mass was recorded. Samples were then placed in a furnace at 550ºC for 4 hours before being cooled in a desiccator and weighed. The percentage mass loss is reported as soil organic matter content. The results include the analysis of four soil sample collections: 16/08/21 after winter barley crop, 29/04/22 after winter cover crop, 03/11/23 after millet and 25/09/23 after spring oats.

4.3.1.2 MICROBIAL BIOMASS CARBON (MBC)

MBC was determined using the chloroform fumigation extraction method (Brookes, 2001; Vance *et al.*, 1987). From each composite sample, duplicate 10 g fresh soil subsamples were processed. One subsample underwent chloroform fumigation with 48-hour dark incubation, followed by extraction of both fumigated and non-fumigated controls with 25 ml of 0.5 M K_2SO_4. Samples were shaken for 1 hour and filtered (Figure 4.1. a) and b).

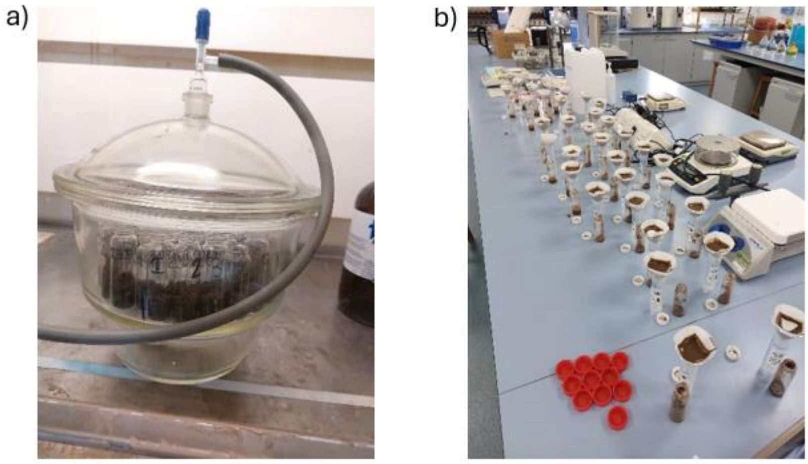


Figure 4. 1 – Sample processing for MBC analysis. **a)** Chloroform fumigation of 10 g soil samples. **b)** Filtration of soil extracts following the addition of 25 ml of 0.5 M K_2SO_4 and 1-hour agitation for subsequent TOC analysis.

The extracts were immediately frozen for subsequent transport to be analysed at Cranfield University. Samples were diluted 5 times prior to analysis using a Shimadzu TOC-L analyser (UK). MBC was calculated as the difference between fumigated and non-fumigated carbon values following equation 4.1:

$$MBC = (F - nF)/K$$

Equation 4.1

Where:

F = fumigated sample,
nF = non-fumigated sample,
K = constant (K = 0.45) (Jenkinson, Brookes and Powlson, 2004).

MBC analyses for collections 3 and 4 were performed via chloroform fumigation at Harper Adams University, with TOC quantification conducted at Cranfield University. Data from collections 1 and 2 were not finalised due to the malfunction of Harper Adams's TOC analyser.

4.4. RESULTS

4.4.1 EFFECTS OF TRAFFIC AND TILLAGE ON SOM

SOM decreased significantly with depth ($p < 0.001$) from 4.63% at 0-10 cm to 3.98% at 10-20 cm and 3.39% at 20-30 cm. The 0-10 cm layer stored 14.03% more SOM than the 10-20 cm layer, which in turn stored 14.82% more than the 20-30 cm layer.

A. SOM AT 0-10 CM DEPTH

The main effect of traffic ($p < 0.001$), tillage ($p < 0.001$) and the interaction between traffic and tillage ($p = 0.001$) on SOM concentrations were all statistically significant.

Within the traffic systems, CTF (4.77%, CV = 5.36%) had significantly higher concentrations of SOM compared to LTP (4.59%, CV = 5.78%) and STP (4.52%, CV = 6.68%). CTF contained a 3.7% higher concentration of SOM compared to LTP and 5.2% higher than STP (Fig. 4. 1).

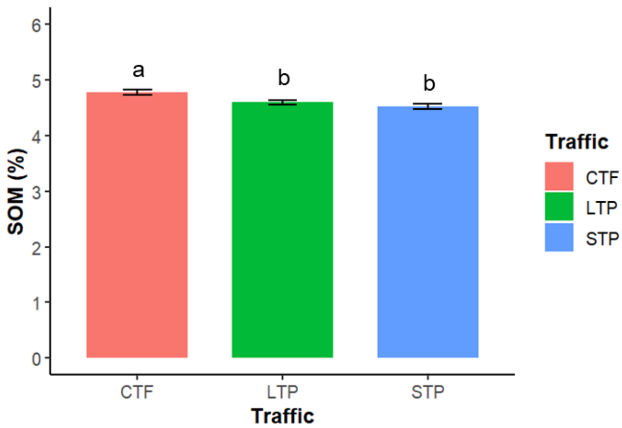


Figure 4. 1 – Main effects of the different traffic systems on SOM (%) at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

Within tillage systems, Zero (4.68%, CV = 8.31%) and Shallow (4.72%, CV = 5.23%) tillage had significantly higher concentrations of SOM compared to Deep (4.48%, CV = 4.27%) tillage. Zero and Shallow tillage systems contained a 4.6% higher concentration of SOM than the Deep tillage treatment (Fig. 4. 2).

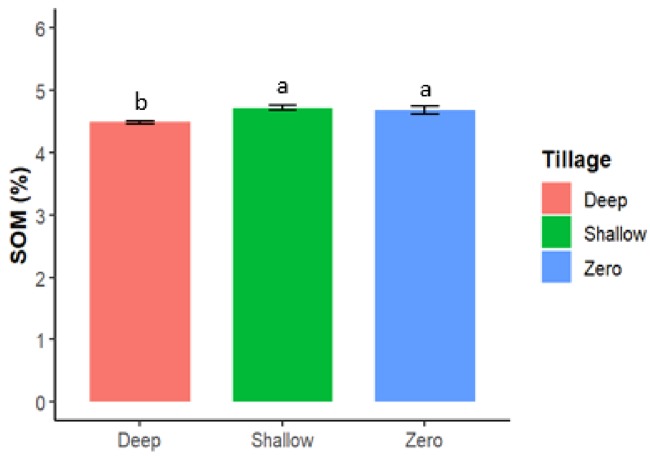


Figure 4. 2 – Main effects of the different tillage systems on SOM concentration at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 48). Bars show standard errors.

There was a significant interaction between the traffic and tillage treatments. CTF Zero (4.97%, CV = 7.49%) and CTF Shallow (4.84%, CV = 5.03%) had the highest concentrations of SOM, which were significantly higher compared to the other treatment combinations. LTP Shallow (4.75%, CV = 4.71%) had significantly higher concentrations of SOM than LTP Deep (4.49%, CV = 5.12%) and STP Deep (4.45%, CV = 4.15%) treatment combinations (Fig. 4. 3).

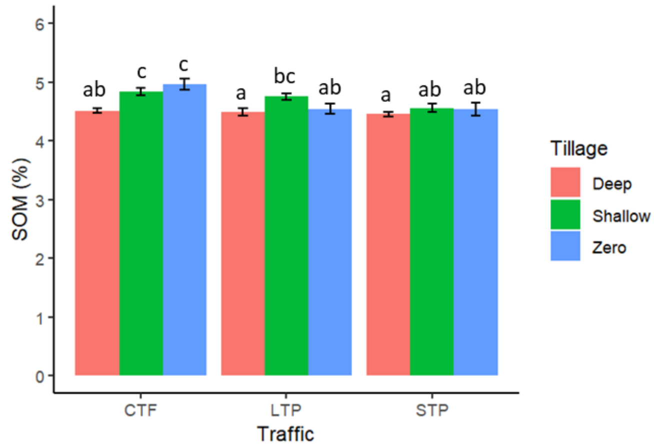


Figure 4. 3 – Main effects of the interaction between traffic and tillage systems on SOM concentration at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 16). Bars show standard errors.

A.1 SOM AT 0-10 CM OVER TIME

The effects of traffic over time: the main effect of traffic ($p < 0.001$) and collection date ($p < 0.001$) led to significant differences in SOM concentration within the top depth of soil (0–10 cm). However, the interaction between traffic and collection date ($p = 0.13$) was not statistically significant.

Within the traffic systems, the observed results were the same as the main traffic effects as above (Fig. 4. 1).

When comparing traffic effects across collection dates, the collection on 16/08/2021 (4.79%, CV = 7.50%) had significantly higher SOM concentrations when compared to the collection on 29/04/2022 (4.52%, CV = 5.59%) and 25/09/2023 (4.55%, CV = 5.62%) (Fig. 4. 4). Collection on 03/11/2022 (4.64%, CV = 6.23%) was not significantly different from Collection 16/08/2021.

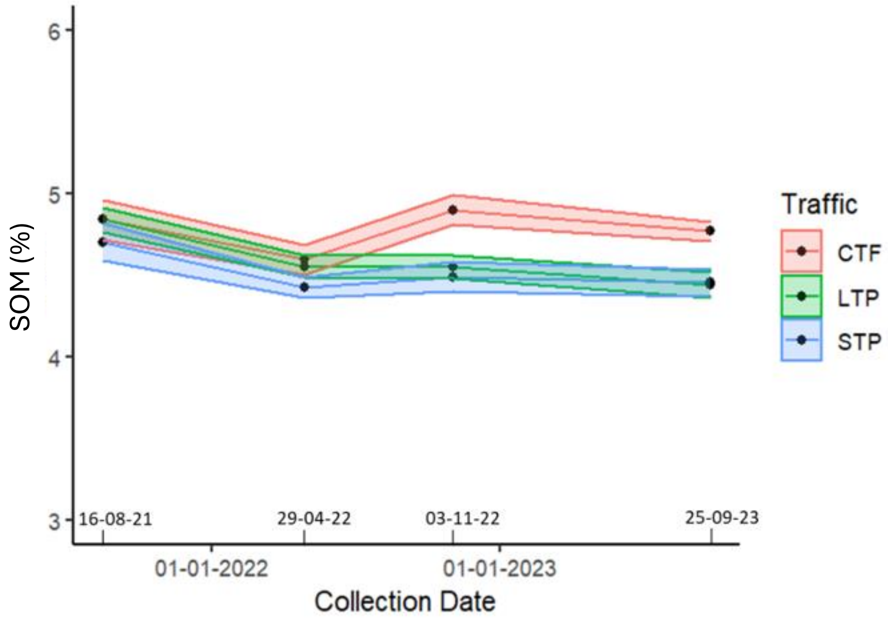


Figure 4. 4 – Main effects of the traffic systems on SOM over time, at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between Traffic systems and Collection dates was not statistically significant (p = 0.13).

The effects of tillage over time: at 0-10 cm both tillage (p < 0.001) and collection date (p < 0.001) had significant effects on SOM concentrations, but the interaction between tillage and collection was not statistically significant (p = 0.87) (Fig. 4. 5)

Within the tillage systems, the results were the same as with the main tillage effects as above (Fig. 4.2).

When comparing tillage effects across different collection dates, the observed results are the same as above.

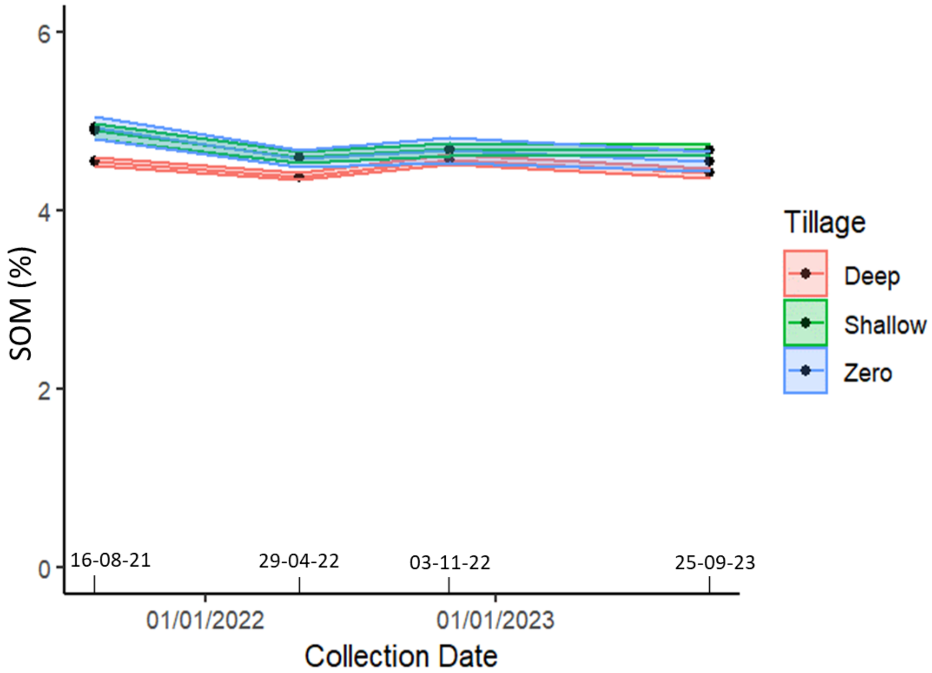


Figure 4. 5 – Main effects of the interaction between the tillage treatments on SOM over time, at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between Tillage systems and collection dates was not significant ($p = 0.87$).

The interaction between traffic and tillage over time: at 0-10 cm there was a significant traffic-tillage interaction ($p < 0.001$) and significant change over time (i.e. collection date; $p < 0.001$). However, the interaction between traffic-tillage and collection date ($p = 0.33$) was not statistically significant (Fig. 4. 6).

Across the collection dates, the sample collection on 16/08/2021 (4.79%, CV = 5.94) had significantly average higher concentrations of SOM than all the other collections.

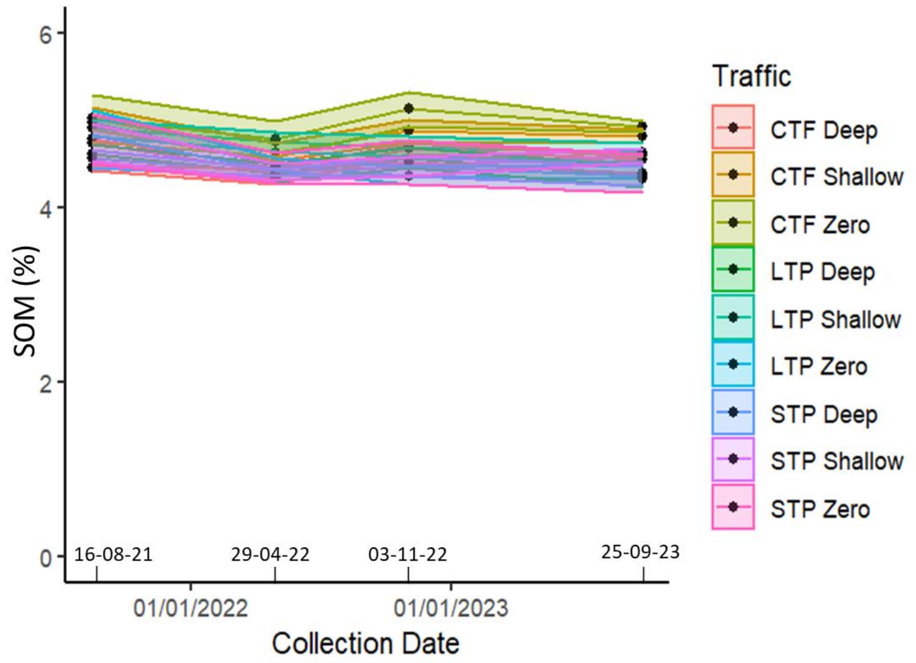


Figure 4. 6 – Main effects of the interaction between traffic and tillage treatments over time on SOM, at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic-tillage and collection dates was not significant ($p = 0.33$).

B. SOM AT 10-20 CM DEPTH

There were no statistically significant changes in SOM concentrations at 10-20 cm depth (traffic $p = 0.49$; tillage $p = 0.77$; the interaction between traffic and tillage $p = 0.58$) (Fig. 4. 7).

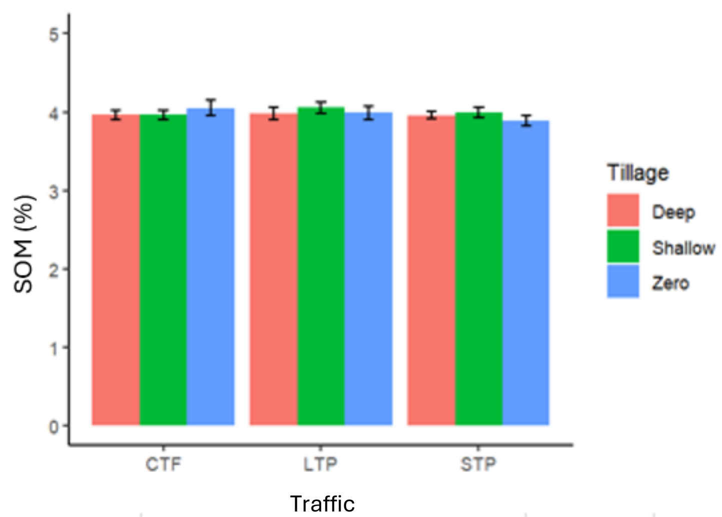


Figure 4. 7 – Main effects of the interaction between traffic and tillage systems on SOM (%) at 10-20 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 16). Bars show standard errors.

B.1. SOM AT 10-20 CM OVER TIME

The effects of traffic over time: significant differences in SOM concentrations were observed at 10-20 cm across collection dates (p = 0.03). Traffic (p = 0.47) and interaction between traffic and collection date (p = 0.21) were not statistically significant (Fig. 4. 8).

Within the collection dates, the collection on 16/08/2021 (4.08%, CV = 7.33%) had significantly higher concentration of SOM when compared to the collection on 29/04/2022 (3.90%, CV = 7.11%).

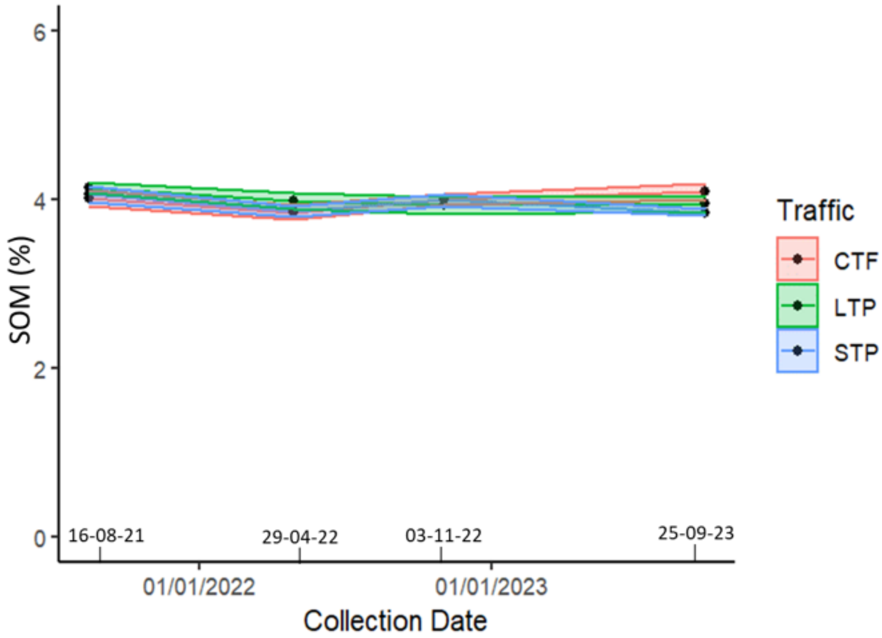


Figure 4. 8 – Main effects of the traffic treatments on SOM over time, at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between traffic systems and collection dates was not significant (p = 0.21).

The effects of tillage over time: significant differences in SOM were observed at 10-20 cm across collection dates (p = 0.04). However, tillage treatment (p = 0.77) and interaction between tillage and collection date was not statistically significant (p = 0.74) (Fig. 4. 9).

Within the collection dates, the observed results were the same as above (traffic x soil sample collection).

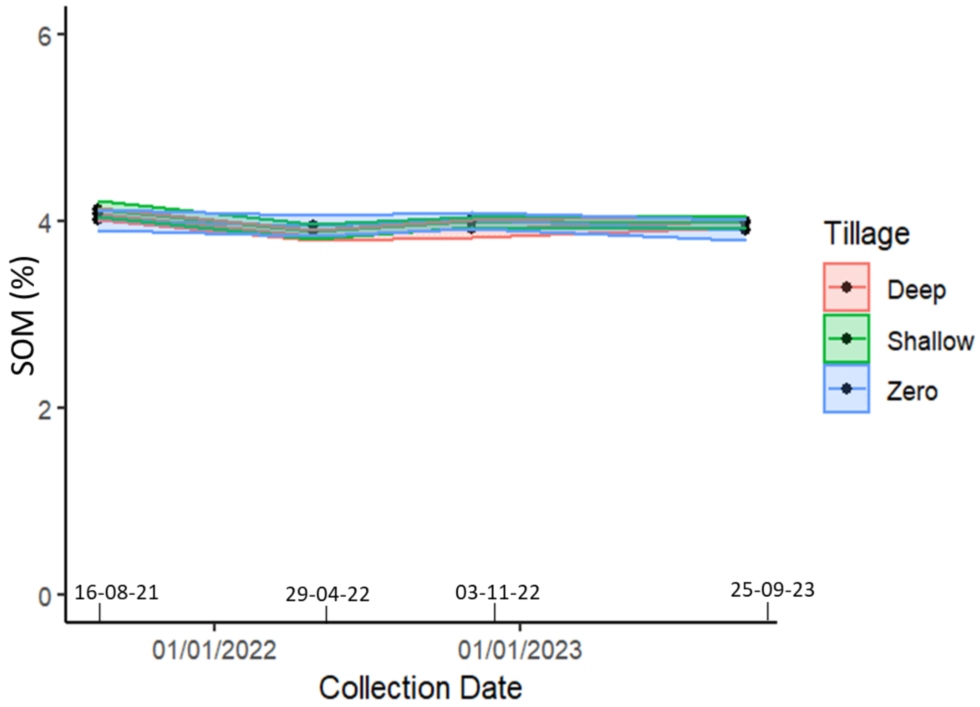


Figure 4. 9 – Main effects of the tillage treatments on SOM over time at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between tillage systems and collection dates was not significant (p = 0.74).

The effects of the traffic-tillage interaction over time: statistically significant differences were observed at 10-20 cm across collection dates (p = 0.05), but traffic-tillage (p = 0.81) and traffic-tillage and collection date interactions (p = 0.57) were not statistically significant (Fig. 4. 10).

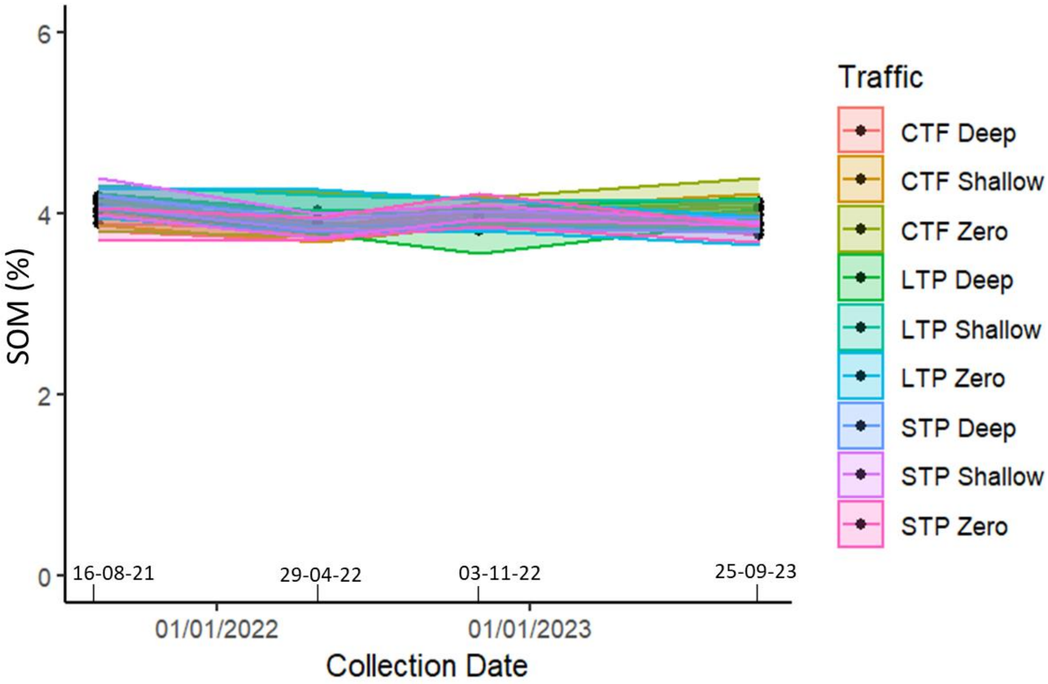


Figure 4. 10 - Main effects of SOM for three traffic systems combined with three tillage systems over four soil sample collections at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection dates was not significant (p = 0.81).

C. SOM (%) AT 20-30 CM DEPTH

Significant differences in SOM concentration were observed at 20-30 cm for tillage (p = 0.016) and the interaction between traffic and tillage (p = 0.006). Traffic did not have a statistically significant effect on SOM concentration at this depth (p = 0.49).

Within tillage systems, SOM concentration in Zero (3.45%, CV=7.82%) tillage was significantly higher when compared to Deep (3.31%, CV=8.45%) tillage systems. Zero tillage exhibited 4.05% higher concentrations of SOM than Deep tillage systems at this depth (Fig. 4. 11).

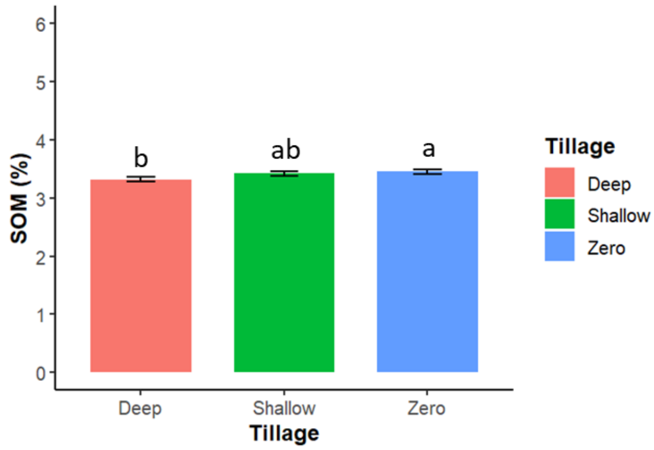


Figure 4. 11 – Effect of the different tillage systems on SOM at 20-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

Within the interactions between traffic and tillage treatments, CTF Zero (3.55%, CV = 6.62%) tillage had significantly higher concentration of SOM when compared to CTF Deep (3.29%, CV = 7.07%) and STP Deep (3.21%, CV = 7.42%) (Fig. 4. 12).

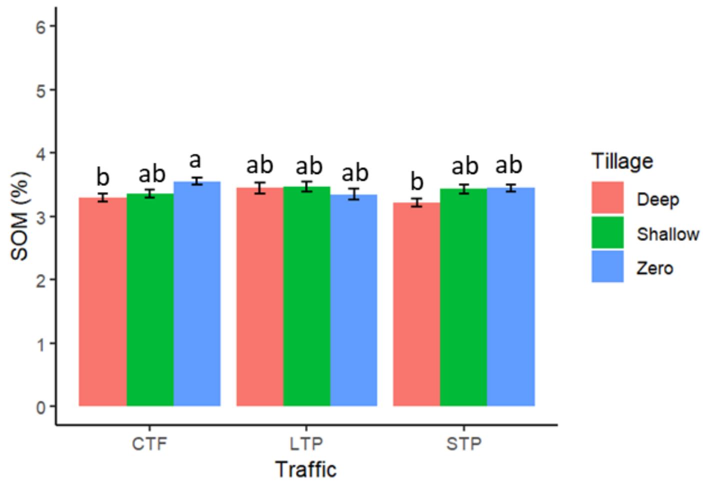


Figure 4. 12 – Effect of the interaction between traffic and tillage systems on SOM at 20-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 16). Bars show standard errors.

C.1 SOM (%) AT 20-30 CM OVER TIME

The effects of traffic over time: Significant differences in SOM concentration were observed at 20-30 cm across the Collection date ($p < 0.001$). The traffic ($p = 0.52$) and interaction between traffic and collection were not statistically significant (p = 0.17) (Fig. 4. 13).

Across the collection dates, the collection on 25/09/2023 had significantly higher concentrations of SOM (3.55%, CV = 6.43%) compared to the collection on 29/04/2022 (3.19%, CV = 7.18%) and 03/11/2023 (3.35%, CV = 7.02%). And the collections on 16/08/2021 (3.48%, CV = 9.15%) and 03/11/2023 (3.35%, CV = 7.02%) had significantly higher SOM concentrations compared to 29/04/2023 (3.19%, CV = 7.18%).

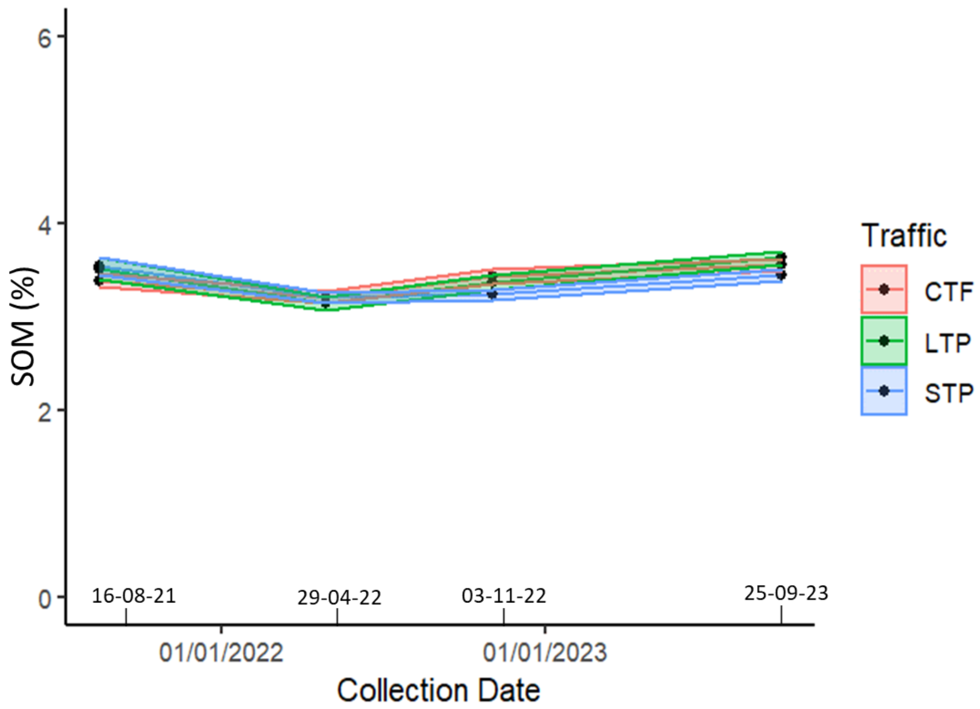


Figure 4. 13 – Main effects of the traffic treatments on SOM over time at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between traffic and collection date was not statistically significant (p = 0.17).

The effects of tillage over time: statistically significant differences in SOM concentrations were observed at 20-30 cm for tillage treatments (p = 0.02) and collection date (p < 0.001). However, the interaction between tillage and collection date was not statistically significant (p = 0.38) (Fig. 4. 14).

Within the tillage treatments, Zero tillage (3.45%, CV = 7.34%) had significantly higher SOM concentrations when compared to Deep (3.31%, CV = 7.88%) tillage treatments (i.e. the same main effects as above). Across the collection dates, the observed results were the same as above for traffic.

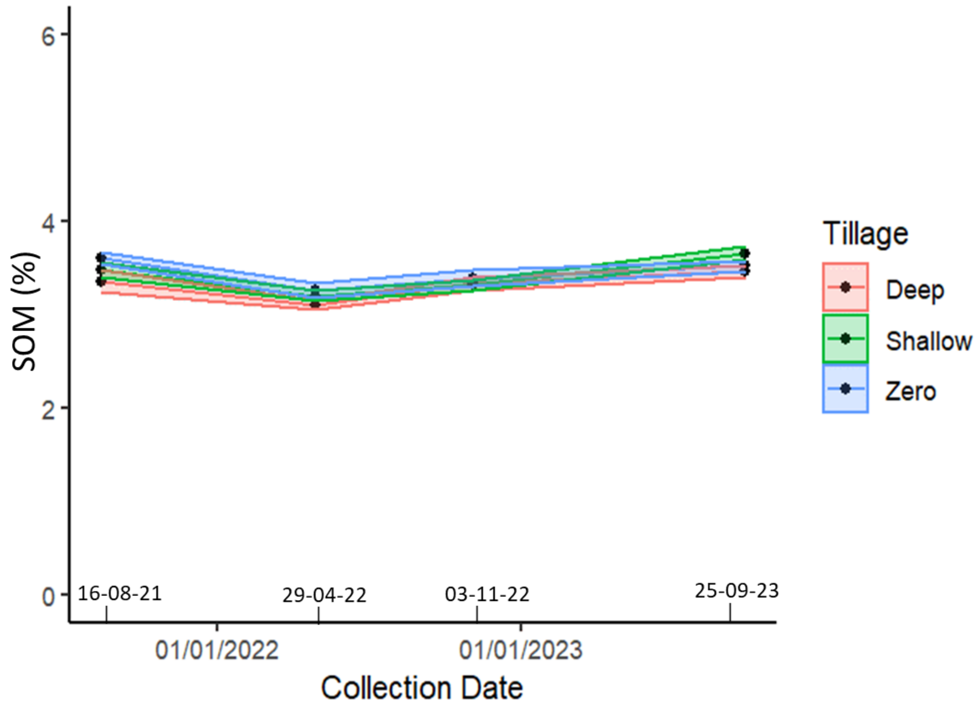


Figure 4. 14 – Main effects of the tillage treatments on SOM over time at 20-30 cm depth. Lines show means (n=12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between tillage and collection was not statistically significant (p = 0.38).

The effects of the traffic-tillage interaction over time: the interaction between traffic and tillage was statistically significant at 20–30 cm depth (p = 0.001). There were significant changes in SOM concentration across collection date (p < 0.001). However, the interaction between traffic and tillage and collection date was not statistically significant (p = 0.10) (Fig. 4. 15).

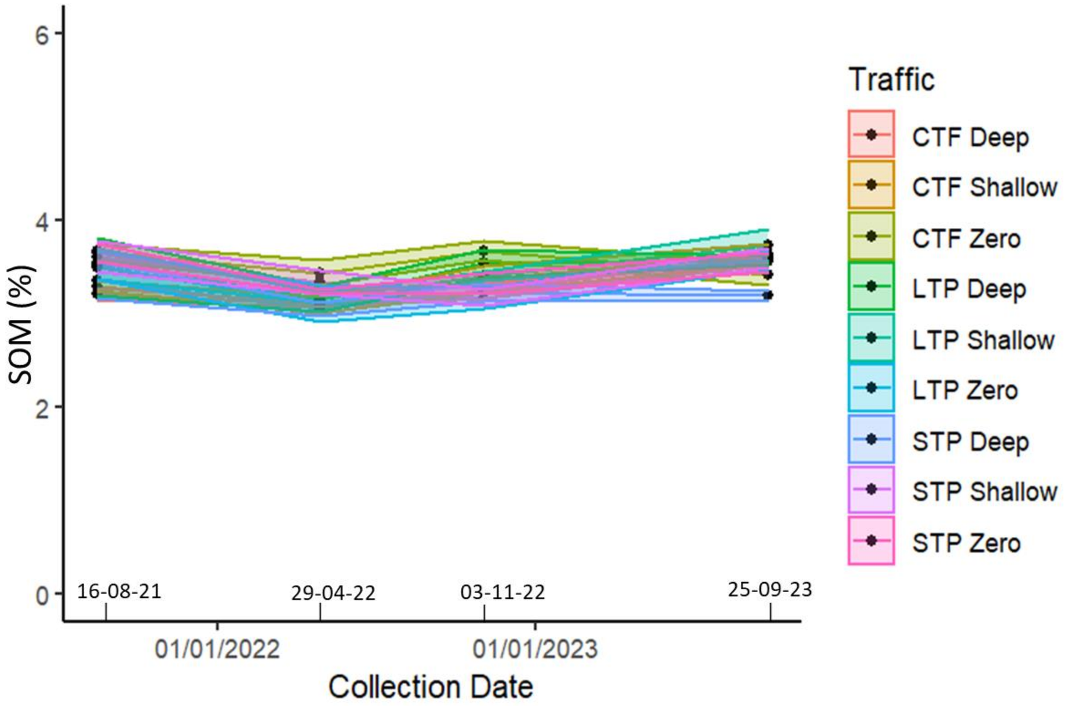


Figure 4. 15 – Main effects of the traffic-tillage interaction on SOM over time at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between traffic and tillage and collection date was not statistically significant ($p = 0.10$).

D. SOM (%) AT 0-30 CM DEPTH

The main effects of traffic ($p < 0.001$), tillage ($p < 0.001$) and depth ($p < 0.001$) are all significant. And the interaction effects between traffic and tillage ($p < 0.001$), traffic and depth ($p < 0.001$) and tillage and depth ($p = 0.02$) were also significant. However, the interaction effect of traffic, tillage and depth ($p = 0.15$) was not significant.

Within the traffic systems at 0-30 cm, CTF (4.05%, CV = 15.53%) had significantly higher SOM concentrations than STP (3.94%, CV = 13.96%). CTF systems had 2.71% more SOM than STP systems (Fig. 4. 16).

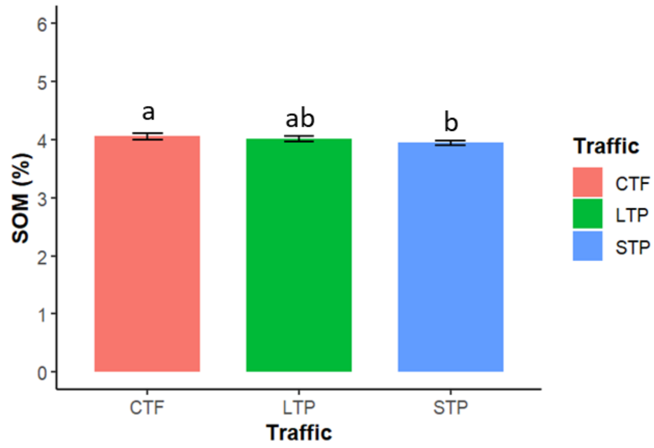


Figure 4. 16 – Effect of the different traffic systems on SOM at 0-30 cm depth. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 144). Bars show standard errors.

Within the tillage systems at 0-30 cm, Zero (4.04%, CV=15.17%) and Shallow (4.05%, CV = 14.81%) tillage systems had significantly higher SOM concentration than Deep (3.92%, CV = 13.81%) tillage systems. Zero and Shallow tillage systems had 2.97% and 3.20% more SOM, respectively, than Deep tillage systems (Fig. 4. 17).

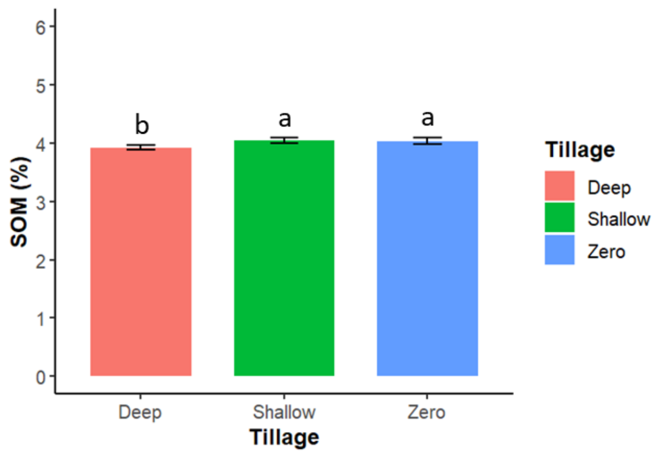


Figure 4. 17 – Effect of the different tillage systems on SOM at 0-30 cm depth. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 144). Bars show standard errors.

Within the different soil depth layers, SOM was significantly greater at 0-10 cm depth (4.63%, CV = 5.94%) than at 10-20 cm depth (3.98%, CV = 7.12%), which was, in turn, significantly greater than at 20-30 cm depth (3.39%, CV = 8.24%) (Fig. 4. 18).

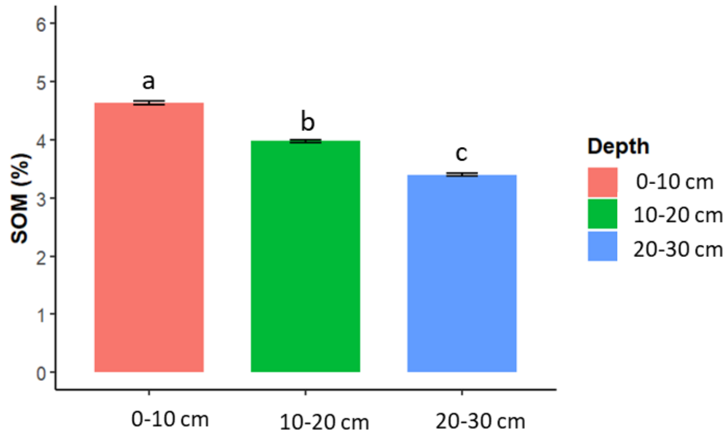


Figure 4. 18 – Effect of the different tillage systems on SOM at 0-30 cm depth. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 144). Bars show standard errors.

Within the traffic and tillage interaction at 0-30 cm, CTF Zero tillage (4.19%, CV = 16.29%) was significantly higher (6.4% more) than the rest of the treatment combinations, except for LTP Shallow and CTF Shallow tillage. LTP Shallow (4.09%, CV = 14.55%) and CTF Shallow (4.05%, CV = 16.41%) were significantly higher than STP Deep (3.87%, CV = 14.25%). And CTF Shallow (4.05%, CV = 16.41%) was significantly higher than CTF Deep (3.92%, CV = 13.88%) (Fig. 4. 19).

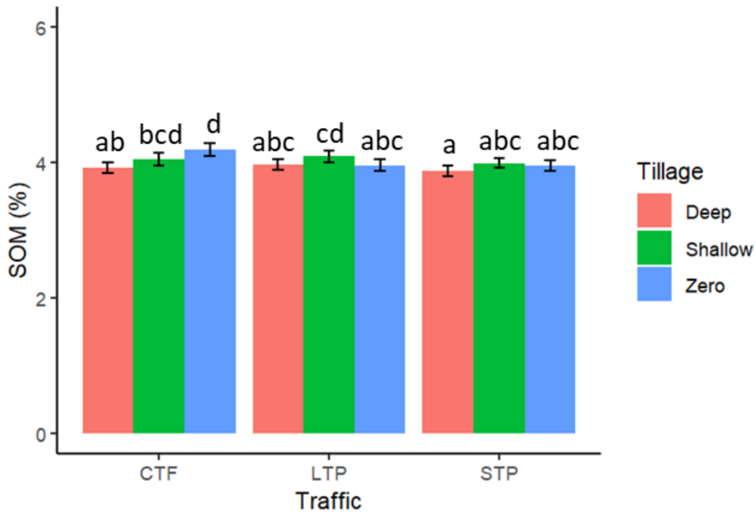


Figure 4. 19 – Main effects of the interaction between traffic and tillage systems on SOM (%) at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 48). Bars show standard errors.

Within the traffic and depth interaction at 0-30 cm, CTF (4.77%, CV = 6.91%) at 0-10 cm was significantly higher than the rest of the traffic systems and depth layers. LTP (4.59%, CV = 6.27%) and STP (4.52%, CV = 7.06%) at 0-10 cm were significantly higher than the rest of the traffic systems and depth layers. CTF (3.99%, CV = 7.57%), LTP (4.00%, CV = 7.76%) and STP (3.94%, CV = 6.19%) at 10-20 cm were significantly higher than all the traffic systems at 20-30 cm (Fig. 4. 20).

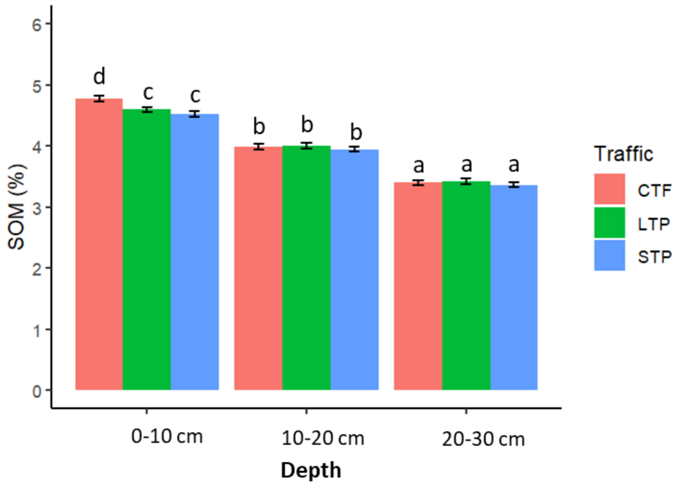


Figure 4. 20 – Main effects of the interaction between traffic systems and depth on SOM (%) at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 48). Bars show standard errors.

Within the tillage and depth interaction at 0-30 cm, Shallow (4.72%, CV = 5.71%) and Zero (4.68%, CV = 9.24%) tillage systems at 0-10 cm were significantly higher than the rest of the tillage systems and depth combinations. Deep (4.48%, CV = 4.26%) tillage at 0-10 cm was significantly higher than the rest of the tillage systems and depth combinations. Zero (3.97%, CV = 8.76%), Shallow (4.00%, CV = 6.53%) and Deep (3.96%, CV = 6.16%) tillage systems at 10-20 cm were significantly higher than the tillage systems at 20-30 cm (Fig. 4. 21).

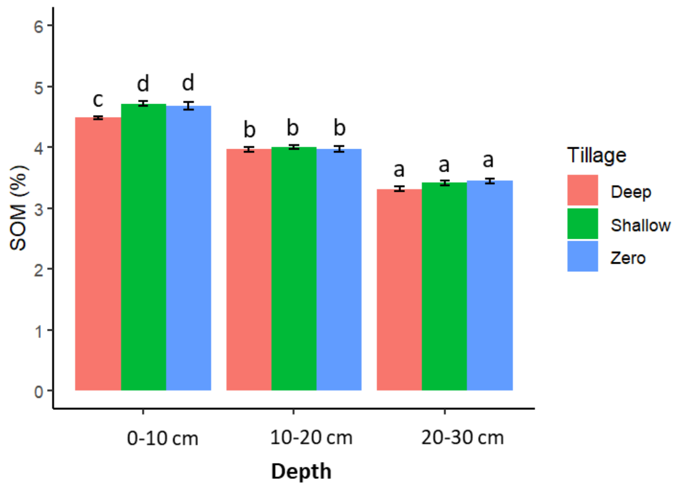


Figure 4. 21 – Main effects of the interaction between tillage systems and depth on SOM (%) at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

4.4.2 EFFECTS OF TRAFFIC AND TILLAGE ON MBC AND ITS RELATION TO SOM CONCENTRATIONS

The results for the third soil sample collection (03/11/2022) showed that only the interaction effect between traffic and tillage systems was statistically significant at 0-10 cm ($p = 0.01$). However, the *Post hoc* analysis revealed only borderline non-significant differences between treatments (data in Appendix A4.2). At 0 – 30 cm only the main effect of depth was significant ($p < 0.001$) with MBC decreasing down the soil profile 0-10, 10-20 and 20-30 cm (310.2 ug/g, CV = 16.5%, 278.3 ug/g, CV = 17.8%, and 204.4 ug/g, CV = 19.5%, respectively).

The results for the fourth soil sample collection (25/09/2022) (analysed only for the 0-10 cm depth interval) showed that there were no statistically significant differences between treatments (traffic $p = 0.19$, tillage $p = 0.39$, traffic and tillage $p = 0.22$) (Results in Appendix 4.3).

The linear regression of MBC as a function of SOM at 0-10 cm (including collections 3 and 4) showed R = 0.49, indicating a moderate positive correlation and $R^2 = 0.24$, meaning that 24% of the variation in MBC was explained by SOM (%) and ($p < 0.001$), indicating that the relationship was significant and moderately strong, but there are also other influencing factors (Fig. 4. 22).

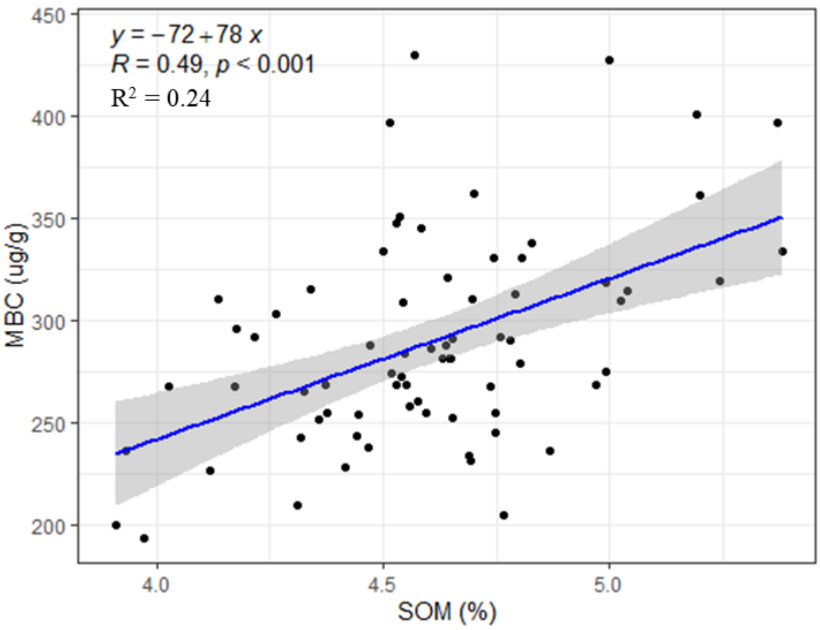


Figure 4. 22 – Simple linear regression for the relationship between SOM and MBC in the surface layer (0-10 cm) across different traffic and tillage systems for the third and fourth soil sample collection (03/11/22 and 25/09/23) in a sandy loam soil (n = 8). The regression equation, line of best fit, regression coefficients (R^2), and statistically significant (*p* value) are shown.

At 10-20 cm the linear regression of MBC as a function of SOM for collection 3, showed R = 0.48, R^2 = 0.23 and p = 0.003, indicating a moderate positive correlation with a significant relationship between the variables (Fig. 4. 23).

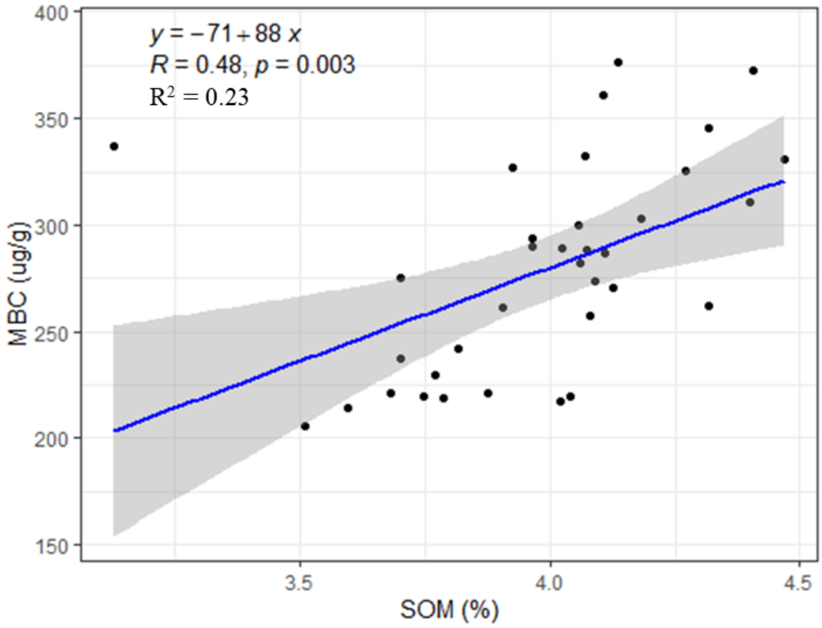


Figure 4. 23 – Simple linear regression for the relationship between SOM and MBC in the surface layer (10-20 cm) across different traffic and tillage systems for the third soil sample collection (03/11/22) in a sandy loam soil (n = 4). The regression equation, line of best fit, regression coefficients (R^2), and statistically significant (*p* value) are shown.

At 20-30 cm, the linear regression of MBC as a function of SOM for collection 3, showed R = 0.44, R^2 = 0.19 and p = 0.007, indicating a moderate positive correlation with a significant relationship between the variables (Fig. 4. 24).

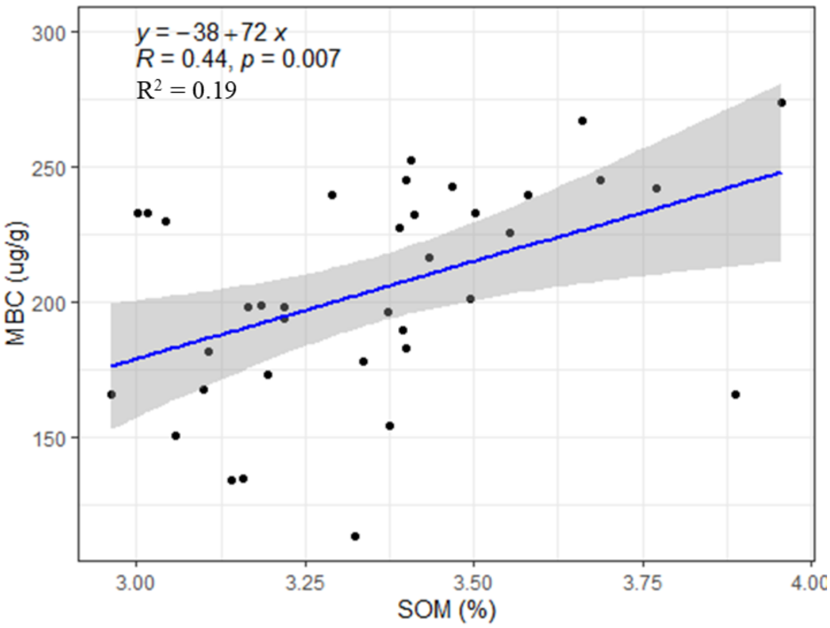


Figure 4. 24 – Simple linear regression for the relationship between SOM and MBC in the surface layer (20-30 cm) across different traffic and tillage systems for the third soil sample collection (03/11/22) in a sandy loam soil (n = 4). The regression equation, line of best fit, regression coefficients (R^2), and statistically significant (*p* value) are shown.

4.5. DISCUSSION

The field sampled in this study has been under the same management practices for the last 12 years. This study focuses on the data collected from 2021- 2023, including 4 soil sample collections. Crop data collection extended to 2024 – Chapter 8; however, soil sampling was not conducted that year as it fell outside the designated study period. Key drivers of SOM levels include C inputs, N fertilisation, vegetation cover, climatic conditions, topography and soil type (Bot and Benites, 2005; Hobley *et al*., 2015; Stcokman *et al*., 2023). Since all these factors were the same across all of the treatments, observed differences in SOM content can be attributed solely to the different traffic and tillage management practices imposed.

Different tillage intensities imposed in cropland can affect SOM dynamics (Haddaway *et al*., 2017), however, the knowledge regarding the effects of different traffic systems and their interaction with different tillage management systems on SOM dynamics remains poorly understood. Understanding these effects is crucial for optimising management practices that maximise SOM building and retention for soil health.

4.5.1. SOM CONCENTRATIONS DOWN THE SOIL PROFILE

The average SOM concentration at 0-10 cm was 4.63%, which is considered a medium-high level for arable soil with a sandy loam texture as in this study (Fento, Albers and Ketterings, 2008). Clay soils are better at protecting organic matter, while sand soils have inherently low SOM due to a reduced protection function. Loamy soils lie somewhere in between, depending on their composition and management practices (Brady, 2008).

Our results showed that SOM concentration decreased with soil depth under all management systems. Continuous cultivation and fertilisation over the last 12 years have resulted in higher SOM concentration in the top layer (4.63% at 0-10 cm) compared to the deeper soil layers (3.39% at 20-30 cm). This is in agreement with other studies (Antony *et al*., 2022; Berhane *et al*., 2020; Petaja *et al*., 2024; Tautges *et al*., 2019) who also reported that the highest SOM concentrations are predominantly observed in surface layers.

4.5.1.1 SOM AT 0-10 CM

4.5.1.1.2 MAIN EFFECTS OF TRAFFIC

At 0-10 cm, CTF stored significantly more SOM than LTP and STP systems. This indicates that the absence of traffic compaction increased the ability of the soil to store SOM, compared to the more compacted soil from the LTP and STP systems. Other studies have shown that CTF systems can develop an improved soil structure, enhancing soil porosity (Guenette *et al*., 2019) and soil biology (Kaczorowska-Dolowy, 2022) with improved SOM levels (Vermeulen *et al*., 2010). The soil pore structure influences many critical soil properties and processes, including water storage and biogeochemical cycling (Rabot *et al*., 2018). It also regulates microbial accessibility to SOM, consequently affecting its decomposition rate, and influencing long-term SOM stabilisation (Dungait *et al*., 2012). The mechanisms associated with this are discussed in more detail in Chapter 6 (POM and MAOM fractions).

Sampling points in the CTF systems represented the non-trafficked crop area of the field. Farmers can typically limit traffic to 12-15% of the field area, leaving approximately 85% non-trafficked. Conversely, the sampling points in LTP and STP systems represented the trafficked area of the field, which had different traffic intensities depending on their interaction with the tillage system (for a detailed account, refer to Chapter 3 Methodology, 3.3. Traffic operations and 3.9. Soil sampling and processing).

At this soil depth (i.e. 0 – 10 cm), LTP systems did not affect SOM concentrations compared to STP systems. Other studies have found that LTP systems had lower bulk density and greater porosity relative to STP systems (Shaheb *et al*., 2024), which can accelerate SOM decomposition (Meurer *et al*., 2020). Although not significant, after 12 years LTP systems stored 1.55% more concentration of SOM than STP systems (0-10 cm). Multiple studies on this site (Kaczorowska-Dolowy, 2022; Millington, 2019; current study, 2021-2023 Chapter 5), reported no significant difference in BD between LTP and STP systems in each soil layer investigated. The absence of BD differences may explain why LTP and STP systems did not affect SOM concentrations at this depth.

4.5.1.1.3 MAIN EFFECTS OF TILLAGE

At 0-10 cm, Zero and Shallow tillage systems stored significantly more SOM (4.91% more) than Deep tillage systems, indicating that SOM increased by decreasing tillage intensity, which provided further evidence for the stratifying effect of reduced tillage on SOM concentrations with depth over time (Kushwa *et al*., 2016). Jacak *et al*. (2023) also reported an increase of SOM in the 0-10 cm layer under reduced tillage intensity. Many studies have found this effect and attribute the SOM increase in the 0-10 cm under reduced tillage to two main factors: minimised redistribution of SOM across the soil profile and reduced aggregate breakage, which slows SOM decomposition rates (e.g. West and Post, 2002; Machado, Sohi and Gaunt, 2003).

Paustian *et al.* (1997) suggested that the higher C content of reduced tillage systems could be due to the increase of SOM concentrated on the surface, more than the mitigated decomposition owing to the lack of soil disturbance. Gelybo *et al*., (2022) reported higher soil respiration and moisture content under zero tillage than under mouldboard ploughing, which suggests an increase in the decomposition of SOM under the zero tillage systems. However, many zero tillage systems also have increased residue return as part of their management regime, so this does not necessarily imply a net SOM loss, but rather suggests enhanced biological activity (supporting bigger microbial, fungi and soil fauna communities) due to improved substrate availability and soil structure in reduced tillage systems.

The results of a long-term field experiment (20 years) in Switzerland on a sandy loam soil by Martinez *et al*. (2016) also concluded that Zero tillage stored significantly higher SOM on the top 0-10 cm compared to mouldboard ploughing. However, they also found that at 15-30 cm layer, mouldboard ploughing had higher SOM concentrations than the Zero tillage treatments. This disagrees with the results presented here, which found no significant difference between the different tillage systems at 10-20, although at 20-30 cm Zero tillage stored higher concentrations of SOM (4% more) than Deep tillage systems. And the interaction between CTF with Zero tillage also led to significantly higher SOM concentrations compared to STP Deep and CTF Deep tillage, which was the opposite effect of Martinez *et* *al.,* (2016). This difference is likely due to the enhanced soil mixing capacity of mouldboard ploughing operations, which invert the soil, compared to the non-inversion tillage system used as the Deep tillage treatment on this study.

4.5.1.1.2 EFFECTS OF THE TRAFFIC AND TILLAGE INTERACTION

Traffic and tillage management systems and their interaction significantly affected SOM concentrations at 0-10 cm. CTF Zero and CTF Shallow tillage had significantly higher SOM when compared to the other treatment combinations. This suggests that reducing the traffic area and tillage intensity increases SOM concentrations on the surface layer of the soil, as these were the two treatments that incurred the lowest levels of disturbance. Surface SOM is essential for enhancing soil structure, biological activity, water infiltration, conservation of nutrients and reducing soil erosion. And all these benefits can contribute to improved crop yields and resilience. Additionally, this combination (CTF with Zero or Shallow tillage systems) can further benefit farmers by reducing fossil fuel consumption and improving efficiency (Vermeulen, 2010).

4.5.A. MAIN EFFECTS OF TRAFFIC OVER TIME

At 0-10 cm, the interaction between traffic systems and soil sample collections was not statistically significant, indicating that the effects of traffic systems on SOM dynamics were consistent over the timeframe of this study. However, the overall effect of all traffic ($p <$ 0.001) and collection dates ($p < 0.001$) were significant, meaning that the different traffic systems and the different collections had a different effect on SOM from each other. Following the increase in crop biomass input after harvest, the least compacted soils (found in CTF systems) stored the highest SOM concentration in the 0-10 cm layer, compared to LTP and STP systems. These findings align with the previous results, indicating that wheel-induced compaction significantly affects SOM storage.

Within the collection dates, the collection on 16/08/21 had significantly higher SOM (4.79%) when compared to the collection on 29/04/2022 (4.52%) and 25/09/2023 (4.55%). Therefore, the average SOM concentration decreased 0.2% after the first soil sample collection. This is relatively small and could be due to normal seasonal fluctuations, due to differences in crop biomass inputs (different crops) and environmental conditions. The low CV = 5% indicates that the sampling and analysis methods were robust.

Crop residues contain carbon (40-45%), nitrogen, potassium, phosphorus and microelements essential for crop growth (Grzyb, Wolna-Maruwka, and Niewiadomska, 2020). The residue quality and environmental conditions (soil moisture and temperature) regulate the rate of SOM decomposition by the soil microbes (Cates *et al*., 2022). When the crop residue is left in the field, only about one-third of its carbon content is sequestered into the soil during the initial year. The majority returns to the atmosphere through microbial respiration. However, crop residue decomposition and nutrient release occur at a slow rate (Aulakh, Khera, and Doran, 2000).

4.5.B. MAIN EFFECTS OF TILLAGE OVER TIME

At 0-10 cm the interaction between tillage and soil sample collections was not statistically significant, indicating that the effects of tillage were consistent across all treatments over the timeframe of this study.

5.5.C. MAIN EFFECTS OF THE TRAFFIC AND TILLAGE INTERACTION OVER TIME

At 0-10 cm, the interaction between traffic and tillage systems and soil sample collections on SOM concentrations was not statistically significant, indicating that the effects of traffic and tillage remained consistent over time.

Increasing SOM content in cropland can take a long time (depending on soil type, initial SOM levels and climate) and it generally requires high levels of SOM inputs. It is generally considered to take at least 5 years before changes in management practices can be detected in SOM concentrations (Smith *et al.*, 2019). At the experimental site used for this study, Kaczorowska-Dolowy (2020) reported that the average SOM at 0-10 cm was 4.30% and at 10-20 cm was 3.64% in Sept 2019. The average SOM at 0-10 cm during 2021-2023 was 4.63% and at 10-20 cm 3.98%. Although not significant (p = 0.27) (see detailed account in Appendix 4), after 4 years of continuous cereal cropping, SOM had an absolute increase of 0.33% SOM at 0-10 cm and 0.34% at 10-20 cm depth. While the SOM showed a positive trend, the magnitude of change could still fall within the range of natural seasonal and spatial variation.

Comparing specific management systems from 2019 to the average of 2021-2023 reveals that over this period, at 0-10 cm depth, CTF Zero and STP Deep systems showed absolute SOM increases of 0.54% and 0.30%, respectively. While not statistically significant, the higher SOM accumulation in CTF Zero compared to STP Deep systems aligns with the expected negative impacts of soil disturbance through compaction and tillage on SOM content. However, these differences may also reflect seasonal variability.

The data from this study also shows the importance of continuous monitoring over an extended timeframe, as the data from 2019-2023 inclusive showed no significant temporal effects on SOM concentrations at 0-10 cm over this period. This agrees with Smith *et al.* (2019), who suggest that the three-year timeframe was insufficient to detect changes over time. This highlights the need for caution when extrapolating from short-term studies to try to gain insights into the effects of management changes on SOM dynamics.

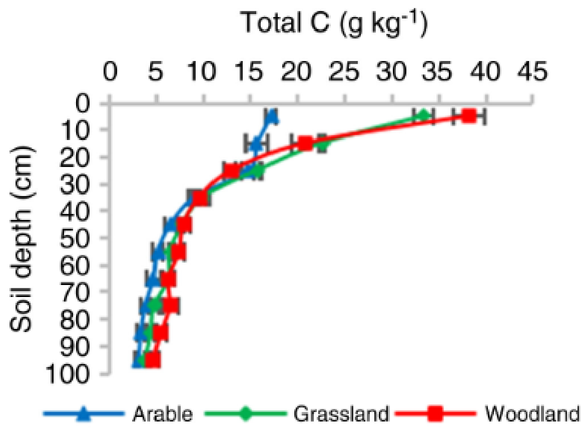
Baseline SOM measurements were not available from the beginning of the field experiment. Before establishing the experimental field, the site had been under cereal and grass crop rotations. For the establishment, a drainage system was installed, suggesting historically wet conditions, likely contributing to already high SOM concentrations at the beginning of the field experiment.

4.5.1.2 SOM AT 10-20 CM

The lack of significant differences within between the main effects of traffic, tillage and the interaction between traffic and tillage systems at this soil depth suggests that these systems stored most of the SOM on the top 0-10 cm with relatively little vertical translocation. Kaczorowska-Dolowy (2022) also reported no significant differences between the different management systems in SOM at 10-20 cm depth in the same site three years prior.

Another study of SOM dynamics in dryland regions of China, also reported that the total effects of management practices on SOM concentrations decreased with increasing soil depth (Zhuo *et al*., 2022). Syswerda *et al*. (2011) also noted no significant difference in SOM at deeper horizons, attributing this to increased spatial variability and lower concentration of SOM with depth.

Antony *et al*. (2022) examined SOM distribution across UK temperate lowland arable, grassland, and woodland soil profiles. Significant inter-land use differences observed at 0-10 cm diminished at 10-20 cm and were negligible at 20-30 cm (Fig. 4.41). While results may vary among different soil horizons, this suggests that even though woodland and grasslands have higher OM inputs than cropland, SOM primarily accumulated in the 0-10 cm layer, having significantly less SOM concentrations at 10-20 cm and 20-30 cm depths.



2**Fig. 4.25** -Total carbon (g kg^{-1}) at 10 cm depth increments to 1 m under arable, grassland and woodland land uses at Sonning farm, Hall farm, and The Vyne. Points represent mean values from all three sites and error bars represent the standard errors of the mean using the three sites as replicates. Source: Antony, *et al.* (2022).

At 10-20 cm depth, traffic, tillage, and their interaction showed no significant temporal effects on SOM content, correlating with non-significant differences between main effects at this depth.

4.5.1.3 SOM AT 20-30 CM

At 20-30 cm depth, SOM was affected by the main effect of tillage and the interaction between traffic and tillage systems.

Within the tillage systems, Zero tillage exhibited 4.05% higher concentrations of SOM than Deep tillage systems at this depth. Within the interaction between the Traffic and Tillage systems, CTF Zero tillage had significantly higher concentrations of SOM than CTF Deep and STP Deep tillage systems, indicating that the lack of soil disturbance in Zero tillage and CTF Zero after 12 years has developed an improved soil structure down the soil profile, facilitating SOM transport. SOM can move down the soil profile through biological processes such as earthworm activity, root litter and exudates (Schrumpf *et al*., 2013), and the movement of dissolved organic matter. The minimal disturbance in these systems likely facilitated higher biological activity, supporting this mechanism. The lack of soil disturbance at this depth could also promote the preservation of protected SOM, resulting in lower decomposition rates compared to Deep tillage systems.

The study mentioned previously by Antony *et al.,* (2022), reported similar subsoil SOM levels across arable, grassland and woodland soils. This suggests that land use and management practices may not significantly influence subsoil SOM concentrations. However, further subsoil sampling is recommended to validate these findings.

4.5.1.1.4 MAIN EFFECTS OF THE INTERACTIONS OVER TIME

At 20-30 cm traffic, tillage, and their interaction showed no significant temporal effects on SOM concentrations. However, due to the diminished effect of management practices on SOM with depth, combined with the increased spatial variability, and slower sequestration rates, detecting significant temporal changes in SOM in deeper soil layers may require long-term studies spanning more than 20 years to see if the SOM might be increasing over time at this soil depth.

4.5.2. SOM IN RELATION TO MBC

MBC represents the living component of SOM. It's made up mostly of fungi and bacteria (90%), archaea, and some meso- and macro-fauna. Although it only constitutes a very small fraction (1-4%) of the SOM, it plays a crucial role in the breakdown of SOM, nutrient availability and carbon sink (Khoshru *et al.,* 2023). It is widely used as an early indicator of changes in soil physical and chemical properties resulting from different management practices in agriculture (das Chagas, Collado and Leite, 2013) and soil health. It is typically higher in undisturbed native soils than in arable land (Gayam *et al*., 2023).

The results showed that the different traffic and tillage systems did not significantly affect MBC down the soil profile. This could be due to the small variation in SOM (±5% between systems) and the low sample size, which might have reduced the statistical power. However, MBC showed a positive linear response ($p < 0.05$; R^2= 0.24-0.19) to the increase of SOM at all soil depths. This agrees with McGonigle and Turner (2017) (McGonigle and Turner, 2017) who similarly observed that MBC had a positive linear response ($p < 0.001$; $R^2 = 0.48$) to SOM in temperate cropland systems.

A global meta-analysis found that conservation tillage (e.g., zero or reduced tillage, minimum of 30% soil surface covered with residues, crop rotations and cover crops) increased MBC by 37% (at 0-20 cm) compared to conventional tillage practices (i.e., ploughing/ harrowing and removal of plant residues). However, the conservation tillage effects were non-significant in sandy soils (Chen *et al*., 2020). Unlike other studies with different SOM inputs into the systems, our long-term study maintained similar SOM inputs across all systems, thus isolating the specific effects of traffic and tillage systems effects on MBC, making the system effects smaller.

Zero tillage systems typically exhibit higher SOM levels (absent differences in SOM inputs between systems) due to aggregate-mediated physical protection that constrains microbial decomposition. In addition, it has been shown that reducing soil disturbance in agricultural systems can enhance microbial carbon use efficiency (CUE) (Chen *et al*., 2020).CUE represents the use of C by the microbes that go towards their growth (biomass production) versus respiration, thus representing a dual control point governing both SOC accumulation and loss (Fen tao et al., 2023). A high CUE is mainly associated with high C storage because the microbe growth will ultimately become microbial necromass (an important constituent of MAOM-C formation -Chapter 6: POM and MAOM-). While a low CUE implies C losses because the microbes need to make a higher investment in acquiring the resources or rebuilding the fungal networks and community structures (broken down in the case of tillage) and less of that C is used for their growth.

CUE likely fluctuates with resource availability, especially during the non-growing season, when microbes are often C-limited, becoming dormant, where the stored C is allocated to metabolic maintenance rather than growth (Tao *et al*., 2023). CUE is also influenced by environmental variables with soil structural properties (BD, soil texture and porosity) being one of the most important (Tao *et al*., 2023). Therefore, a well-structured soil such as CTF Zero tillage system should promote microbial activities, increasing CUE and SOC formation and persistence, which agrees with the SOM and SOC stocks results (Chapter 5) even if it is not picked up by the MBC.

On top of that the different agricultural management practices can also change the fungal and bacterial community composition, and this might also influence the rates of soil C storing and loss (Zhang *et al*., 2013).

As SOM is decomposed by the microbes, most of it gets released back into the atmosphere and a very small proportion gets stabilised (as microbial necromas or microbial transformations). With constant OM inputs, we cannot increase both SOM concentrations and microbial activity at the same time. This leads to the question by Janzen *et al.,* (2006), shall we hoard it or use it? Currently, there is an emphasis on storing SOC in agricultural soils as a climate change mitigation strategy. To increase SOM storage, it is important to reduce soil disturbance. However, most of the C stored when disturbance is reduced is in the form of POM, a labile component of SOM (Chapter 6) which could also be easily decomposed by the microbes if the farmer starts ploughing the soil again.

4.5.3. SOM 0-30 CM

While the above sections have explored SOM dynamics as impacted by the different traffic and tillage management systems in 10 cm layers moving down through the topsoil, most plant roots and much of the soil biota access the whole depth of the topsoil rather than specific layers. So, to gain insights into the soil health impacts of the different traffic and tillage systems in terms of SOM concentrations, it is also necessary to consider the whole topsoil (i.e. 0–30 cm) SOM concentrations.

Within the traffic treatments, CTF had significantly higher (2.71% more) SOM concentration than STP systems. However, LTP systems were not statistically significantly different from CTF or STP. These results did not fully support our first hypothesis, which expected greater SOM in both CTF and LTP systems.

Within the tillage treatments, Zero and Shallow tillage both had significantly higher (3.08% more) SOM concentrations than Deep tillage on average across the combined soil layers. The similarity between Zero and Shallow tillage systems may be attributed to our Zero tillage system having more disturbance than expected due to our drill used discs (down to ~5 cm) to break the surface crop residues left on the soil from the previous crop and enhance the tilth. These results support our second hypothesis, which expects that reduced tillage systems will store more SOM.

While increasing the tillage intensity is often associated with SOM depletion, this study highlighted the critical role of preventing wheel-induced compaction in maximising SOM storage. Both traffic compaction and tillage intensity affected the SOM. However, it was the interaction between both systems (CTF and Zero tillage systems) that stored significantly higher (5.01% more) SOM concentrations than the other treatment combinations at 0-30 cm over 12 years. LTP Shallow and CTF Shallow tillage stored 3.19% more SOM concentrations than the rest of the treatment combinations. STP Deep had the lowest SOM concentrations. This supports the third hypothesis, which expected significant interactions between the traffic and tillage management systems.

However, this small SOM increase was likely not gradual. SOM can increase faster when the levels are low and at the beginning of the management change and slow down over time as the system reaches an equilibrium (specific to the soil type and farming system) (BSSS, 2023). A long-term experiment in Denmark on a sandy loam soil with cereal crops showed that the effect of straw addition and ryegrass cover crop on SOC sequestration peaked after 10-15 years when a new equilibrium was reached (Jensen *et al.*, 2022). This study also showed that there were no statistically significant temporal effects on SOM concentrations from 2019 to 2021-2023. Therefore, it is possible that the systems are approaching an equilibrium after 12 years, resulting in no SOM increase or the SOM increase has slowed down because the system is close to reaching the equilibrium, and the smaller increase fell below our statistical detection threshold (or statistical power).

The yearly accumulation rate would also depend on the initial SOM content of the soil at the beginning of the experiment (i.e. 12 years ago). The initial SOM content at the start of the experimental field was unknown, but all the treatments began at the same level and accumulated varying amounts of SOM over time. Therefore, if we compare conventional management systems such as STP Deep (3.87% SOM) with CTF Zero (4.19% SOM), CTF Zero stored 8.26% more SOM compared to STP Deep systems. The higher SOM concentration in CTF Zero systems may be attributed to the increased C protection in soil aggregates due to the reduced soil disturbance, but also to an enhanced OM input due to CTF systems increasing the grain yield by 4% over the last 8 year-period (Kaczorowska, 2022). Zero tillage systems had the lowest yields at the beginning, but improved with time, producing the same or higher yields than Deep and Shallow tillage systems (Godwin *et al*., 2022). The enhanced SOM may also increase crop yield, creating a virtuous circle (Lal, 2004).

Increasing SOM concentrations in the soil has been linked with increased crop yield. For example, according to Pan *et al*. (2009), an increase of 1% of SOM could increase cereal productivity by 0.43 Mg ha^{-1}. And Ma *et al.* (2023) concluded that the global production of wheat, maize and rice could increase by 4.3% through increasing current SOM to optimum levels (between 12.7 and 43.9 g SOC kg $soil^{-1}$), although current available management practices would increase crop production by only 0.7%. In contrast, other authors have also shown that crop yields can be maximised without high SOM concentrations as long as the soil has sufficient supplies of nutrients (mineral fertilisers) and water (Hijbeek *et al*., 2016; Oelofse *et al*., 2015). In any case, even if the SOM increase does not promote a crop yield increase, it can have large beneficial impacts such as improving nitrogen management and decreasing direct and indirect nitrous oxide emissions (Powlson *et al.,* 2018).

Increasing SOM concentrations in the top 0-30 cm over the last 12 years compared to “business as usual” is likely to be a meaningful improvement in soil health. The next chapter explores the consequences of this increase in terms of SOC stocks in Mg/ha.

4.5.4. LIMITATIONS

Accurate quantification of SOM is challenging due to its high spatial variability. However, the consistency of SOM levels over time and the low coefficient of variation between soil sample collections suggest a robust and reliable sampling methodology was applied here.

At the beginning of the field experiment in 2011, the soil pH was 6.6, declining to 5.8 in 2019 (Kaczorowska-Dolowy, 2022) and 5.4 (0-30 cm) by 2021 (current study). The optimum pH for arable cropping is 6.5 (AHDB, 2024). Agricultural practices such as crop growth and nitrogen fertiliser applications acidify soil over time. When pH falls below 6.5, some nutrients become less available (e.g., phosphorus, potassium, sulphur, calcium, and magnesium), potentially impacting crop yield and quality. Most beneficial soil bacteria prefer a pH range of 6-7, therefore, bacterial activity may be slightly reduced, although fungal populations might be favoured. The experimental site has not had any liming applications to avoid the formation of inorganic carbon, affecting SOC measurements. Therefore, the low soil pH may influence SOM content through two contrasting mechanisms: reducing the OM inputs into the soil due to lower crop yields, slowing SOM decomposition and reduced microbial activity, possibly leading to slightly higher SOM retention over time compared to an “average” UK arable system. Therefore, extrapolation of these results to other system should be taken with care.

4.6. CONCLUSIONS

The initial hypothesis that “soil compaction from agricultural vehicles has a negative effect on SOM, therefore reduced traffic and wheel pressure will lead to higher depth-specific SOM concentrations and MBC content” was partially supported by the results of this study. At 0-30 cm, CTF systems stored 2.8% higher SOM concentrations than STP systems. However, there were no significant differences between the traffic treatments for MBC content.

The second hypothesis that “soil disturbance by tillage increases SOM decomposition, therefore reduced tillage will lead to higher depth-specific SOM concentrations and MBC content” was partially supported by the results, which showed that at 0-30 cm, Zero tillage stored 2.8% and Shallow 3% higher SOM concentrations than Deep tillage systems. However, MBC content was not significantly affected by the tillage treatments at each soil depth.

The results also partially confirmed the third hypothesis that “the interaction between traffic and tillage systems will impact SOM content and MBC at different depths”. At 0-30 cm, the combination of CTF with Zero tillage stored 6.4% more SOM concentration than the rest of the treatment combinations, except for LTP Shallow and CTF Shallow. LTP Shallow and CTF Shallow stored 3.3% more SOM concentration than the rest of the treatment combinations. The interaction between traffic and tillage systems did not significantly affect MBC content at each soil depth. Only at 0-30 cm the overall effect of soil depth was significant with MBC content decreasing down the soil profile 0-10, 10-20 and 20-30 cm.

This study emphasises the critical role of preventing wheel-induced compaction (STP) and deep tillage in maximising SOM storage and shows that the main effect of Traffic management systems and Tillage practices and their interaction can have significant effects on SOM concentrations. This highlights the potential to improve soil health, as quantified by SOM concentrations, by changing the soil management system to minimise disturbance. This is likely to have beneficial effects in terms of improving the sustainable use of soils with benefits for food security (see Chapter 7 for crop production) as well as assisting agriculture on the pathway to Net Zero (see Chapter 5 for C sequestration and storage).

CHAPTER 5

THE EFFECT OF TRAFFIC AND TILLAGE MANAGEMENT SYSTEMS ON SOIL ORGANIC CARBON CONCENTRATION, SOIL BULK DENSITY AND SOIL ORGANIC CARBON STOCKS

5.1. INTRODUCTION

As discussed in previous chapters, there has been an increased interest in SOM in recent years due to using soils as a climate change mitigation strategy by sequestering carbon into the soil (Chabbi *et al*., 2017; Janzen, 2015). SOC is a fraction of SOM, and changes in this fraction determine the level of C stored in soil. Therefore, this chapter will analyse SOC concentrations and compare and contrast them with the SOM results. Changing the focus to SOC permits the quantification of soil carbon storage in soils, usually referred to as SOC stocks (Simth *et al*., 2020).

Changes in agricultural management practices can affect both soil bulk density and SOC concentrations at different depths. Quantifying SOC stocks can capture the changes caused by the interaction between these variables more accurately than quantifying SOC concentrations alone. This can provide deeper insights into the impact of those management practices on the SOC dynamics for a given soil. The Intergovernmental Panel on Climate Change (IPCC) also proposed in their guidelines the quantification of soil carbon stocks to a depth of 30 cm for assessing carbon sequestration potential (Penman *et al.,* 2003).

Accurate quantification of SOC stock changes in response to agricultural management practices remains a significant challenge, primarily due to difficulties in precise SOC (Stanley *et al.,* 2023) and soil bulk density (Zhou *et al.,* 2019; Poeplau, Vos and Don, 2017) quantification. Many studies have calculated SOC stocks either by fixed depth (FD) approach or equivalent soil mass (ESM) methodologies (Chaplot and Smith, 2023; Hubbard, Strickland and Phatak, 2013; Meurer *et al*., 2018). FD approach is prone to error because different management practices in cropland can have different effects on soil BD (Wendt and Hauser, 2013). For example, trafficking a field compacts the soil which means that a greater mass of soil may be present at the chosen depth than was present before the compaction event. This can lead to the strange conclusion that trafficking soil can lead to increased SOC stocks, which is an artefact of the approach rather than a true change in the amount of SOC in a soil.

The ESM method calculates SOC stocks based on equivalent soil mass rather than fixed soil volume (Ellert, Janzen and McConkey, 2001). The ESM method quantifies the SOC stock stored in a reference soil mass (e.g., control treatment, initial experimental mass, or "business as usual" treatment) (Von Haden, Yang, and DeLucia, 2020; Wendt and Hauser, 2013) to circumvent issues such as compaction events leading to the incorrect conclusion of increased SOC stocks. Therefore, the ESM approach is recommended (Von Haden, Yang and DeLucia, 2020).

ESM approaches can also vary, encompassing both modelling (e.g. cumulative coordinate) and non-modelling techniques (e.g. direct calculation of SOC stock) (Peng *et al.,* 2024). Furthermore, the selection of an optimal reference soil mass can be unclear. Therefore, this study comparatively evaluated three SOC stock estimation approaches using two distinct reference soil masses to assess their relative efficacy (Ferchaud, Chlebowski and Mary, 2023).

In recent decades, there has been an increased focus on the quantification of SOC stocks in conservation tillage systems, particularly zero tillage and reduced tillage (He *et al*., 2023; Cooper *et al*., 2021; Ogle *et al*., 2019; Haddaway *et al.,* 2017). Tillage reduction is one of the recommended measures to increase SOC sequestration to build SOC stocks (Krauss *et al*., 2022; Six *et al.,* 2000), although to reach its full potential, it should be accompanied by other measures that increase the SOM input into the system such as crop rotation and cover crops (including diversification of plant species and permanent soil cover with living roots) and the integration of animals (FAO, 2022). However, the effects of different traffic and low-ground tyre pressure systems on SOC stocks remain unknown. Thus, long-term traffic and tillage studies are needed to assess their effects on SOC stocks within different soil profiles and climatic conditions. Quantifying SOC concentrations alone is insufficient to provide in-depth insights into the impacts of these management practices on SOC dynamics; quantification of the changes in SOC stocks is essential for understanding how soils will respond to those management system changes.

5.2. AIM AND HYPOTHESIS

This chapter aims to quantify the effects of alternative traffic systems and their interaction with different tillage systems on SOC concentrations, soil bulk density and SOC stocks in a long-term field experiment over the last three years (2021-2023).

The hypotheses for this chapter are:

4. Agricultural traffic increases soil BD and affects SOC content and SOC stocks; therefore, CTF and LTP systems will not increase soil BD as much as STP, storing higher depth-specific SOC content and SOC stocks.
5. Soil disturbance by tillage increases soil BD and SOC decomposition; therefore, reduced tillage will lead to lower soil BD and higher depth-specific SOC content and SOC stocks.
6. The interaction between traffic and tillage systems will affect BD, SOC content and SOC stocks at different depths.

5.3. METHODOLOGY

5.3.1 LABORATORY ANALYSIS

5.3.1.1 SOIL ORGANIC CARBON CONTENT

SOC (%) concentrations were determined by the Dumas dry combustion method (Bertsch and Ostinelli, 2019) using a LECO CN.828 Carbon/Nitrogen macro combustion analyser (LECO Instrument Ltd, UK). For the first soil sample collection (16/08/2021), the soil was sieved to 4 mm, and 1.5 mg of soil was taken for carbon analysis. For subsequent sample collections, the soil was initially sieved to pass 2 mm, followed by further sieving to pass 50 µm. The soil that passed through the 50 µm sieve (i.e. < 50 µm) was considered to contain the MAOM fraction of SOM (Midwood *et al*., 2021, after Poeplau *et al*., 2018). The soil that remained on the sieve was considered to contain the POM fraction of SOM (i.e. 2000–50 µm) (The fractionation method is explained in Chapter 6, POM AND MAOM). Both fractions were independently milled using a ball mill for 2 minutes to a fine flour consistency. The SOC concentrations of the fractionated samples were determined with the same LECO CN analyser as above and both fractions were summed to give the total SOC.

Soil pH was determined after the first soil sample collection (detailed results in Appendix 5, Table 5.4). The low soil pH (mean 5.4) at 0-30 cm depth indicated the absence of soil carbonates. Thus, total soil C was assumed equivalent to SOC (Mikha *et al*., 2010).

5.3.1.2. SOIL pH

The soil pH was analysed on air-dried samples mixed with distilled water in the 1:2.5 soil-water ratio (volumetric proportion 1:2.5 and analysed with a Jenway 3510 pH meter as suggested by Carter and Gregorich (2008) (Fig. 5.1).

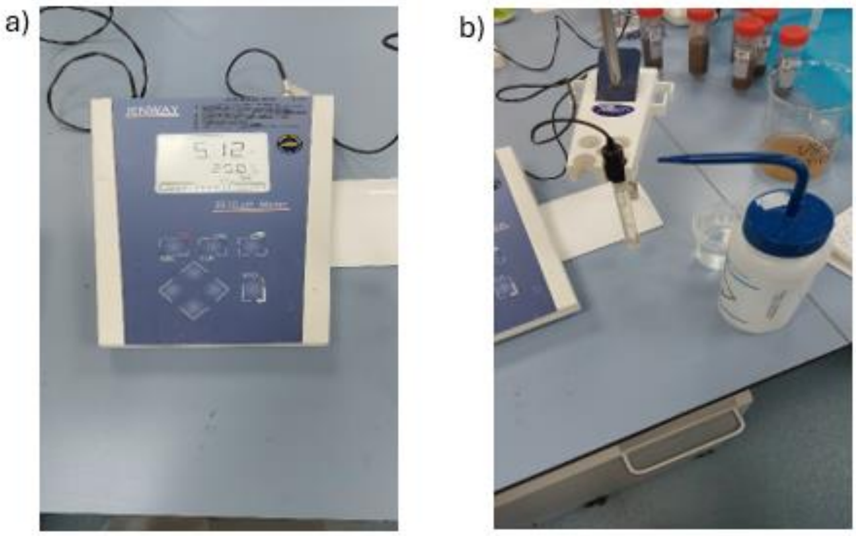


Fig. 5. 1. a) and b) the pH meter (Jenway 3510) used to analyse air-dried samples mixed with distilled water 1:2.5 soil-water ratio).

5.3.1.3. SOIL BULK DENSITY (BD)

For more details on the type of auger used and time of sampling, please refer to Chapter 3, Methodology (*3.9.2. Soil sampling for dry bulk density analysis*).

One sample was collected from each plot, reflecting the general sampling approach discussed previously (i.e. CTF, at the centre of the plot, representing the non-trafficked crop area, and STP and LTP – from the permanent wheel way).

Undisturbed core samples (30 cm long and 5 cm in diameter) were collected and sectioned into 10 cm increments from the surface (Fig. 5 b). When the final increment depth deviated from 10 cm, the precise measured depth was incorporated into the bulk density calculations. Stones were manually removed from the soil and weighed prior to drying the soil samples in a fan-assisted oven for 3–4 days until a constant mass was achieved. The dry weights were used to determine dry bulk density at 0-10, 10-20, and 20-30 cm depths per plot.

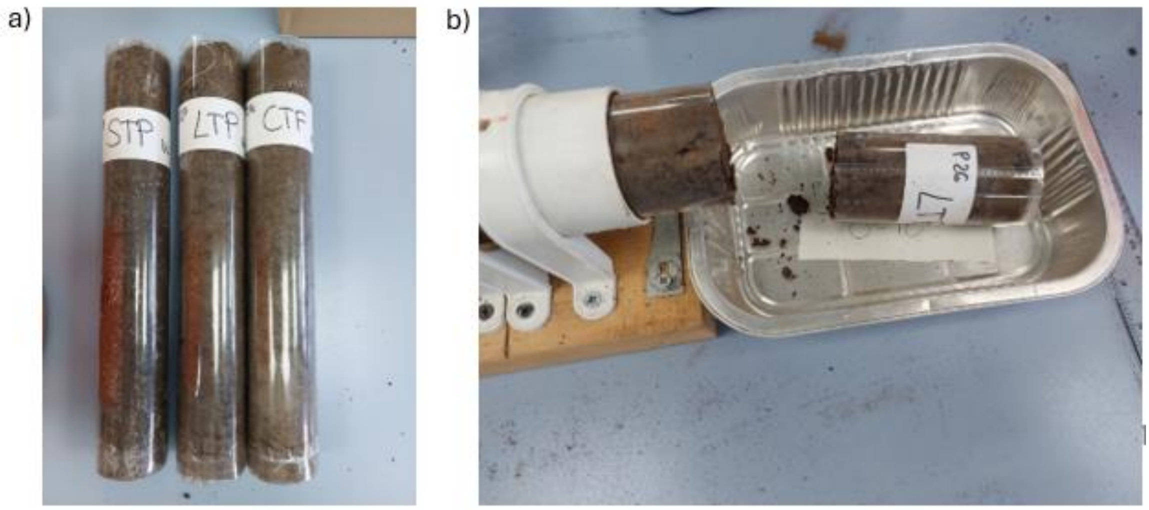


Figure 5. 1 – a) Liners (5 cm diameter and 30 cm length) for dry bulk density measurements extracted with the Royal Eijkelkamp auger (04.15.SB soil 68, Netherlands) **b)** Liners were sectioned into 10 cm depth increments, with stones manually separated and weighed prior to oven-drying the soil samples.

Bulk density was determined by dividing the weight of the dry soil by the volume of the core occupied by the soil after correction for stoniness (Poeplau, Vos and Don, 2017) calculated as presented in Equation 5.1.

$$\text{Bulk density } (g\ cm^{-3}) = \frac{\text{Mass of dry soil} - \text{Mass of stones}}{\text{Volume of soil} - \left(\frac{\text{Mass of stones}}{\text{Density of stones } (2.6\ g\ cm^{-3})}\right)} \qquad (5.1)$$

The BD data from the first soil sample collection was excluded from the analysis of the effects of traffic and tillage systems and their interaction on BD. This is because the time of sampling differed from the other collections (having extra compaction on the wheel ways due to the compaction treatments and drilling operations), and stones were not removed, making the BD data higher than normal, meaning that these data were not readily comparable. However, this data set was included in the evaluation of how BD has evolved over time.

5.3.2. SOIL SAMPLING STRATEGY

Please refer to "*Chapter 3 Methodology. 3.9. Soil sampling and processing and 3.9.1. Soil sampling for soil carbon analysis*".

5.3.3. SOC STOCKS ASSESSMENT

This study calculated the SOC stocks for the last three soil sample collections in three different ways, using the SimpleESM: R script (Ferchaud, Chlebowski and Mary, 2023):

i. a fixed depth method (FD),
ii. a “classical” **ESM** $_{\text{non-linear model}}$: The soil profile is segmented into 1 mm layers. Each is then assigned a soil mass and SOC concentration based on corresponding measured layers for each sampling location and date. The SOC stock calculation depth (z) is determined by incrementally adding 1 mm layers until approximating the reference soil mass (Autret *et al.,* 2016; Mary *et al*., 2020).
iii. an “alternative” **ESM** $_{\text{cubic spline model}}$: where a cubic spline model is fitted on the relationship between cumulative soil mass and cumulative SOC stock at each sampling location. This non-linear model is then used to interpolate the SOC stocks values at the reference soil masses (Wendt & Hauser, 2013; Von Haden *et al*., 2020).

And with two different and contrasting reference soil masses:

i. as a control treatment: CTF Zero tillage
ii. as a business as usual: STP Deep tillage

The carbon stocks of the three soil depths (0-10, 10-20, and 20-30 cm) were summed to calculate the cumulative carbon stocks (Mg ha^{-1}) (0-30 cm) per treatment.

5.4. RESULTS

5.4.1 EFFECTS OF TRAFFIC AND TILLAGE ON SOC

SOC concentration decreased significantly with depth (p < 0.001). The average SOC concentration at 0-10 cm was 20.87 g/kg. At 10-20 cm, it was 17.17 g/kg. And at 20-30 cm, it was 13.69 g/kg. The 0-10 cm layer had a 17.7% higher SOC concentration than the 10-20 cm layer, which in turn had a 22.7% higher concentration than the 20-30 cm layer.

5.4.1.1 COMPARISON OF THE RESULTS FROM SOC AND SOM

The results of SOC concentration analysis (Appendix 5) followed the same trends as the SOM concentrations, although with minor discrepancies, which are detailed below:

At 0-10 cm depth, traffic ($p < 0.001$), tillage ($p < 0.001$), and their interaction ($p < 0.001$) significantly influenced both SOM and SOC concentrations (Appendix A.5.1), with the following exceptions:

For the overall effect of all traffic systems, the CTF contained significantly higher SOC concentration (5.5% more) compared to LTP systems and (7.5% more) than STP systems (Fig 5. 1- Appendix A.5.1). However, this proportion was lower for SOM, where CTF contained significantly higher SOM concentration (3.7% more) compared to LTP and (5.2% more) than STP (Fig. 4.1- Chapter 4).

For the overall effect of all tillage systems, Zero and Shallow tillage systems contained significantly higher SOC (5.1% more) (Fig.5.2- Appendix A.5.1) and SOM (4.6% more) (Fig. 4.2 -Chapter 4) concentration compared to Deep tillage systems.

For the interaction between the traffic and tillage systems, CTF Zero (23.19 g/kg, CV = 9.50%) had significantly higher SOC concentration compared to all the other treatment combinations. The second highest was CTF Shallow (21.62 g/kg, CV = 6.59%) and the third highest was LTP Shallow (21.42 g/kg, CV = 5.74%) (Fig. 5. 3- Appendix A.5.1). While for SOM concentrations both CTF Zero (4.97%, CV = 7.49%) and CTF Shallow (4.84%, CV = 5.03) had the highest concentrations of SOM, followed by LTP Shallow (4.75%, CV = 4.71%) (Fig. 4.3 -Chapter 4).

At 10-20 cm depth, the results for the SOC and SOM concentration were the same. There were no statistically significant differences in SOC (Fig. 5. 7 -Appendix A.5.2) or SOM concentrations across treatments (Fig. 4. 7 -Chapter 4).

At 20-30 cm depth, there were some discrepancies between the effects of the different traffic and tillage systems on SOM and SOC concentrations. For SOC, only the interaction effect between traffic and tillage systems ($p = 0.005$) significantly affected SOC concentration (Fig. 5. 11- Appendix A.5.3). While for SOM, both the main effect of tillage ($p = 0.016$) and the interaction between the traffic and tillage systems ($p = 0.006$) significantly affected SOM concentrations (Fig. 4.11 and Fig. 4.12 -Chapter 4-).

For the interaction between traffic and tillage systems, CTF Zero tillage (14.92 g/kg) had significantly higher SOC concentration when compared to CTF Shallow (13.08 g/kg) and STP Deep (12.95 g kg) (Fig. 5. 11- Appendix 5.3.1). And for SOM concentrations, CTF Zero tillage (3.55%) had a significantly higher concentration of SOM when compared to CTF Deep (3.29%) and STP Deep (3.21%) (Fig. 4.12 Chapter 4).

The results of SOC concentrations combined at 0-30 cm (Appendix A.5.4) followed the same trend as the SOM concentrations over the same depth profile.

5.4.1.2 COMPARISON OF THE RESULTS FROM SOC AND SOM CONCENTRATIONS OVERTIME

At 0-10 cm depth, only the interaction between the main traffic systems and collection date ($p = 0.006$) significantly affected both SOC and SOM concentrations, meaning that the different traffic systems significantly affected those concentrations differently over time (Fig. 5.4- Appendix A.5.1.1). However, for SOM concentrations the interaction between Traffic and Collection date ($p = 0.13$) was not significant.

At 10-20 cm and 20-30 cm depth, neither SOC concentrations (Appendix A.5.2.1 and A.5.3.1) nor SOM concentrations (B.1. and C.1. –Chapter 4) were significantly affected over time.

5.4.2 EFFECTS OF TRAFFIC AND TILLAGE ON SOIL BD

The results showed that BD increased with soil depth. The average BD at 0-10 cm was 1.25 g/cm^3, at 10-20 cm was 1.34 g/cm^3 and at 20-30 cm was 1.36 g/cm^3.

5.4.2.1. BD AT 0-10 CM DEPTH

Only the effect of traffic ($p < 0.001$) was statistically significant; the effect of tillage ($p = 0.24$) and the interaction between traffic and tillage on BD ($p = 0.90$) were not statistically significant.

Within the traffic systems, CTF (1.14 g/cm^3, CV = 7.20%) had significantly lower BD compared to LTP (1.31 g/cm^3, CV = 7.14%) and STP (1.30 g/cm^3, CV = 8.25%) systems. Therefore, CTF had 14% lower BD compared to LTP and STP systems (Fig. 5. 2).

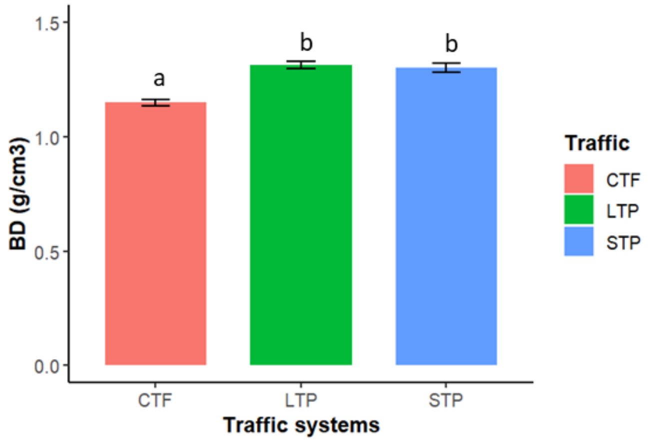


Figure 5. 2 – Main effects of the different traffic systems on BD (g/cm^3) at 0-10 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

5.4.2.2. BD AT 0-10 CM OVER TIME

-The effects of traffic over time: traffic ($p < 0.001$), collection date ($p < 0.001$) and the interaction between traffic and collection on BD ($p = 0.005$) were all statistically significant.

Within the traffic systems, the results were marginally higher than the overall effects of traffic displayed above (Fig. 5.1) due to the incorporation of collection 1, which had higher BD values due to the lack of accounting for stoniness. CTF (BD=1.20 g/cm^3, CV = 9.26%) had significantly lower BD compared to LTP (BD=1.40 g/cm^3, CV = 7.08%) and STP (BD = 1.40 g/cm^3, CV = 8.16%) systems.

Within the collection dates, the collection on 16/08/2021 (i.e. collection 1) (BD = 1.58 g/cm^3, CV = 10.18%) had significantly higher BD when compared to the rest of the collections: on 17/05/2022 (BD = 1.27 g/cm^3, CV = 6.47%), on 31/10/2022 (1.23 g/cm^3, CV = 8.47%) and 18/09/2023 (1.25 g/cm^3, CV = 7.55%) (Fig. 5. 3).

Within the interaction of traffic and collection, there were complex interactions as seen in Figure 5.3. STP and LTP (16/08/2021) had significantly higher BD than the rest of the traffic treatments and collection dates (with BD = 1.70 and 1.67 g/cm^3, respectively). CTF (16/08/21), LTP (17/05/22) and LTP (18/09/23) with BD = 1.35, 1,33 and 1.34 g/cm^3 respectively, had significantly higher BD than CTF from (17/05/22, 31/10/22 and 18/09/23) (BD = 1.17, 1.14 and 1.13 g/cm^3 respectively). And STP (17/05/22 and 31/10/22) with BD = 1.31 and 1.31 g/cm^3, respectively, had significantly higher BD than CTF (31/10/22 and 18/09/23) with BD = 1.14 and 1.13 g/cm^3, respectively (avg. CV = 8.17%).

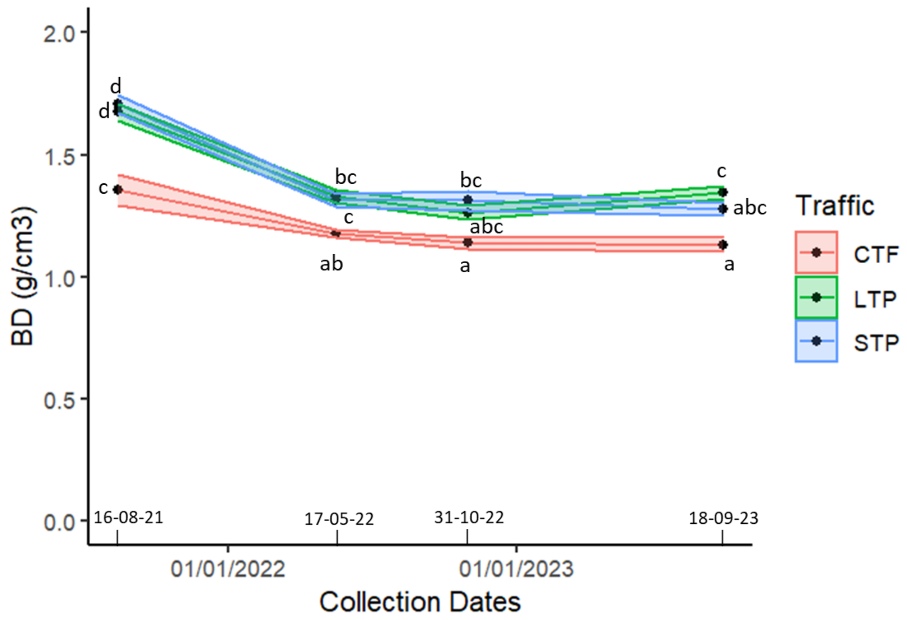


Figure 5. 3 – Main effects of the traffic systems on BD over time, at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors.

-**The effects of tillage over time**: at 0-10 cm tillage ($p = 0.15$) and the interaction between tillage and collection date ($p = 0.46$) were not statistically significant. However, BD varied significantly across collection dates ($p < 0.001$).

Within the collection dates, the results were the same as above; the collection on 16/08/2021 (BD = 1.58 g/cm^3, CV = 13.59%) had significantly higher BD when compared to the rest of the collections: on 17/05/2022 (BD = 1.27 g/cm^3, CV = 8.53%), on 31/10/2022 (1.23 g/cm^3, CV = 9.98%) and on 18/09/2023 (1.25 g/cm^3, CV = 10.3%) (Fig. 5. 4).

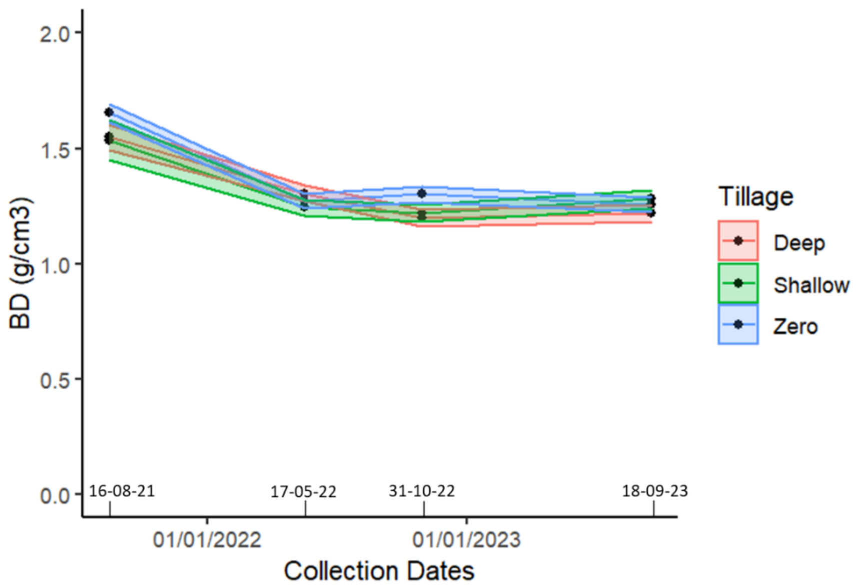


Figure 5. 4 – Main effects of the interaction between the tillage treatments on BD over time, at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between tillage systems and collection dates was not significant (p = 0.46).

-The effects of the traffic-tillage interaction over time: at 0-10 cm traffic-tillage (p < 0.001), collection date (p < 0.001) and the interaction between traffic-tillage and collection date on BD (p = 0.005) were all statistically significant (Fig. 5. 5).

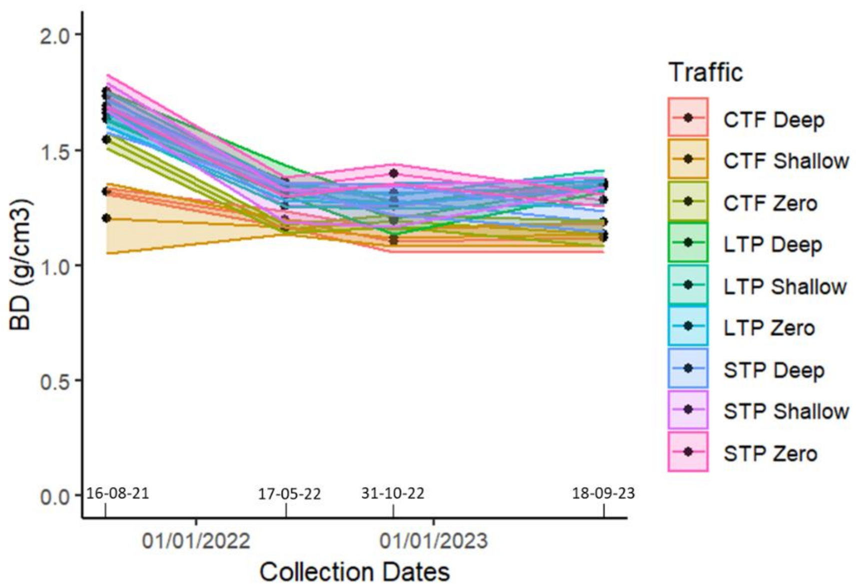


Figure 5. 5 – Main effects of the interaction between traffic and tillage treatments over time on BD, at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors.

5.4.2.3. BD AT 10-20 CM DEPTH

The effect of traffic ($p < 0.001$), tillage ($p = 0.04$), and the interaction between traffic and tillage on BD ($p = 0.04$) were all statistically significant.

Within the traffic systems, the CTF (1.26 g/cm^3, CV = 5.82%) had significantly lower BD compared to LTP (1.39 g/cm^3, CV = 5.25%) and STP (1.36 g/cm^3, CV = 4.70%) systems. Therefore, CTF systems had 8.7% lower BD compared to LTP and STP systems (Fig. 5. 6).

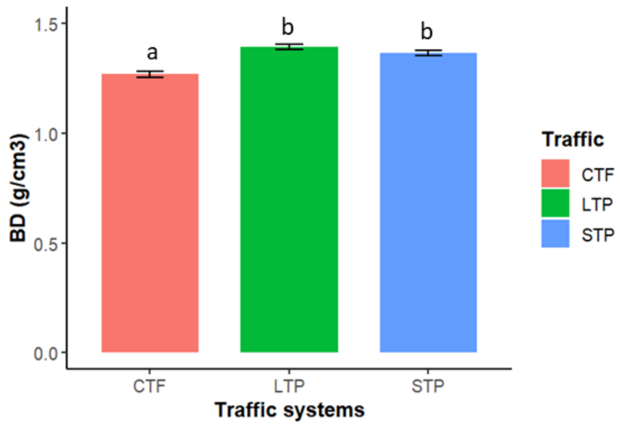


Figure 5. 6 – Main effects of the different traffic systems on BD at 10-20 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

Within the tillage systems, Deep tillage (1.32 g/cm^3, CV = 6.30%) had significantly (*Post hoc* $p = 0.05$) lower BD compared to Zero (1.35 g/cm^3, CV = 3.59%) tillage systems.

Within the traffic and tillage systems, LTP Zero (1.39 g/cm^3, CV = 2.71%) and LTP Shallow (1.42 g/cm^3, CV = 6.58%) had significantly higher BD compared to CTF Zero, Shallow and Deep (1.30 g/cm^3, CV = 4.22%; 1.28 g/cm^3, CV = 5.80%; 1.21 g/cm^3, CV = 7.46%, respectively). STP Zero (1.37 g/cm^3, CV = 3.85%) and STP Deep (1.37 g/cm^3, CV = 4.98%) had significantly higher BD than CTF (Shallow and Deep) (1.28 g/cm^3, CV = 5.80%; 1.21 g/cm^3, CV = 7.46%, respectively). And LTP Deep (1.37 g/cm^3, CV=6.45%) and STP Shallow (1.34 g/cm^3, CV = 5.28%) had significantly higher BD than CTF Deep (1.21 g/cm^3, CV = 7.46%) (Fig. 5. 7).

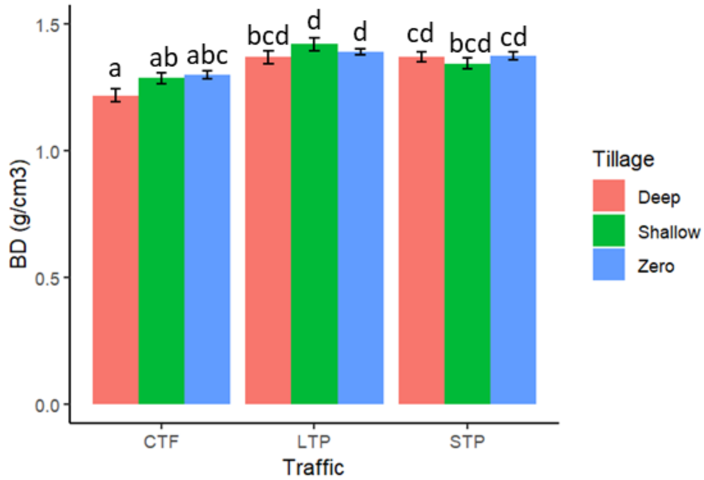


Figure 5. 7 – Main effects of the interaction between the different traffic and tillage systems on BD at 10-20 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 16). Bars show standard errors.

5.4.2.4. BD AT 10-20 CM OVER TIME

-The effects of traffic over time: traffic ($p < 0.001$) and collection date ($p < 0.001$) both had statistically significant effects on BD, but the interaction between traffic and collection on BD ($p = 0.90$) was not statistically significant (Fig. 5. 8).

Within the traffic systems, CTF (1.34 g/cm^3, CV = 6.49%) had significantly lower BD compared to LTP (1.47 g/cm^3, CV = 5.14%) and STP (1.44 g/cm^3, CV = 5.72%) systems. As discussed previously, the increased BD values were due to including the 1st soil sample collection for the temporal analysis.

Within the collection dates, the results were the same as those reported above; the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023.

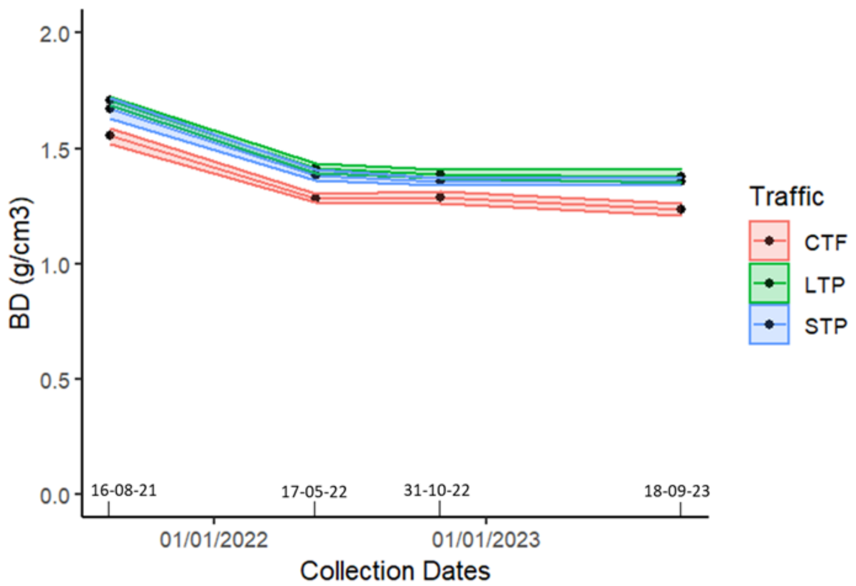


Figure 5. 8 – Main effects of the traffic systems on BD over time, at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between Traffic and Collection was not significant (p = 0.90).

-**The effects of tillage over time**: at 10-20 cm tillage (p = 0.40) and the interaction between tillage and collection date (p = 0.18) were not statistically significant. But the different sample collection dates had BDs that were statistically significantly different (p < 0.001).

Within the collection dates, the results were the same as above, the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023 (Fig. 5. 9).

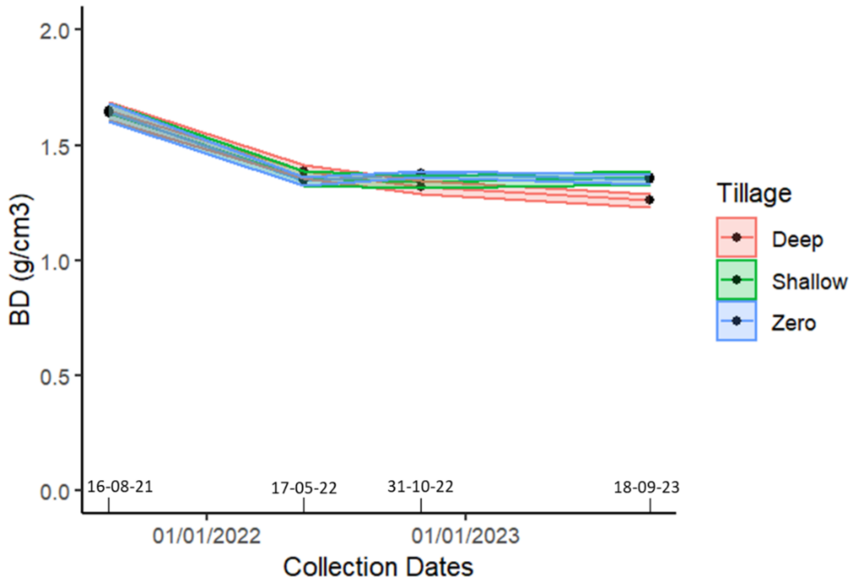


Figure 5. 9 – Main effects of the tillage systems on BD over time, at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between Tillage and Collection was not significant ($p = 0.18$).

-The effects of the traffic-tillage interaction over time: at 10-20 cm traffic-tillage ($p < 0.001$) and collection date ($p < 0.001$) were statistically significant, but the interaction between traffic-tillage and collection date on BD ($p = 0.32$) was not statistically significant (Fig. 5.10).

Within the collection dates, the results were the same as those reported above, the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023 (Fig. 5. 10)

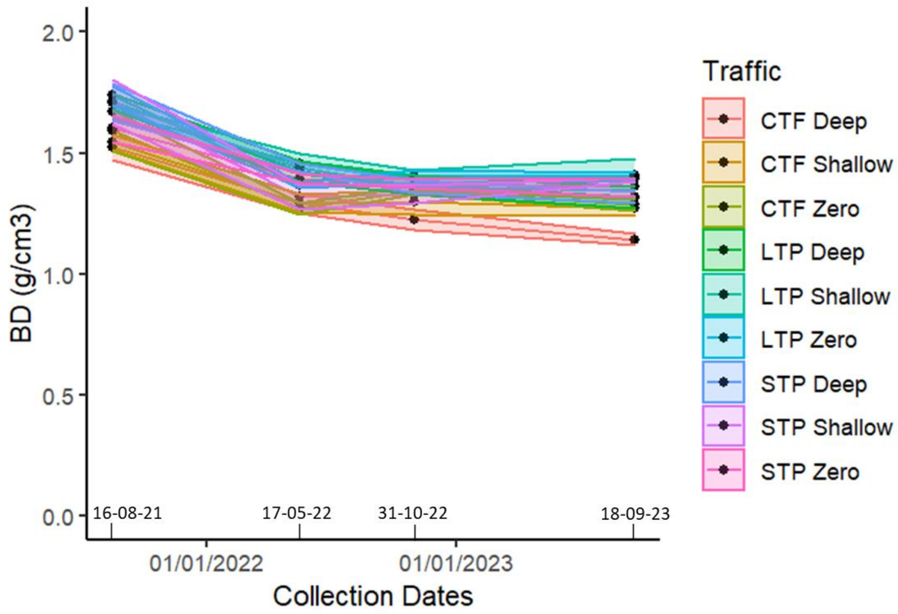


Figure 5. 10 – Main effects of the interaction between traffic and tillage treatments over time on BD, at 10-20 cm depth. Lines show means (n = 12). Ribbons show standard errors.

5.4.2.5. BD AT 20-30 CM DEPTH

Only the effect of traffic ($p < 0.001$) was statistically significant; tillage ($p = 0.19$) and the interaction between traffic and tillage ($p = 0.25$) were not statistically significant.

Within the traffic systems, CTF (1.33 g/cm^3, CV = 5.96%) had significantly lower BD compared to LTP (1.41 g/cm^3, CV = 4.57%) and STP (1.39 g/cm^3, CV = 5.32%) systems. CTF systems had 6% lower BD compared to LTP systems and 4.5% lower BD compared to STP systems (Fig. 5. 11).

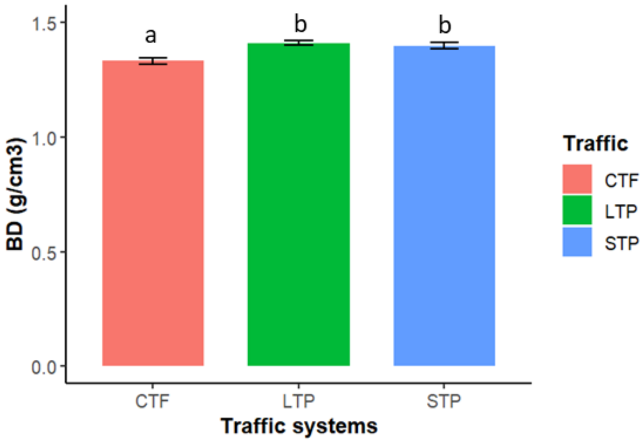


Figure 5. 11 – Main effects of the different traffic systems on BD at 20-30 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

5.4.2.6 BD AT 20-30 CM OVER TIME

-**The effects of traffic over time**: traffic ($p < 0.001$) and collection date ($p < 0.001$) were statistically significant, but the interaction between Traffic and Collection on BD ($p = 0.36$) was not statistically significant (Fig. 5.12).

Within the traffic systems, CTF (1.38 g/cm^3, CV = 6.07%) had significantly lower BD compared to LTP (1.47 g/cm^3, CV = 5.41%) and STP (1.44 g/cm^3, CV = 7.34%) systems. The increased BD values are due to including the 1st soil sample collection for the temporal analysis.

Within the collection dates, the results were the same as above, the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023.

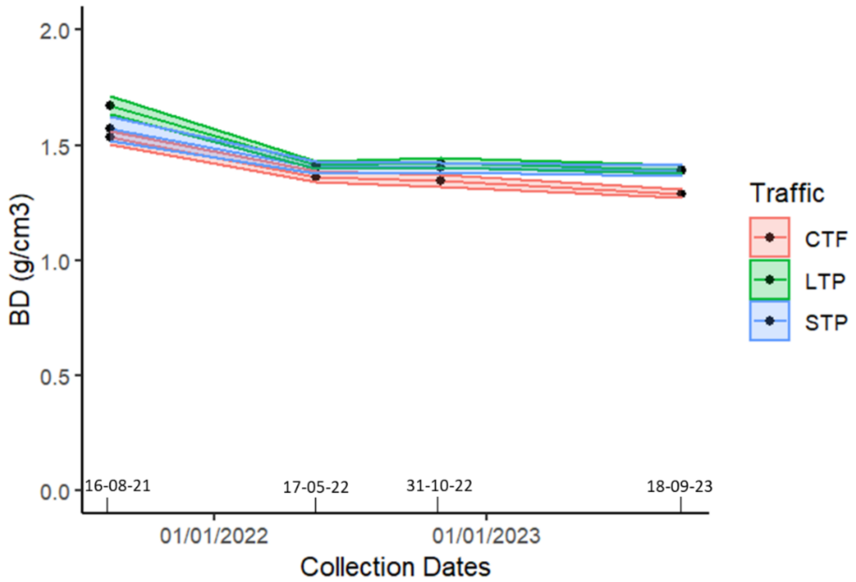


Figure 5. 12 – Main effects of the traffic systems on BD over time, at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between traffic and collection was not significant ($p = 0.36$).

-The effects of tillage over time: at 20-30 cm tillage ($p = 0.23$) and the interaction between tillage and collection date ($p = 0.92$) were not statistically significant. But the BD changed significantly between the collection dates ($p < 0.001$).

Within the collection dates, the results were the same as above, the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023 (Fig. 5.13).

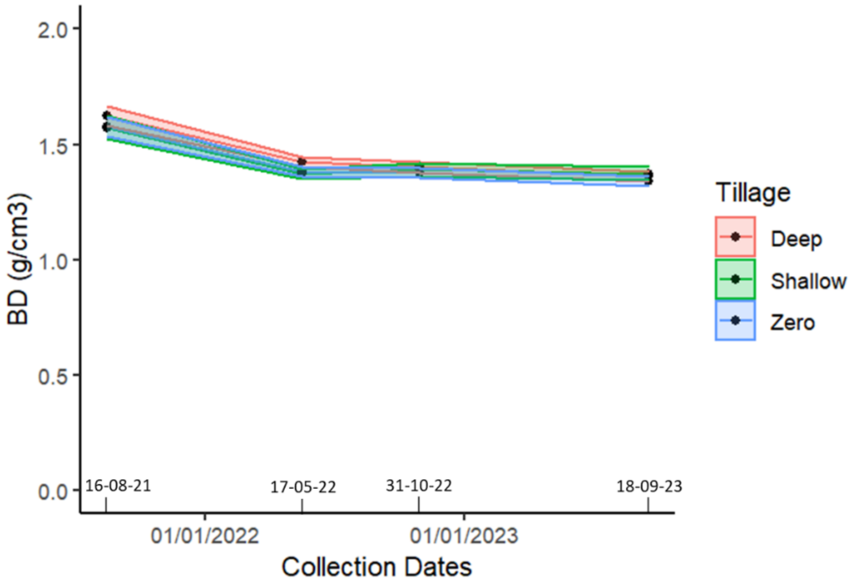


Figure 5. 13 – Main effects of the Tillage systems on BD over time, at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between Tillage and Collection was not significant ($p = 0.92$).

-The effects of the traffic-tillage interaction over time: at 20-30 cm, traffic-tillage (p < 0.001) and collection date (p < 0.001) were statistically significant, but the interaction between traffic-tillage and collection date ($p = 0.42$) was not statistically significant.

Within the collection dates, the results were the same as above, the collection on 16/08/2021 had significantly higher BD when compared to the rest of the collections: 17/05/2022, 31/10/2022 and 18/09/2023.

Within the traffic-tillage interaction, LTP Deep had significantly higher BD (1.49 g/cm^3, CV = 11.4%) compared to CTF (Zero, Shallow and Deep) tillage (avg. 1.38 g/cm^3, CV = 8.9%). And STP Deep (1.49 g/cm^3, CV = 11.4%) had significantly higher BD than CTF Zero tillage (1.37 g/cm^3, CV = 11.3%) (Fig. 5.14).

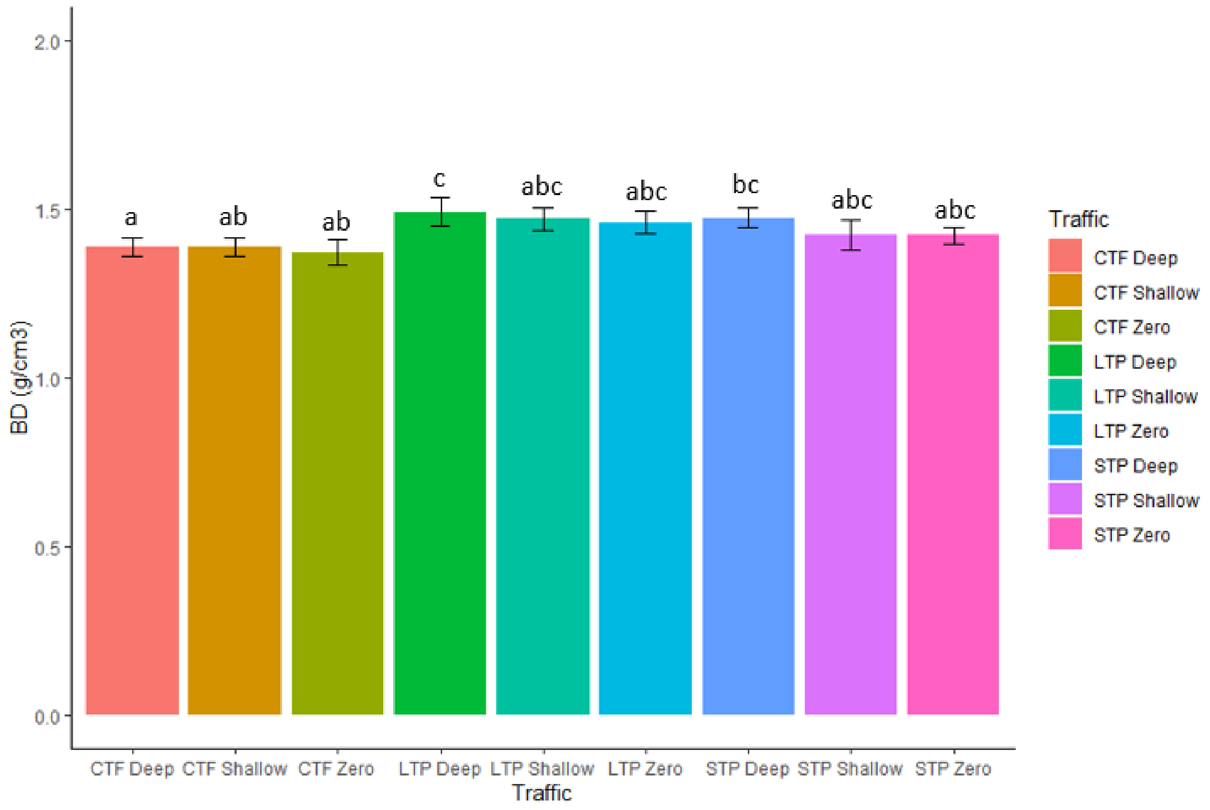


Figure 5. 14 – Main effects of the interaction between traffic and tillage treatments on BD, at 20-30 cm depth. Lines show means (n = 16). Letters indicate significant differences based on ($p < 0.05$).

5.4.2.7. EFFECTS OF TRAFFIC ON BD ACROSS THE SOIL PROFILE

Soil BD was significantly influenced by the traffic ($p < 0.001$) treatments throughout the study (2021-2023) across all depths (0-30 cm). CTF had significantly lower BD than LTP and STP traffic systems across all sample depths. No statistically significant differences in BD were observed between LTP and STP systems at any sampled depth.

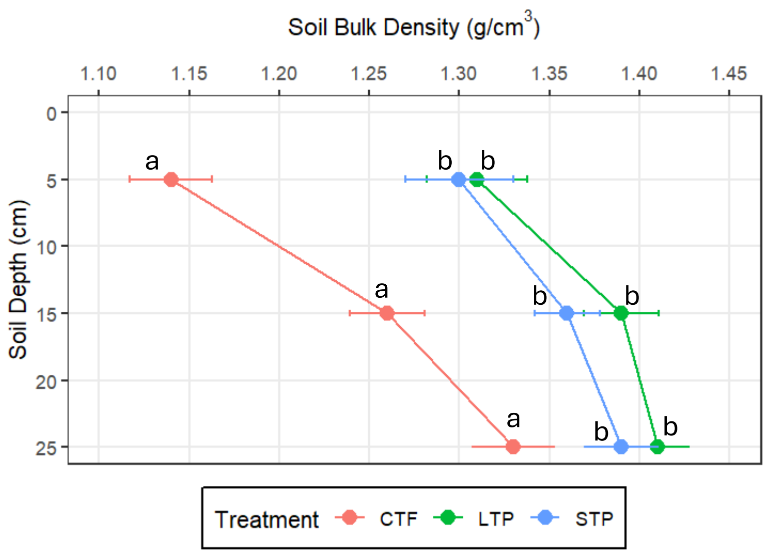


Figure 5. 15 – Main effects of the different traffic systems on BD at 0-30 cm in 10 cm increments. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Points show means from each 10 cm increment (n = 12). Horizontal lines represent standard errors.

BD increased with depth ($p < 0.001$), with 20-30 having significantly higher BD than 10-20 cm, which also had significantly less BD than 0-10 cm. CTF exhibiting the highest increase between 0-10 cm (1.14 g/cm^3) to 10-20 cm (1.26 g/cm^3) (Fig. 5.16).

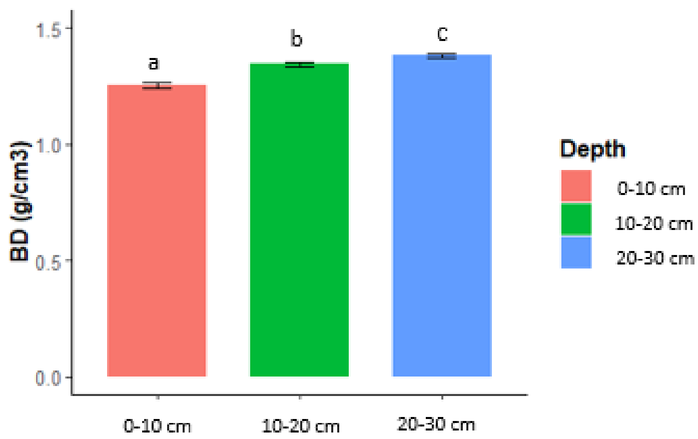


Figure 5. 16 – Main effects of the different Traffic systems on BD at 0-30 cm in 10 cm increments. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Points show means from each 10 cm increment (n = 12). Horizontal lines represent standard errors.

5.4.2.8. EFFECTS OF TILLAGE ON BD ACROSS THE SOIL PROFILE

Soil BD was not significantly influenced by the tillage (p = 0.38) treatments over the course of the study (2022-2023) across all depths (0-30 cm depth) (Fig. 5.17).

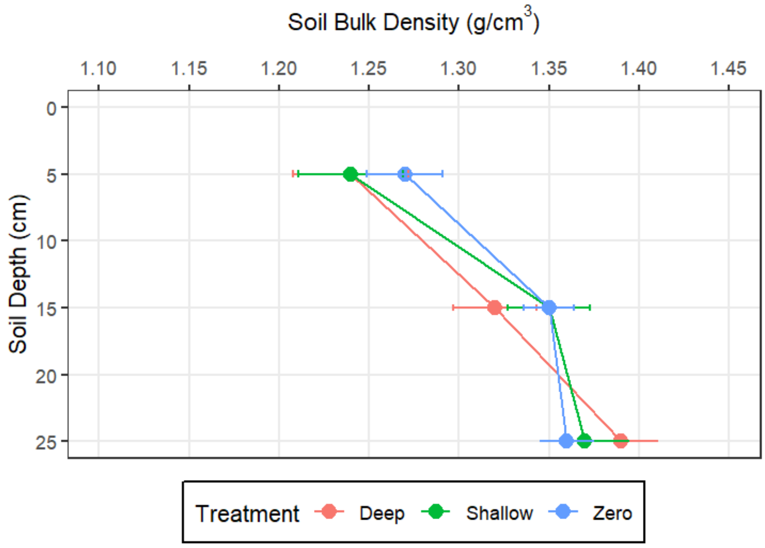


Figure 5. 17 – Main effects of the different Tillage systems on BD at 0-30 cm in 10 cm increments. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Lines show means (n = 12). Horizontal lines represent standard errors.

5.4.2.9. BD AT 0-30 CM DEPTH

The main effects of traffic ($p < 0.001$), depth ($p < 0.001$), the interaction between traffic and depth ($p = 0.003$) and the interaction between tillage and depth ($p = 0.04$) were statistically significant. The main effects of tillage ($p = 0.38$), the interaction between traffic and tillage ($p = 0.35$) and the interaction between traffic-tillage-depth ($p = 0.38$) were not significant.

Within the traffic systems, CTF (1.23 g/cm^3, CV = 6.38%) had significantly lower BD compared to STP (1.35 g/cm^3, CV = 6.09%) and LTP (1.37 g/cm^3, CV = 5.74%) systems.

Within the different depth layers, 0-10 cm (1.25 g/cm^3, CV = 7.62%) had significantly lower BD compared to 10-20 cm (1.34 g/cm^3, CV = 5.26%) and both of these layers had significantly lower BD than 20-30 cm (1.38 g/cm^3, CV = 5.28%).

Within the interaction between traffic and depth, LTP and STP at 20-30 cm and LTP at 10-20 cm had significantly higher BD (avg. 1.40 g/cm3, CV = 5.2%) than all the other treatments and depth combinations except for STP at 10-20 cm. The lowest BD was observed in CTF at 0-10 cm (1.14 g/cm^3, CV = 7.1%) and CTF 10-20 cm (1.26 g/cm^3, CV = 6.4%) (Fig. 5.17). At each depth, CTF exhibited the lowest observed BD of the treatments (Fig. 5.18).

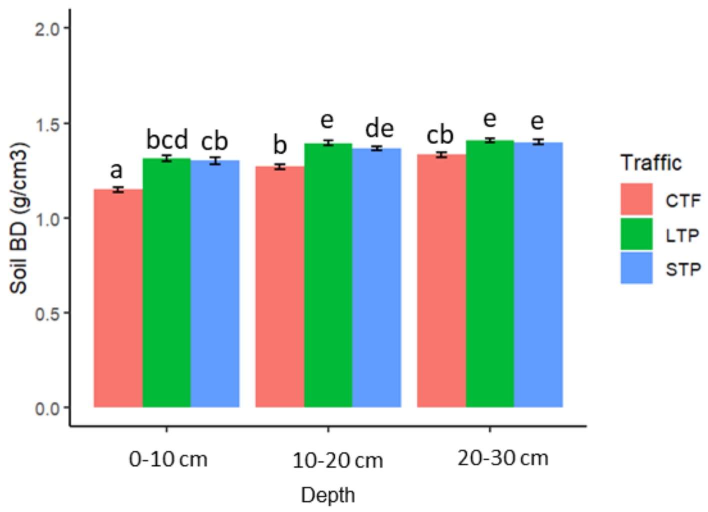


Figure 5. 18 – Main effects of the interaction between the main effect of traffic and depth on BD at 0-30 cm. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

Within the interaction between tillage and depth, Deep and Shallow tillage at 20-30 cm (1.38 g/cm^3, CV= 6.18%) had significantly higher BD compared to Deep at 10-20 cm and Deep, Shallow and Zero at 0-10 cm (avg. 1.27 g/cm^3, CV= 9.3%). The lowest BD values were observed on Deep and Shallow at 0-10 cm (avg. 1.24 g/cm^3, CV = 10.3%) (Fig. 5.19).

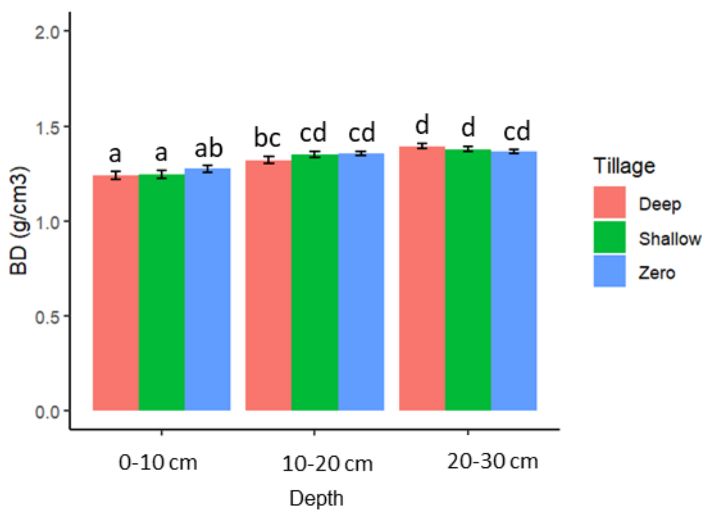


Figure 5. 19 – Main effects of the interaction between the main effect of tillage and depth on BD at 0-30 cm. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

5.4.3. EFFECTS OF TRAFFIC AND TILLAGE ON SOC STOCKS

5.4.3.1. ESM METHOD COMPARISON AND SELECTION

The fixed depth method resulted in higher SOC stocks on LTP and STP systems than the “classical” and “alternative” methods. This is to be expected because those systems had the highest soil BD and therefore contained more soil within the same volume. Both the “classical” and the “alternative or cubic spine model” ESM methods yielded the same outcomes. The only difference observed was when a different reference soil mass was used. Both reference soil masses yielded the same results in terms of treatment significance, but when STP Deep tillage was used as a reference, the SOC stocks were consistently 6 Mg/ha higher for all treatments compared to using CTF Zero tillage as a reference soil mass. Therefore, for comparing the effects of different traffic and tillage systems on SOC stocks, either could be chosen as long as the chosen soil reference mass is kept consistent throughout the experiment and long-term comparison of SOC stocks.

Peng *et al.* (2024) compared different ESM approaches and concluded that the ESM cubic spline model was preferred to estimate SOC stocks. Therefore, the ESM “alternative or cubic spline model”, together with STP Deep tillage as a reference soil mass, was chosen for this study.

Data from the first soil sample collection were excluded from the analysis of the different Traffic and Tillage systems and their interaction on SOC stocks. This is because this collection had higher BD data, due to taking the samples after drilling, using a different auger and not quantifying the stones in the analysis.

5.4.3.2. SOC STOCKS AT 0-30 CM DEPTH

SOC stocks (Mg/ha) (0-30 cm) per treatment were calculated as the sum of the three soil depths (i.e. 0-10, 10-20, and 20-30 cm).

The average SOC stocks were 69.13 Mg/ha. Only the effect of the interaction between traffic and tillage (p = 0.002) had a statistically significant effect on SOC stocks. The overall effect of traffic (p = 0.14) and tillage (p = 0.13) individually did not significantly affect SOC stocks.

Within the interaction between the traffic and tillage systems, CTF Zero tillage (73.95 Mg/ha, CV = 8.72%) had significantly higher SOC stocks compared to CTF Deep (67.39 Mg/ha, CV = 4.26%), CTF Shallow (68.28 Mg/ha, CV = 6.77%), LTP Zero (68.25 Mg/ha, CV = 10.12%), STP Deep (68.09 Mg/ha, CV = 9.29%), STP Shallow (68.28 Mg/ha, CV = 7.72%), STP Zero (67.67 Mg/ha, CV = 8.40%) (Fig. 5.19). LTP Shallow (71.67 Mg/ha, CV = 6.94%) and LTP (Deep) (68.53 Mg/ha, CV = 5.83%) were not statistically significant (Fig. 5. 20).

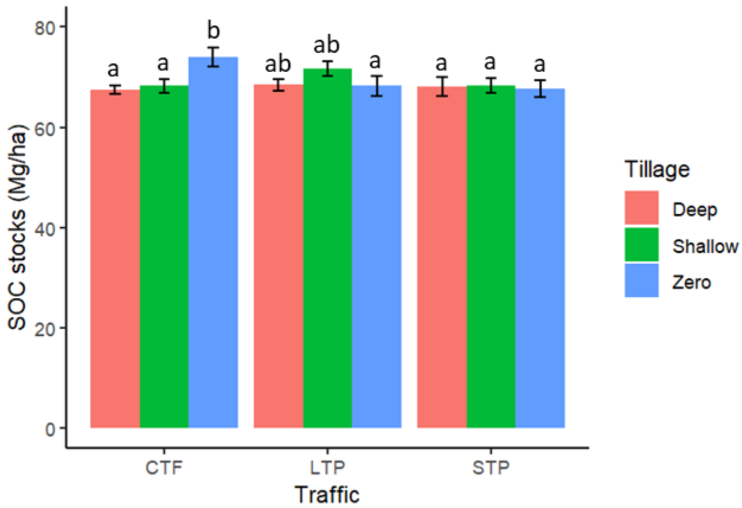


Figure 5. 20 – Main effects of the interaction between traffic and tillage systems on SOC stocks (Mg/ha) at 0-30 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 12). Bars show standard errors.

5.4.3.2.1. SOC STOCKS AT 0-30 CM OVER TIME

-**The effects of traffic over time**: traffic ($p = 0.18$) and the interaction between traffic and collection date ($p = 0.14$) did not significantly affect SOC stocks. Only the main effect of the collection date ($p = 0.02$) was statistically significant.

Within the collection dates, the collection on 03/11/2022 (70.16 Mg/ha, CV = 8.06%) had significantly higher SOC stocks when compared to the collection on 25/09/2023 (67.43 Mg/ha, CV = 6.62%) (Fig. 5.21).

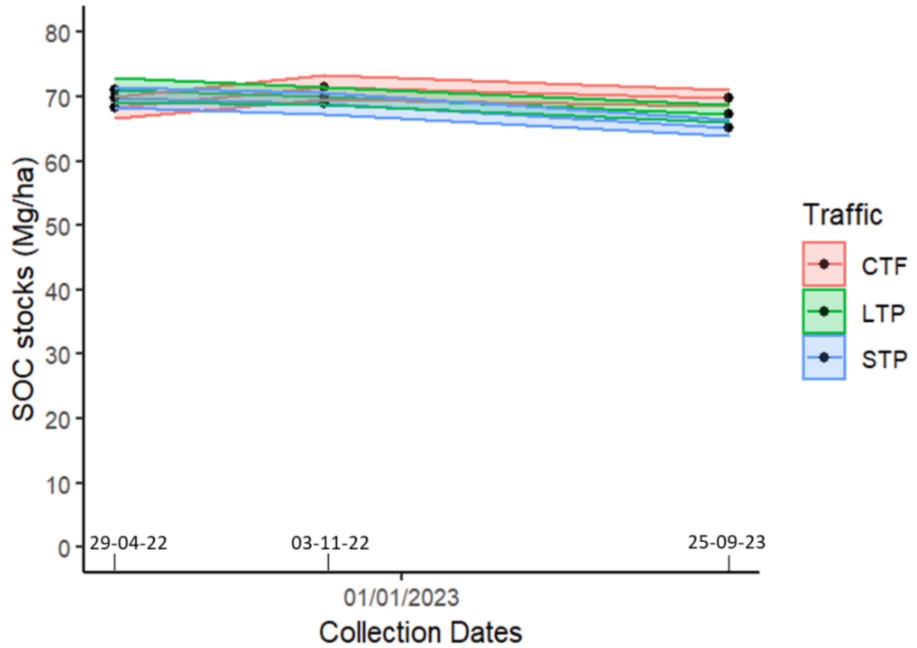


Figure 5. 21 – Main effects of the traffic systems on SOC stocks over time, at 0-30 cm depth (over traffic treatments). Lines show means (n = 12). Letters indicate significant differences based on ($p <$ 0.05). Ribbons show standard errors. The interaction between Traffic and Collection was not significant (p = 0.14).

-**The effects of tillage over time**: at 0-30 cm tillage (p = 0.17) and the interaction between tillage and collection date (p = 0.28) were not statistically significant. But SOC stocks varied significantly between collection dates (p = 0.02).

Within the collection dates, the results were the same as above, the collection on 03/11/2022 (70.16 Mg/ha, CV = 7.76%) had significantly higher SOC stocks when compared to the collection on 25/09/2023 (67.43 Mg/ha, CV = 6.76%) (Fig. 5. 22) (same as above).

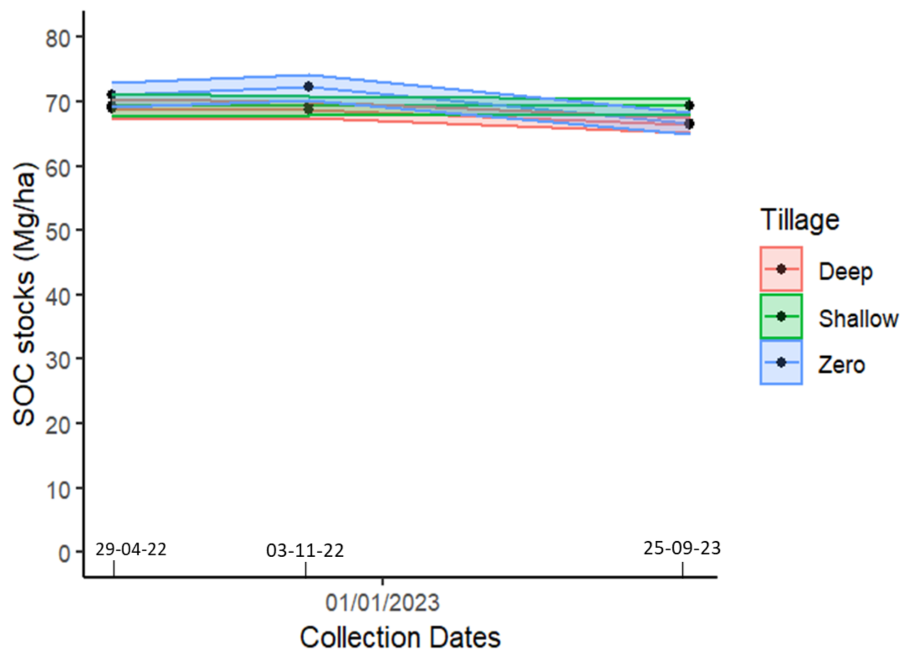


Figure 5. 22 – Main effects of tillage systems on SOC stocks over time, at 0-30 cm depth (over the tillage treatments). Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors. The interaction between tillage and collection was not significant (p = 0.28).

-The effects of the traffic-tillage interaction over time: at 0-30 cm, the main effect of the traffic-tillage interaction (p < 0.002) and Collection date (p = 0.01) significantly affected SOC stocks. However, the interaction between traffic and tillage systems and Collection date (p = 0.26) was not statistically significant (Fig. 5.23).

Within the traffic-tillage interaction, the results were the same as above on the main effects of the interaction between traffic and tillage where CTF Zero (73.95 Mg/ha, CV = 8.70%) had significantly higher SOC stocks compared to all the other treatments (avg. 68.07 Mg/ha, CV = 7.24%) except to LTP Shallow (71.67 Mg/ha, CV = 6.91%).

Within the collection dates, the collection on 25/09/2023 (67.43 Mg/ha, CV = 6.53%) had significantly less SOC stocks compared to the collections on 29/04/2022 (69.79 Mg/ha, CV = 7.76%) and 03/11/22 (70.16 Mg/ha, CV = 7.69%).

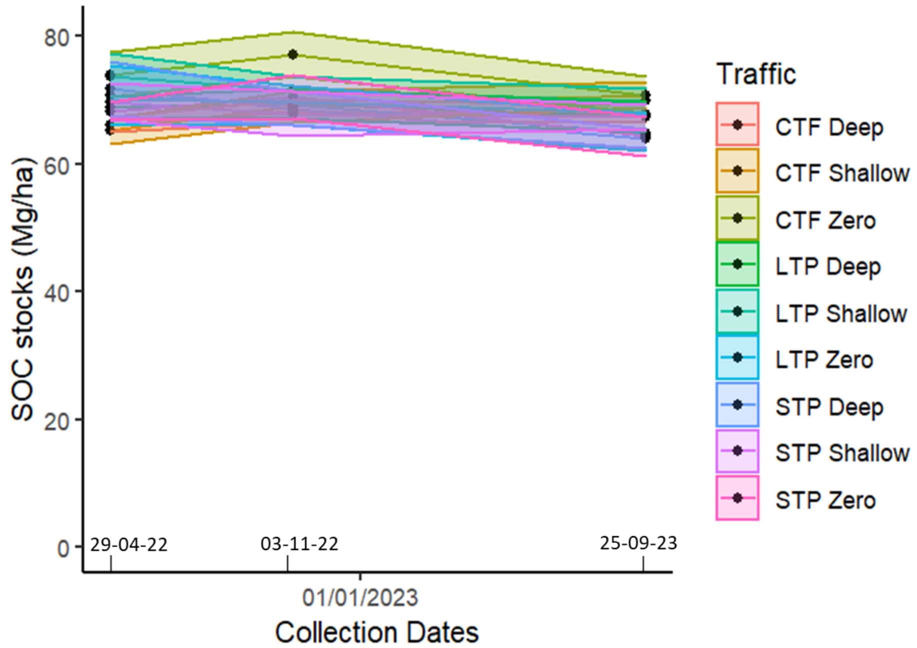


Figure 5. 23 – Main effects of the interaction between traffic and tillage treatments over time on SOC stocks, at 0-30 cm depth. Lines show means (n = 4). Ribbons show standard errors. The interaction between the traffic and tillage systems and collection date (p = 0.26) was not significant.

5.5. DISCUSSION

The sampling strategy used for this analysis aimed to account for key factors such as SOC spatial variation, soil BD, SOC concentrations, and SOC changes over time. SOC changes may occur very slowly over many years or decades, so the long-term nature of this field experiment is important to detect the potential effects of the different traffic and tillage systems on SOC over time. This is because SOC content is highly spatially variable, and changes between years are usually relatively small. Therefore, long periods, often exceeding 5 years (Smith *et al.,* 2020), are needed to be able to detect the signal (i.e. treatment effects) against the background noise (i.e. spatial heterogeneity).

To assess potential differences in SOC concentrations between the 4 mm (i.e. the first soil sample collection) and 2 mm (i.e. the rest of the soil sample collections) sieved soils, both sieved soil fractions were analysed in the final sampling collection. At 0-10 cm depth, a marginally non-significant interaction (p = 0.08) was observed between the interaction of the Traffic-Tillage systems and sieve sizes. At 10-20 and 20-30 cm depths, interactions between the Traffic-Tillage systems and the sieved sizes were not statistically significant (p = 0.83 and p = 0.99, respectively), suggesting consistency in carbon content across both sieving methods at all depths (detailed analysis in Appendix 5. Table 5.1, 2 and 3). However, the SOC stock analysis excluded this initial soil sample collection (4mm sieve) due to methodological inconsistencies in the bulk density measurements: absence of stone correction, sampling after drilling (adding extra compaction) and use of different auger equipment.

5.5.1. INVESTIGATION OF THE SOM TO SOC PEDOTRANSFER FUNCTION

SOC concentration is one of the three metrics required to quantify SOC stocks. Soil scientists have historically estimated SOC from SOM using the Van Bemmelen factor (1.724) where SOC (%) = SOM (%)/ 1.724. However, different conversion factors have been posited, with values varying between 1.4 and 2.5 (based on the assumption that SOM is 40-58% SOC, e.g. Chatterjee *et al.* (2009) and Pribyl (2010). These conversion factors are variable; they are influenced by the amount of SOM, its composition, the degree of decomposition, the amount of clay in the soil, vegetation cover and soil depth (Pribyl, 2010). Therefore, applying any conversion number universally to calculate SOC from SOM, or vice versa, has the potential for introducing error when estimating the C content of a soil (Pribyl, 2010).

This study demonstrated that the use of the Van Bemmelen factor here would lead to an overestimation of SOC content down the soil profile. In the soil used for this experiment, the SOM-to-SOC conversion factor increased with depth: at 0-10 cm, it was approximately 2.09; at 10-20 cm, it increased to 2.31; and at 20-30 cm, it further increased to 2.47. These variations further demonstrate the problematic nature of using a SOM-to-SOC conversion factor and strongly suggest that that approach should not be used; direct measurements of either SOM or SOC are required, depending on the research question or hypothesis being tested.

5.5.2. SOC DOWN THE SOIL PROFILE

There is an increasing global interest in understanding SOC dynamics in cropland because of the potential of using soils as a climate change mitigation strategy (Lal *et al.*, 2018). While the extent of this potential remains debated (Amundson and Biardeu, 2018; Smith *et al.*, 2005), increasing SOC concentrations can also offer additional benefits for soil health (Lavallee, Soong and Cotrufo, 2020), ecosystem services (Smith *et al.*, 2015), and crop yield (Oldfield *et al.*, 2019) and stability (Xu *et al.*, 2019).

The comparison between the SOM (Chapter 4) and SOC concentrations revealed similar trends but also some discrepancies, which further reiterates the need to quantify SOC concentrations directly for calculating SOC stocks.

At 0-10 cm, CTF Zero tillage had significantly higher SOC concentrations than the rest of the treatment combinations. CTF Shallow tillage had the second-highest SOC concentrations. However, when CTF was implemented in conjunction with Deep tillage systems, the potential benefits to SOC concentrations associated with less trafficked soil were negated. This is likely because deep tillage systems redistribute SOC throughout the whole profile, as well as breaking soil aggregates and exposing protected SOC to decomposition. This promotes a relatively faster turnover of SOM compared to less disturbed soils (Balesdent, Chenu and Balabane, 2000; Liang *et al*., 2009). In contrast, under Zero tillage, the SOC accumulates at the surface as part of SOM, which leads to slower decomposition as the material is less exposed to microbial decomposition compared to material that is incorporated into the soil. Additionally, keeping crop residue on the surface protects the soil surface from raindrop impact and reduces soil erosion, and so is considered a beneficial component of sustainable soil management (Zheng *et al.,* 2018*).*

This research showed that 61.2% of the total SOC in the 0-30 cm layer was concentrated in the top 10 cm, on average, across all treatments. This aligns with findings from the Government of Australia (2022) for the south-west region of Western Australia, where approximately 60% of the organic matter within the top 30 cm of the soil was also reported to be located in the uppermost 10 cm of the soil.

5.5.2.1 SOC OVER TIME MAIN

At 0-10 cm, CTF systems stored significantly more SOC in the last two soil sample collections compared to LTP and STP systems. This could be attributed to the effects of the previous crops. Prior to the 03/11/2022 soil collection, a cover crop was grown and left to decompose in the field, followed by a millet crop. However, due to late planting of the millet the crop was not taken to yield and was instead mowned, with the whole crop biomass left in the field (see Chapter 7). These two crops increased the crop residue left on the soil surface compared to the previous years. The last two soil sample collections had significantly higher overall soil SOC than previous collection dates, probably due to the increased amount of crop residue returned. This observation aligns with the previously mentioned increase in SOC in CTF systems.

At 10-20 cm, the different traffic and tillage systems did not significantly affect SOC concentrations. This research also showed that the BD differences between CTF and LTP/STP systems diminished at 10-20 compared to 0-10 cm; it increased across all systems at this soil depth compared to the 0-10 cm layer, likely enhancing the negative effects of soil compaction on SOC sequestration. There is a two-way interaction between SOC concentrations and soil BD, as the amount of SOC concentrations in the soil can affect soil physical properties such as soil porosity and BD (Esmaeilzadeh and Ahangar, 2014), and soil BD can also affect the decomposition rate of SOM, by reducing the pore space and aeration and making it much less accessible to microorganisms (Dungait *et al.*, 2012; Carlesso *et al.*, 2019). However, other biotic and abiotic factors are also at play. Some modelling studies with different SOC concentration levels have tried to incorporate soil porosity and BD into their models and have shown a negative correlation between SOC concentrations and BD (Meurer *et al.,* 2020; Ruehlmann and Körschens, 2009), however, there are not many studies on this area and more research is needed.

At 20-30 cm depth, CTF Zero tillage showed unexpectedly higher SOC content (+12.7%) compared to both STP Deep and CTF Shallow treatments. It also had higher SOM concentrations (+9.23%) compared to CTF Deep and STP Deep tillage systems. This could be due to the enhanced soil structure (Millington, 2016) over the years, promoting better conditions down the soil profile for root development (Kaczorowska-Dolowy, 2022; Kaczorowska-Dolowy *et al.*, 2019), habitat for soil microorganisms, fungal networks and earthworm populations (Kaczorowska-Dolowy *et al.*, 2019), which ultimately enhance the vertical transport of dissolved and particulate organic matter and/or root exudates. Soil aggregates are also not disturbed at this depth, meaning that protected SOC within aggregates remains protected and so is retained, facilitating build-up over time.

5.5.3. SOIL BD DOWN THE SOIL PROFILE

Both agricultural traffic and tillage systems can affect soil bulk density, but the interaction between these factors is complex. It can vary depending on soil type, moisture, organic matter content, vegetation cover, soil fauna activity, etc. However, as these factors were similar across all the plots, with the experiment designed to control for spatial heterogeneity (Chapter 3), observed differences in BD were attributed to the traffic and tillage management practices imposed.

The findings showed that BD increased with soil depth ($p < 0.001$). The average BD at 0-10 cm was 1.25 g/cm^3, at 10-20 cm was 1.34 g/cm^3 and at 20-30 cm was 1.36 g/cm^3. The soil BD values in the 10-20 cm soil layer were 7.2% higher compared to the 0-10 cm layer and the 20-30 cm soil layer were 2.9% higher than the 10-20 cm layer. This agrees with Panagos *et al.* (2024), who also reported that soil BD values in the 10-20 cm soil layer were 5-10% higher compared to the 0-10 cm layer for European cropland and grassland. This increase likely occurs due to the mass of the soil in the topmost layer pressing down onto the deeper layers, combined with less disturbance at this depth from both mechanical (e.g. min till) and biological factors (e.g. microarthropods and earthworms). On top of that, the influence of rainfall and cycles of wetting and drying can also gradually turn the soil to settle, promoting the collapse of macropores and particle reorientation into densification (Blanco-Canqui and Ruis, 2018).

5.5.3.1. MAIN EFFECTS OF TRAFFIC ON BD

BD increased with depth across all traffic treatments. At each soil depth layer, CTF had significantly lower BD than STP and LTP systems across all sampled depths (0-10 cm, 10-20 cm and 20-30 cm). The CTF offered an overall BD improvement of 10.5% compared to LTP and STP traffic systems at 0-30 cm. No significant differences in BD were observed between LTP and STP traffic systems at any depth.

The significant improvement in BD within CTF treatments compared to LTP and STP treatments aligns with the findings of previous studies on the same site (Kaczorowska-Dolowy, 2022; Millington, 2019; Smith, 2017). They also found that LTP and STP treatments were not significantly different down the soil profile, however Smith, (2017), after only 2 years of treatments, found much higher values than the rest: STP (1.62 g/cm^3), LTP (1.59 g/cm3) and CTF untrafficked (1.48 g/cm^3) CTF trafficked (1.68 g/cm^3). These higher values could be due to a slumping effect after all the field was subsoil to 600 mm using Flatlift followed by ploughing to 250 mm (Smith, 2017), previous to the study.

The average BD results of the traffic treatments 0-30 cm within this study (CTF 1.23 g/cm^3, LTP 1.37 g/cm^3 and STP 1.35 g/cm^3) were also lower than the ones reported by Kaczorowska-Dolowy (2022) (CTF 1.31 g/cm^3, LTP 1.42 g/cm^3 and STP 1.45 g/cm^3) and Millington (2019) (CTF 1.33 g/cm^3, LTP 1.41 g/cm^3 and STP 1.47 g/cm^3). This can, however, be explained because they all fail to account for the stones when calculating bulk density. Although the rock fragment within this study was not very big (~6%), it can lead to some overestimation of the bulk density values.

Within this study, LTP had a 40% reduction in wheel pressure compared to STP treatments, while Kaczorowska-Dolowy (2022), Millington (2019) and Smith (2017) had a 30% reduction. The lack of significant differences in BD between STP and LTP could be due to the low number of samples, i.e. one per treatment, and the location chosen to represent the treatment, i.e. the permanent traffic lane (which represents 2-3 wheel passes depending on the tillage treatment, over the growing season). Most of the compaction happens after the first wheel pass, so if only one pass were used, there might be a greater difference between LTP and STP in BD. However, multiple wheel passes may potentially negate the soil compaction mitigation effects of using LTP on BD, as the repeated traffic introduces additional compaction to the already compacted soil, diminishing their difference. Other studies comparing the effects of LTP with STP (low ground pressure tyres with standard pressure tyres) in cropland have reported significantly lower BD values under LTP after “a single pass” of the tyres (Antille *et al.,* 2013), and lower soil penetration resistance (associated with lowed BD) for three tillage systems and two crop rotations (maize and soybean) (Shaheb *et al*., 2024).

5.5.3.2. MAIN EFFECTS OF TILLAGE ON BD

The effects of tillage on BD were not significant among all the soil depths, meaning that the effects of tillage on BD in the agricultural systems used in this study, under the local experimental conditions, were only temporary; by the end of the growing season (when the samples were taken), the soil has settled to similar BD values between the treatments regardless of the crop or how many years the treatments have been applied. This lack of observed significant differences in the tillage treatments on BD was also reported by Kaczorowska-Dolowy, (2022) and Millington, (2019) on the same field site study, as well as other authors such as Jabro *et al*., (2016) and Martinez *et al.,* (2008). However, other authors have also stated that zero and reduce tillage practices increased soil BD when compared to other conventional and deep tillage practices (Tian *et al*., 2022; Ji *et al.,* 2013; Gathala et al., 2011; Lampurlanés and Cantero-Martinez, 2003). This difference could be due to different reasons, such as soil type (some soils might be more prone to compaction after zero and shallow tillage than others), weather conditions (variations in rainfall, temperature, freeze-thaw cycles, can affect soil structure and how it responds to tillage), types of crops and its rotations, use of cover crops, residue management, different equipment used and timing of sampling, measuring methods, or the intensity of the tillage applied as “conventional” and “deep” tillage are not well-defined terms and can involve different levels of soil disturbance.

4.5.3.3. MAIN EFFECTS OF THE INTERACTION BETWEEN TRAFFIC AND TILLAGE ON BD

The main effects of the traffic and tillage interaction on BD were not statistically significant at 0-10 or 20-30 cm but were statistically significant (p = 0.04) at 10-20 cm. At 10-20 cm, there were complex interactions between the traffic and tillage systems, with LTP Shallow and Zero tillage systems showing significantly higher BD compared to CTF Zero, Shallow and

Deep tillage systems and STP Zero and Deep tillage systems also showing significantly higher BD than CTF Shallow and Deep tillage systems. This was because CTF treatments were sampled in the non-trafficked crop area (representing 70% of the plot area), while LTP and STP were sampled in the permanent wheel ways. This sampling strategy was designed to represent field-scale conditions (where CTF systems can achieve up to 85% non-trafficked crop area) despite the constraints of plot-scale experimentation. LTP Shallow at 10-20 cm showed the highest soil BD 1.42 g/cm^3. This could be because the Shallow tillage treatments were only tilled to 10 cm depth. Therefore, a harder pan layer could be forming at 10-20 cm with the lower pressure from the wheelings not compacting the soil as much to deeper layers. While for the STP Shallow tillage treatments, the compaction might be more uniform across the soil profile, developing a harder pan layer down to 30 cm due to the increased pressure from the wheelings.

4.5.3.4. MAIN EFFECTS OF BD OVER TIME

The BD results of the last three soil sample collections (17/05/2022, 31/10/2022 and 18/09/2023) showed no statistically significant differences, meaning that the BD results were relatively consistent over time. The first soil sample collection on 16/08/2021 had significantly higher BD than all the rest of the collections due to methodological measurements: i.e. the different sample time within the cropping season compared to the other sample collections (adding two extra compaction events). This collection also failed to account for the stones in the analysis.

5.5.4. SOC STOCKS (0-30 CM)

5.5.4.1 SOC STOCKS ASSESSMENT

To quantify the potential of the different agricultural management systems to sequester carbon, this study compared SOC stocks calculated using various methods: site-specific fixed depth (FD) versus equivalent soil mass (ESM) approaches. For the FD approach, SOC stocks are calculated as the product of soil bulk density, depth/ area and SOC concentration. However, this method can overestimate SOC in the treatments with greater bulk densities (Wend and Hauser, 2013).

The ESM approach has been widely discussed as the more appropriate method, as different management practices, such as traffic and tillage, influence bulk densities and soil masses. However, there is no standardised protocol for the modelling procedure, and they vary considerably (e.g. Ellert and Bettany, 1995; Von Haden *et al.,* 2020; Wendt and Hauser, 2013).

The results showed that the fixed depth method resulted in higher SOC stocks in LTP and STP systems compared to the ESM “classical” and “alternative” methods, attributable to the higher BD of LTP and STP systems, containing more soil mass within the same volume. However, the EMS “classical” and “alternative cubic spline model” produced identical outcomes.

5.5.4.2. SELECTION OF THE REFERENCE SOIL MASS(ES)

ESM-based SOC stock calculations require selecting a “reference soil mass” for each depth. However, no standardised criteria exist for the reference mass selection. When comparing several treatments, the reference soil mass can be calculated as the average mass of soil samples taken from a baseline (Ferchaud, Chlebowski and Mary, 2023), control or “business-as-usual” treatment (von Haden, Yan and DeLucia, 2020). Some studies have used the ploughed plots as a reference soil mass when comparing reduced tillage with conventional ploughing (Krauss *et al.,* 2022).
To elucidate the potential implications arising from using any of these different and contrasting soil reference masses, our study calculated the ESM approaches using both: a control (the un-trafficked soil of CTF Zero tillage) and the “business-as-usual” conventional plough treatment (STP with Deep tillage) as reference soil masses.
Both reference soil masses yielded the same results in terms of treatment significance. However, using STP Deep tillage as reference consistently produced SOC stocks 6 Mg/ha higher across all treatments, versus the CTF Zero tillage reference. For valid traffic and tillage system comparisons, either reference soil mass is suitable, provided it remains consistent throughout experimental and long-term SOC stock analyses.

5.5.4.3. SOC STOCKS (0-30 CM)

The highest SOC stocks, reported on an EMS basis following the alternative or cubic spline model with STP Deep as a reference soil mass, were observed in CTF Zero tillage, at 73 Mg/ha, storing 5 Mg/ha (or 7.3%) more than all the other treatment combinations, except for LTP Shallow (71.7 Mg/ha) and Deep tillage (68.5 Mg/ha) that were not significantly different. The lowest SOC stocks were observed in STP Zero tillage, at 67.7 Mg/ha and CTF Deep tillage, at 67.4 Mg/ha. The lack of significant differences in SOC stock between LTP (Shallow and Deep) and CTF Zero tillage and the rest of the treatment combinations suggests that LTP systems occupy an intermediate position in terms of soil carbon storage, though this may also reflect insufficient statistical power to detect real differences. However, this positioning could indicate that LTP (Shallow and Deep) might offer a compromise management strategy with lower implementation costs than CTF Zero tillage systems. It can also suggest that occasional tillage under low tyre pressure conditions may be less detrimental to SOC storage than conventional approaches. Long-term monitoring will be essential to confirm whether these systems maintain SOC storage over time.

These results are consistent with Martinez *et al*. (2016) who also reported that the EMS SOC stocks (0-50 cm) on a sandy loam soil and long-term field experiment (20 years) in Switzerland, were lowest for a deep till system (mouldboard plough; 70 Mg/ha) and highest in a no till system (73 Mg/ha). These findings also align with Cooper *et al.* (2021), who reported a 6 Mg/ha increase in SOC stocks under zero tillage compared to conventional tillage after 6-10 years. Similarly, in a systematic review, Haddaway *et al*. (2017) also concluded that soils under zero tillage for more than 10 years, stored 4.6 Mg/ha more SOC (0-30 cm) than those under conventional agricultural practices. However, these findings did not specify where the samples were taken within the system. Zero tillage systems typically result in 45% of the field area being trafficked compared to 85% in conventional systems (Kroulik, 2009). Depending on the sampling strategy or the number of samples taken, most of the samples could have been taken from a non-trafficked area inside the Zero tillage system. If this was the case, then the results would be similar to our CTF Zero tillage systems.

This investigation revealed that only the interaction between traffic and tillage was statistically significant, meaning that traffic or tillage alone did not significantly affect SOC stocks but their interaction did. This aligns with Rosinger *et al*. (2023), who reported that tillage intensity on its own was a weak predictor of SOC sequestration potential. Another study by Mary *et al.* (2020) in Northern France also reported that SOC storage and mineralisation rates were affected more by the carbon inputs rather than tillage intensity in a 47-year old tillage experiment. Martinez *et al*. (2016) in Switzerland also reported no significant differences in SOC stocks between no-till and mouldboard plough tillage treatments after 20 years. These findings mirror other studies in central and northern Europe (i.e. also with cold and temperate climates) such as Schjønning and Thomsen, (2013), and Hermle *et al.* (2008), corroborating the limited or variable role of tillage intensity on SOC storage when considered in isolation from other related system variables such as residue management or cover cropping.

This study showed that combining CTF with Zero tillage (with residue return and two winter cover crops over a 12-year period) on sandy loam soil, under UK climatic conditions, resulted in an additional storage of 5 Mg/ha SOC stocks (0 - 30 cm). Comparatively, Rosinger *et al.* (2023) reported a 14.3 Mg/ha increase in SOC stocks in the top 35 cm of soil under conservation agriculture compared to conventional farming systems over a mean period of 26 years in North-Eastern Austria. These findings show the critical importance of considering the temporal aspect of SOC storage following a management system change. The rate of SOC sequestration is not constant, typically being highest in the first years after a management change is introduced and slowing down over time as the soil approaches a new equilibrium (BSSS, 2023). This scenario was calculated by Bayene *et al*. (2023), where 5 Mg/ha of organic residues were applied to the soil every year for 50 years in a temperate climate. The results showed that the rate of increase of SOC stocks, while initially high, progressively diminished over time until it reached a constant value after approximately 20 years. This equilibrium is derived from the balance of SOC inputs (e.g. crop residues and rhizodeposition) and outputs (primarily resulting from the microbial decomposition of SOM) from soil systems, which are also influenced by different environmental factors such as temperature, soil moisture and texture. It seems likely that in cropland, C inputs are usually not high enough and/or the mineralisation rate, erosion, or leaching are too fast due to disturbances such as tillage, to achieve a significant accumulation over time. There is always a limit on the amount of C inputs that a farmer can feasibly incorporate into the system from crop biomass (Janzen *et al*., 2022) and organic amendments, as well as important socio-economic constraints such as farmers being incentivised to sell straw when prices are high rather than returning it to the soil.

Another important consideration is that if the farmer stops incorporating SOM into the field, the SOC storage will decline progressively faster at the beginning, and a slower the decline over time (Bayene *et al*., 2023).

If the management systems of this study, with crop residue retention, cover cropping, and diverse crop rotation, are compared to another system with mouldboard plough, with no cover crops or crop residues, a greater difference in SOC stocks could potentially be observed.

This study investigated the single-measure effects of traffic and tillage systems and their interaction on SOC storage, by maintaining consistency in other influential factors affecting SOC (e.g. soil type, climate, N fertiliser and SOM input: crop rotation, crop residues, cover crops or organic fertiliser) across all plots. However, SOC stocks are affected by various interacting factors as discussed above. Variations in these factors could lead to differences in SOC storage. Rosinger *et al.* (2023) identified soil texture and initial C content of the soil as the strongest predictors of SOC stock differences between conservation and conventional farming systems in long-term studies (26 years). In particular, coarse-texture soils were more responsive to a system change towards conservation agriculture, sequestering significantly more SOC than on fine and medium-texture soils. Therefore, the rate of SOC sequestration with the adoption of the recommended traffic and tillage management practices will depend on many variables such as time since the new management practice was introduced, soil texture and structure, climate, type of crop, and other management practices used (Lal, 2004).

This research showed complex interactions between the traffic and tillage systems. If applied together, CTF with Zero tillage significantly increased SOC stocks. However, CTF systems combined with Shallow or Deep tillage systems didn't increase the SOC stocks. In this case, the tillage-induced soil disturbance of Shallow or Deep tillage systems seems to negate the beneficial effects of reduced soil compaction of CTF systems on SOC stocks. Similarly, in STP systems combined with Zero or Shallow tillage, the increased soil compaction likely offsets the SOC sequestration benefits of reduced tillage.

LTP shallow tillage, although not significantly different from CTF Zero, had the second highest SOC stocks. This could be attributed to the relatively similar soil disturbance depths between Shallow tillage (10 cm) and the disc used in zero tillage systems (5 cm).

Globally, there is a slow shift from conventional to conservation or regenerative agricultural practices (Kassam, Friedrich and Derpsch, 2019). Although there are different definitions of regenerative agriculture, often the traffic component in soil disturbance is omitted (Newton *et al.,* 2020). Our findings demonstrate complex interactions between the different traffic and tillage systems on SOC storage, suggesting that to maximise SOC sequestration, agricultural management practices using CTF combined with Zero tillage should be adopted, together with other conservation management practices.

5.5.4.3.1 SOC STOCKS (0-30 CM) OVER TIME

The main effects of traffic and tillage systems over the last 3 years showed that only the main effect of the soil sample collections was statistically significant, which means that SOC stocks were affected by the different traffic and tillage systems equally over this time frame, as no statistically significant treatment effects were observed.

On average, across all treatments, from the collection on 03/11/22 (70.16 Mg/ha, CV = 8.06%), there was a 4% decrease in SOC stocks by the time of the next crop and last soil sample collection on 25/09/2023 (67.43 Mg/ha, CV = 6.62%). This observed decrease correlates with the SOC concentration results. It can also be attributed to the higher SOC stocks on CTF Zero tillage (on collection 03/11/2022). While SOC stock changes typically occur over long periods, this decrease over time could be associated with the sampling time and type of crop. The soil sample collection on 03/11/2023 coincided with the flowering stage of millet, which was planted late in the season. The green millet crop could have potentially contributed to higher biomass, resulting in increased litter and rhizodeposition compared to other cereal crops in post-harvest conditions.

5.5.5. LIMITATIONS

As discussed in Chapter 4, accurate measurements of SOM or SOC are challenging due to their spatial heterogeneity (both vertically and horizontally; Poeplau and Greorich, 2023). Temporal variability due to changing climatic conditions (having wetter or drier years) also affects SOC sequestration rates (Mitchell *et al*., 2024). A large number of samples are needed to account for spatial variability and detect small, significant differences between the different systems. However, this is not always viable due to practical and cost constraints. In a recent study by Mitchell *et al*. (2024), analysing the carbon credit projects in Australia it was also concluded that "carbon credit schemes should have a minimum measurement period of at least five years to reduce the impact of interannual rainfall variability on SOC accumulation and reduce the risk of reporting false SOC gains". This also agrees with the conclusions drawn by Smith *et al.* (2020), who reported that long periods of time, often exceeding 5 years, are necessary to reliably detect the signal (i.e. treatment effects) against background noise (i.e. spatial heterogeneity).

5.5.6. FUTURE RESEARCH

As mentioned previously, SOC storage can vary depending on many factors such as the amount of SOM inputs and types, soil type, climatic conditions, vegetation cover, and management practices such as traffic and tillage. Developing a robust understanding of soil carbon storage and monitoring techniques is important for developing future policies. However, there are still many challenges, such as the slow rate of SOC change and uncertainty regarding the persistence of sequestered SOC over time (Wiese *et al*., 2021). Furthermore, systems eventually reach an equilibrium between carbon inputs and outputs and can also saturate. However, the period required to reach this equilibrium point and the SOC concentration of the equilibrium vary between sites, as it is affected by many factors.

Therefore, further research is required to better understand SOC dynamics long-term and be able to tailor management practices to local conditions and needs.

5.6. CONCLUSIONS

The initial hypothesis that "agricultural traffic increases soil BD and affects SOC content and SOC stocks; therefore, CTF and LTP systems won't increase soil BD as much as STP, storing higher depth-specific SOC content and SOC stocks" was partially supported by our results, which showed that soil BD increased with soil depth under all treatment combinations. CTF systems had significantly lower BD at all soil depth layers compared to LTP and STP systems. There was a 10.5% improvement in BD at 0-30 cm when using CTF systems compared to LTP and STP systems. There were no significant differences in BD between LTP and STP at all soil depth layers. However, the overall effect of the traffic systems did not significantly affect SOC stocks on ESM at 0-30 cm.

The results partially supported the second hypothesis that "soil disturbance by tillage increases soil BD and SOC decomposition; therefore, reduced tillage will lead to lower soil BD and higher depth-specific SOC content and SOC stocks". The overall effect of tillage systems was only significantly affected at 10-20 cm depth, where Deep tillage systems had significantly lower BD than Zero tillage systems. However, at 0-30 cm, soil BD was not significantly influenced by the tillage treatments over the course of the study (2022-2023). The overall effect of tillage systems did not significantly affect SOC stocks on ESM at 0-30 cm.

The findings partially align with the third hypothesis that "the interaction between traffic and tillage systems will affect BD, SOC content and SOC stocks at different depths". The interaction between traffic and tillage systems only affected soil BD at 10-20 cm depth layer, where LTP with Zero and Shallow tillage had significantly higher BD than CTF (Zero, Shallow and Deep). And STP Zero and Deep tillage systems had significantly higher BD than CTF Shallow and Deep tillage systems.

SOC stocks on ESM at 0-30 cm showed significant interactions between the traffic and tillage systems. After 12 years of continuous traffic and tillage practices, together with other conservation management practices, the combination of CTF with Zero tillage management systems stored 5 Mg/ha more SOC stocks than the rest of the treatment combinations, except for LTP Shallow and Deep tillage systems, which were not statistically significant. Therefore, this study showed that by combining CTF with Zero tillage SOC stocks could be increased in the top 30 cm of sandy loam soil, in temperate Europe.

Neither traffic nor tillage systems showed significant main effects on SOC stocks, suggesting that these individual management practices alone were insufficient to influence soil carbon storage. This study also highlighted that the effectiveness of CTF systems for SOC storage depended on the tillage intensity. After 12 years at 0-30 cm, CTF systems, if combined with Shallow or Deep tillage systems, showed no significant improvement in SOC stocks, possibly because of higher SOC mineralisation.

CHAPTER 6

THE EFFECT OF TRAFFIC AND TILLAGE MANAGEMENT SYSTEMS ON SOIL ORGANIC MATTER FRACTIONS (POM AND MAOM)

6.1. INTRODUCTION

There is currently a strong focus on increasing carbon farming management practices to maintain or increase SOC stocks. Chapter 5, examined how SOC stocks were affected by the different traffic and tillage management systems. However, to better understand how the different management practices affect SOC dynamics and their stabilisation over time, we need to look at the distribution of SOC into the different SOM fractions (POM and MAOM).

Arable soils with annual crops store most C in the MAOM fraction. In contrast, the POM fraction generally exhibits relatively low levels of C due to the harvesting and removal of plant biomass, low levels of root input due to growing annual crops, and frequent tillage destroying soil aggregates and aerating soils, which exposes POM-C to decomposition, promoting a fast turnover of any crop input into the soil (Cambardella and Elliott, 1992; Lugato *et al.,* 2018, 2021). Therefore, the POM fraction is considered more dynamic and vulnerable to decomposition than the MAOM fraction and can be more easily affected by a change in management practices (Lavallee, Soong and Cotrufo, 2020). This is why the POM fraction has also been identified as a sensitive indicator of the agronomic function at field scale *(*Schipanski, Drinkwater and Russelle, 2010*)* and for short-term changes in SOC stock following land use change *(*Eze *et al.,* 2023). In contrast, the MAOM fraction is more stable and less susceptible to decomposition, although it can also be affected by the steady-state/ equilibrium, positive priming effects and saturation (Castellano *et al*., 2015; Kuzyakov, Friedel and Stahr, 2000; West and Six, 2007).

Both POM and MAOM fractions are important for the effective management of SOC stocks in cropland because they are formed, protected, and lost through different pathways (Lavallee, Song and Cotrufo, 2019), so they respond differently to environmental changes (e.g., Salonen *et al*., 2024; Angst *et al*., 2023; Rocci *et al*., 2021) and management practices (Sampson *et al.,* 2020). Some of the recommended strategies to increase SOM in cropland systems include: (1) organic and inorganic amendment; (2) crop straw retention; (3) reduced tillage intensity (Lin *et al*., 2023) and other regenerative agriculture practices such as cropping diversity, integrating crop-livestock systems and keeping the soil covered and with living roots through the year *(e.g.,* (Prairie, King and Cotrufo, 2023; MacLaren, et al., 2022*).* However, not much is mentioned about reducing traffic-induced soil compaction by including different traffic management approaches. No prior research appears to have explored the main effects of different traffic systems and their interaction with different tillage systems on SOM fractions (POM and MAOM). This data could provide insights on how to enhance SOC sequestration, improve soil health and optimise crop production while transitioning towards more sustainable agricultural practices.

Carbon sequestration is affected by N availability (Tian *et al*., 2006); therefore, the C/N ratio will also be analysed for POM and MAOM fractions to investigate their formation pathway and how it might be affected by the different treatments.

6.2. AIM AND HYPOTHESIS

This chapter aims to quantify the effects of alternative traffic systems and their interaction with different tillage systems on the SOM fractions (POM-C and MAOM-C) dynamics in a long-term field experiment over the last three years (2021-2023).

The hypothesis for this chapter are:

1. Traffic-induced soil compaction will store less POM-C and MAOM-C content, therefore, reduced traffic and wheel pressure will lead to depth-specific higher storage of POM-C and MAOM-C content.
2. Tillage-induced soil disturbance increases SOM decomposition, therefore, reduced tillage will lead to depth-specific higher storage of POM-C and MAOM-C content.
3. The interaction between traffic and tillage systems will affect POM-C and MAOM-C content at different depths.

6.3. METHODOLOGY

6.3.1. LABORATORY ANALYSIS

6.3.1.1 SOM FRACTIONING

POM and MAOM fractionation were undertaken using the simplified method of Midwood *et al*. (2021) after Poeplau *et al*. (2018). In short, 50 g of air-dried 2 mm-sieved soil from each depth and treatment was placed in a glass jar with 150 ml of ultra-pure water and seven 5-mm diameter glass beads. Jars were then shaken at 200 rpm for more than 16 h. The soil suspension was then separated using a 50 μm sieve. Both fractions (i.e., the fraction that passed through the 50 μm sieve, and the fraction that did not) were collected in separate trays and dried in the oven at 60°C for more than 16 h (Fig. 6.1).

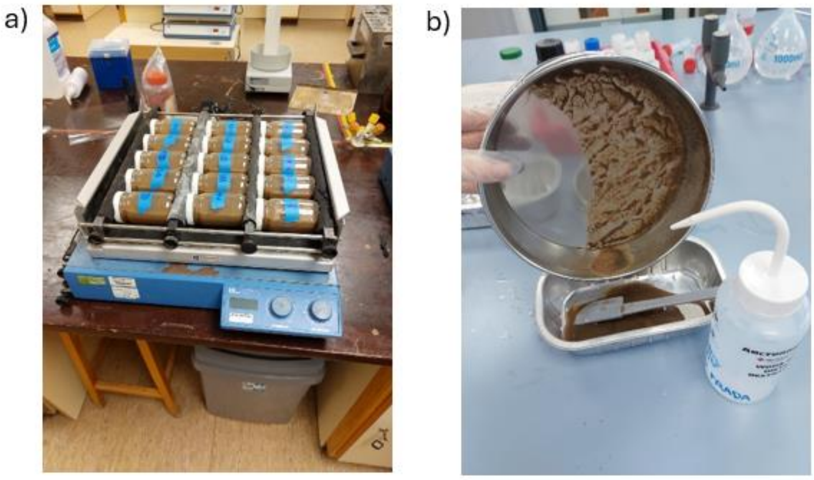


Figure 6. 1 – **a)** Glass bottles with 50 g of sieved soil (<2 mm) plus 150 ml ultra-pure water, shaking > 16 hr and **b)** Collecting the POM fraction (2000 mm – 50 μm) retained on top of the 50 μm sieve. The MAOM fraction (< 50 um) is the soil solution that passed through the 50 μm sieve.

The first soil sample collection (21/08/2021) was excluded from this analysis due to the absence of SOM fractionation.

6.3.1.2 POM-C AND MAOM-C EVALUATION

The POM and MAOM fractions, from all treatments and depths, were individually ball-milled to the consistency of flour and homogenised using a TissueLyser II (QIAGEN) with a ball cartridge prior to analysis (Fig.6.2). The POM-C and MAOM-C were evaluated using a subsample of 0.15 g in a tin cup on a Carbon/ Nitrogen analyser LECO CN828 series (LECO Instrument Ltd, UK) through direct combustion (950˚C). Due to the large number of samples, only one sample per treatment was analysed unless the sample got stuck or exhibited anomalous C levels, random repetition checks were performed at the end of every analysis.

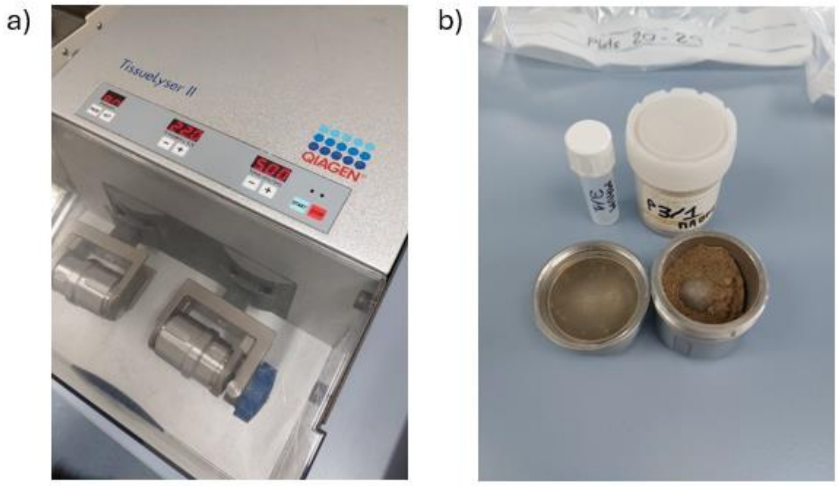


Figure 6. 2 – **a)** Ball-mill cartridge used TissueLyser II (QIAGEN) to mill soil samples to flour consistency. **b)** Opened ball cartridge with soil after milling.

6.3.1.3 POM-N AND MAOM-N AND C/N RATIO EVALUATION

POM-N, MAOM-N and the C/N ratio of the fractioned samples were determined with the same CN analyser as above. POM C/N ratio was calculated as POM-C divided by POM-N and MAOM C/N ratio as MAOM-C divided by MAOM-N. The atomic mass of C and N were incorporated into the calculations.

6.4 RESULTS

6.4.1 EFFECTS OF TRAFFIC AND TILLAGE ON SOM FRACTIONS

The results from the physical fractionation of SOM from 50 g of air-dried, 2 mm-sieved soil samples across treatments and depths (2022-23) showed that on average, the POM fraction constituted 69.5% and the MAOM fraction 29.53% of the sampled soil weight at 0–30 cm. On average the accumulated weight of the two separate fractions POM (2000–50 µm) and MAOM (> 50 µm) accounted for 99.09% of the initial soil weight, illustrating good overall recovery.

All the measured C was taken to represent OC, due to the low soil pH (mean 5.4, at 0-30 cm), indicating the absence of carbonate minerals (Mikha *et al.*, 2010).

The average SOC concentration of both SOM fractions (POM-C and MAOM-C) decreased with soil depth (Fig. 6.3). The MAOM-C fraction contributed to 80.20% and POM-C contributed to 19.78% of the total SOC at 0-30 cm. However, if we look at the individual soil depth layers, the MAOM-C contribution to the total SOC increased with soil depth.

At 0-10 cm, MAOM-C contributed 74.77% of the total SOC, and POM-C contributed 25.22%. At 10-20 cm, MAOM-C contributed 81.27% and POM-C 18.72% of the total SOC, and at 20-30 cm, MAOM-C contributed 84.57% and POM-C 15.42% of the total SOC.

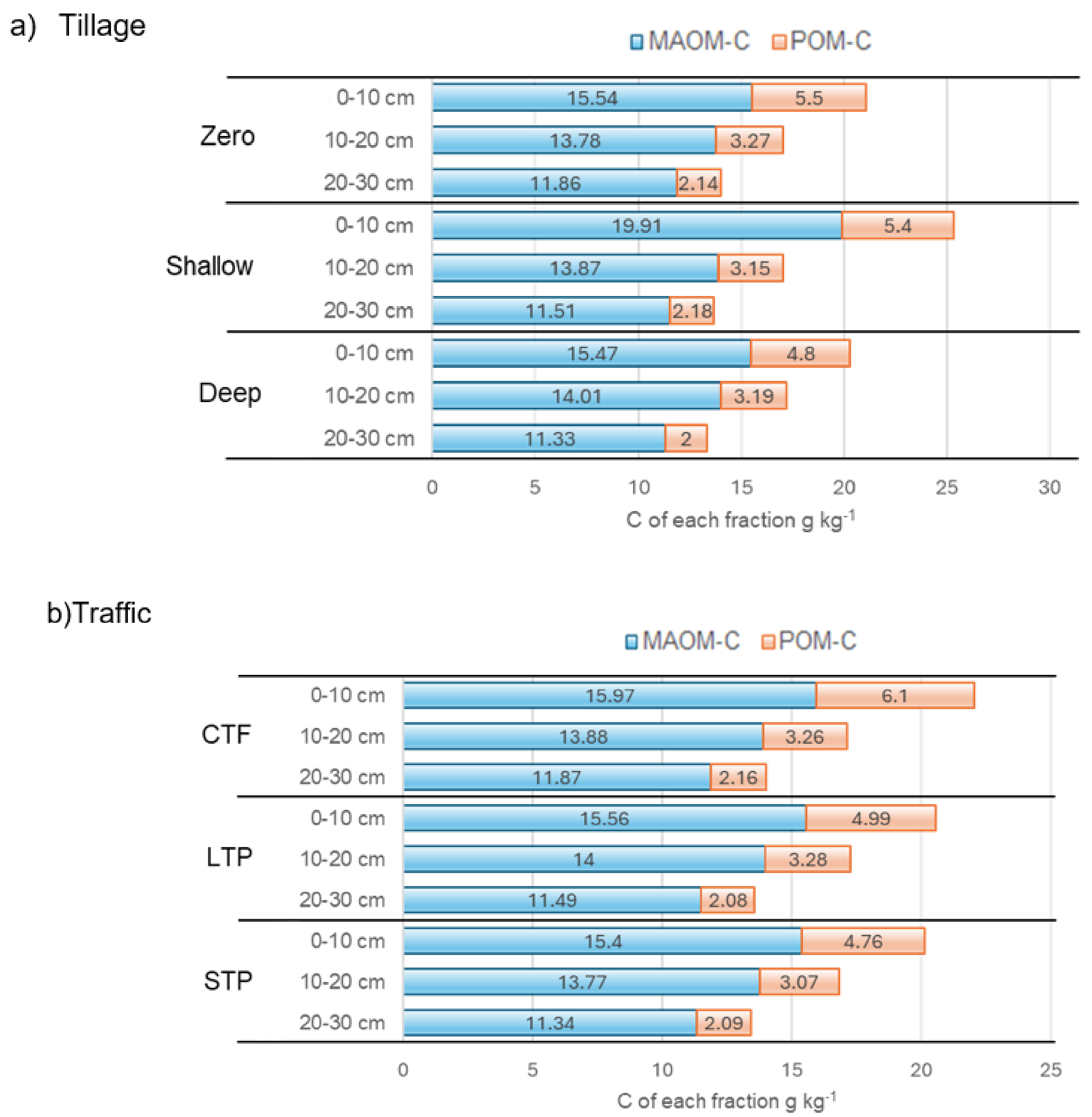


Figure 6. 3. – **a)** Average soil organic carbon (SOC) concentration in g kg^{-1} of SOM fraction for the tillage treatments (Deep, Shallow and Zero). **b)** Average soil organic carbon (SOC) concentration in g kg^{-1} of SOM fraction for the traffic treatments (CTF, LTP and STP). The average CV (%) for POM (0-30 cm) was 20.67% and for MAOM (0-30 cm) was 9.32%.

A. POM-C AT 0-10 CM DEPTH

The overall effect of traffic ($p < 0.001$), tillage ($p = 0.001$), and the interaction between traffic and tillage ($p = 0.013$) were all significant. The average POM-C at 0-10 cm was 5.28 g/kg.

Within the traffic systems, the permanent crop bed of CTF (6.10 g/kg, CV = 11.16%) had significantly higher POM-C concentration compared to LTP (4.99 g/kg, CV = 21.86%) and

STP (4.76 g/kg, CV = 16.67%) systems. CTF systems had 25.25% more POM-C than LTP and STP systems at 0-10 cm (Fig. 6.4).

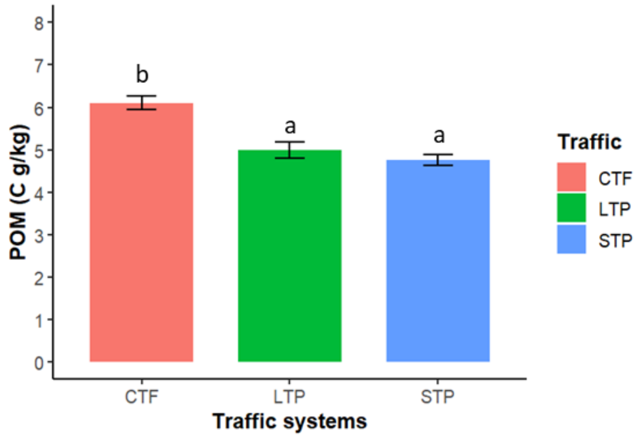


Figure 6. 4 – Main effects of the different traffic systems on POM-C at 0-10 cm. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 12). Bars show standard errors.

Within the tillage systems, Zero (5.55 g/kg, CV = 25.15%) and Shallow (5.41 g/kg, CV = 19.48%) had significantly higher POM-C compared to Deep (4.88 g/kg, CV = 15.10%) tillage systems. Deep tillage systems had 12.29% less POM-C than Zero and Shallow tillage systems at 0-10 cm (Fig. 6.5).

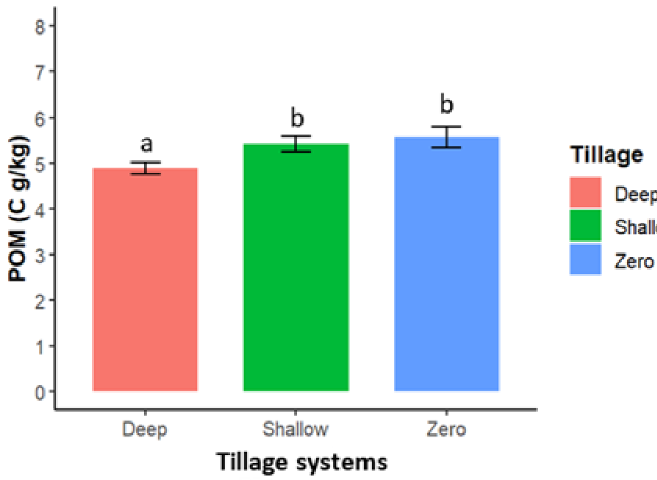


Figure 6. 5 – Main effects of the different tillage systems on POM-C at 0-10 cm. Data from 2022-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 12). Bars show standard errors.

Within the interaction between the traffic and tillage systems, CTF Zero tillage (6.87 g/kg, CV = 13.66%) had significantly higher POM-C than the rest of the treatment combinations, except CTF Shallow. And CTF Shallow (5.99 g/kg, CV = 11.52%) had significantly higher POM-C compared to STP Zero (4.83 g/kg, CV = 18.37%), STP Shallow (4.79 g/kg, CV = 16.60%), STP Deep (4.65 g/kg, CV = 15.03%) and LTP Deep (4.55 g/kg, CV = 15.76%) (Fig. 6.6).

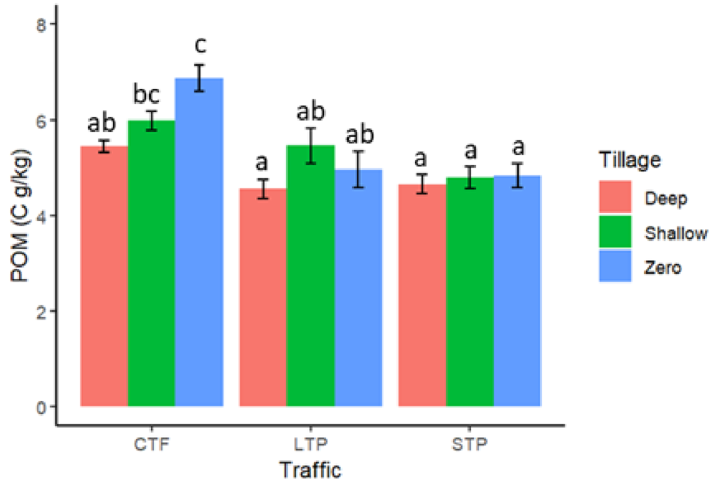


Figure 6. 6 – Effects of the interaction between the traffic and tillage systems on POM-C at 0-10 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 12). Bars show standard errors.

A.1. POM-C AT 0-10 CM OVER TIME

The effects of traffic over time: the main effect of traffic ($p < 0.001$), collection ($p < 0.001$) and the interaction between traffic and collection ($p < 0.001$) were all significant.

Within the traffic systems, the results were the same as the main traffic treatments above (Fig. 6.4).

Within the collection dates, the collection on 29/04/2022 (5.83 g/kg, CV = 16.99%) had significantly more POM-C than the collection on 03/11/2022 (5.05 g/kg, CV = 12.40%) and 29/09/2023 (4.96 g/kg, CV = 13.88%).

Within the traffic and collection dates interaction, CTF on (03/11/2022, 25/09/2023 and 29/04/22) and LTP on 29/04/2022 had significantly higher POM-C than the rest of the traffic treatments and date combinations, except for STP on 29/04/2022 (Fig. 6.7).

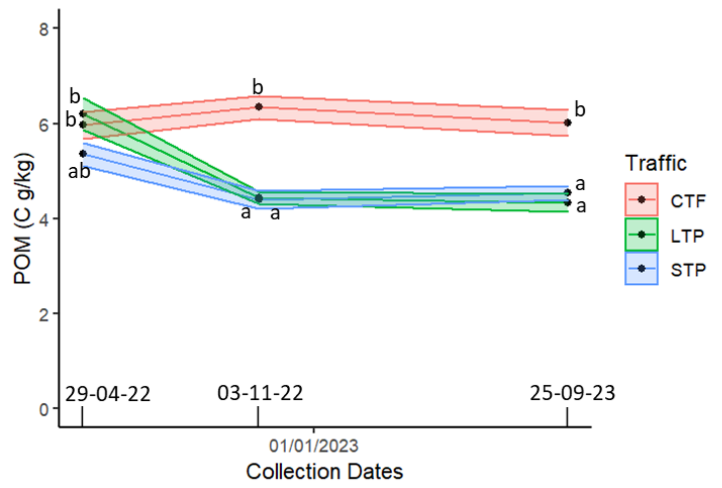


Figure 6. 7 – Main effects of the interaction between the traffic systems and collection dates on POM-C at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors.

The effects of tillage over time: at 0-10 cm, the overall effect of tillage (p = 0.01) and collection (p < 0.001) were significant, but the interaction between tillage and collection (p = 0.80) was not significant (Fig. 6.8).

Within the tillage systems, the results were the same as the main traffic treatments above (Fig. 6.5).

Within the collection dates, the results were the same as above on the effects of traffic systems over time.

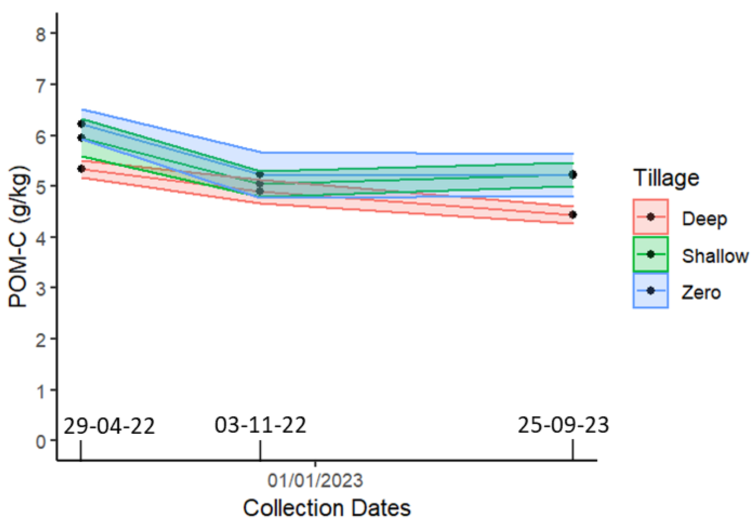


Figure 6. 8 – Main effects of the interaction between the tillage systems and collection dates on POM-C at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection was not significant (p = 0.80).

The effects of the traffic-tillage interaction over time: 0-10 cm, the overall effect of traffic-tillage ($p < 0.001$), collection ($p < 0.001$) and the interaction between traffic-tillage and collection ($p < 0.001$) were all significant (Fig. 6.9).

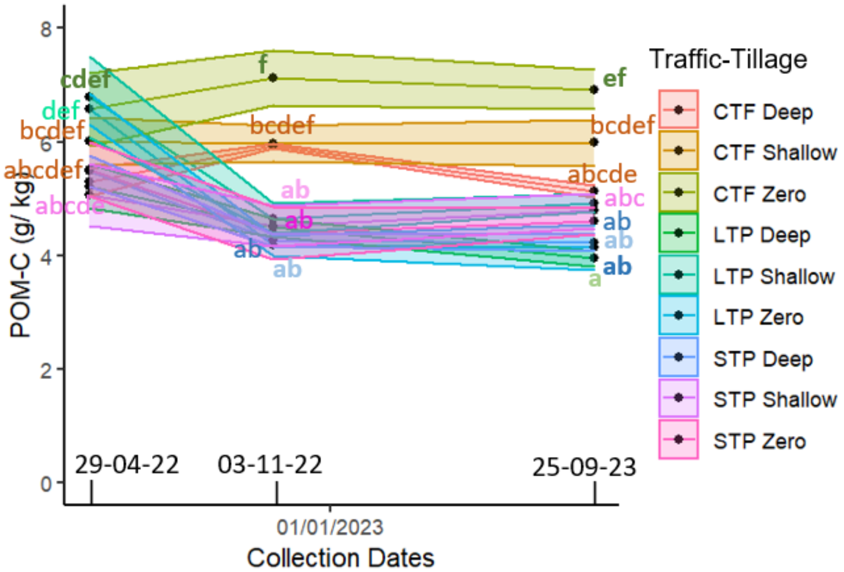


Figure 6. 9 – Main effects of the interaction between the traffic-tillage systems and collection dates on POM-C at 0-10 cm depth. Lines show means (n = 4). Ribbons show standard errors. Letters indicate significant differences based on ($p < 0.05$).

B. POM-C AT 10-20 CM DEPTH

The overall effect of traffic ($p = 0.38$), tillage ($p = 0.75$), and the interaction between traffic and tillage ($p = 0.30$) were not significant (Fig. 6.10). The average POM-C at 10-20 cm was 3.20 g/kg.

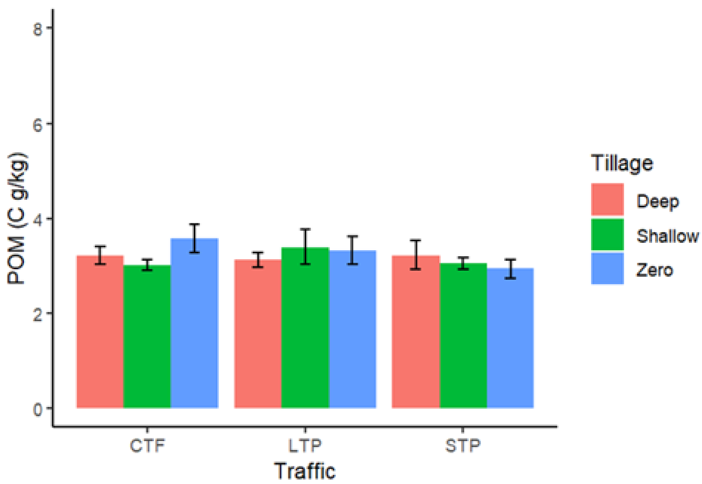


Figure 6. 10 – Effects of the interaction between the traffic and tillage systems on POM-C at 10-20 cm. Data from 2022-2023. Columns show means (n = 12). Bars show standard errors. The interaction between traffic systems and collection was not significant ($p = 0.30$).

B.1. POM-C AT 10-20 CM OVER TIME

The effects of traffic over time: the main effect of traffic (p = 0.37) and the interaction between traffic and collection (p = 0.37) were not significant. Only the main effect of collection (p < 0.001) was statistically significant (Fig. 6.11).

Within the collection dates, the collection on 29/04/2022 (3.61 g/kg, CV = 29.94%) had significantly more POM-C than the collection on 03/11/2022 (3.07 g/kg, CV = 20.65%) and 25/09/2023 (2.93 g/kg, CV = 15.84%).

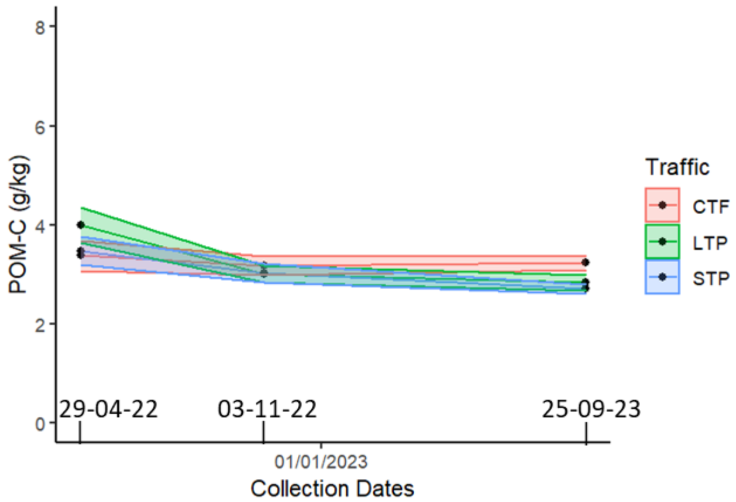


Figure 6. 11 – Main effects of the interaction between the traffic systems and collection dates on POM-C at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p = 0.37).

The effects of tillage over time: at 10-20 cm, the main effect of tillage (p = 0.75) and the interaction between tillage and collection (p = 0.39) were not significant. Only the main effect of collection (p < 0.001) was statistically significant (Fig. 6.12).

Within the collection dates, the results were the same as above within the effects of traffic over time.

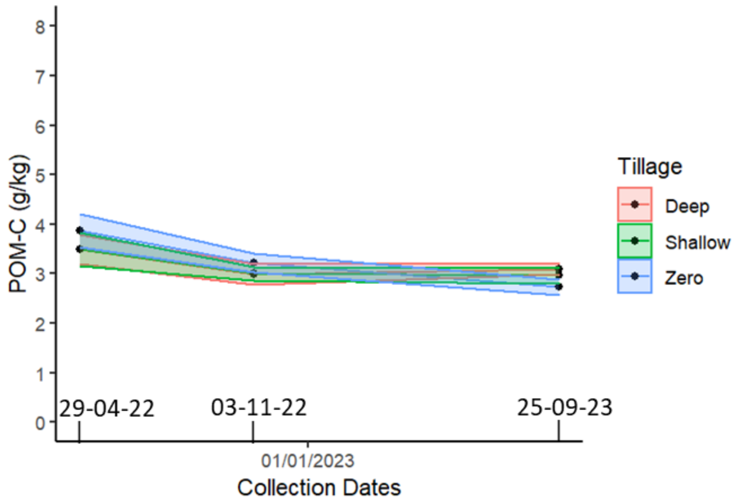


Figure 6. 12 – Main effects of the interaction between the tillage systems and collection dates on POM-C at 10-20 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection was not significant (p = 0.39).

The effects of the traffic-tillage interaction over time: at 10-20 cm the main effect of collection (p < 0.001) and the interaction between traffic-tillage and collection (p = 0.03) were significant (Fig. 6.13). However, the main effect of traffic-tillage (p = 0.40) was not significant.

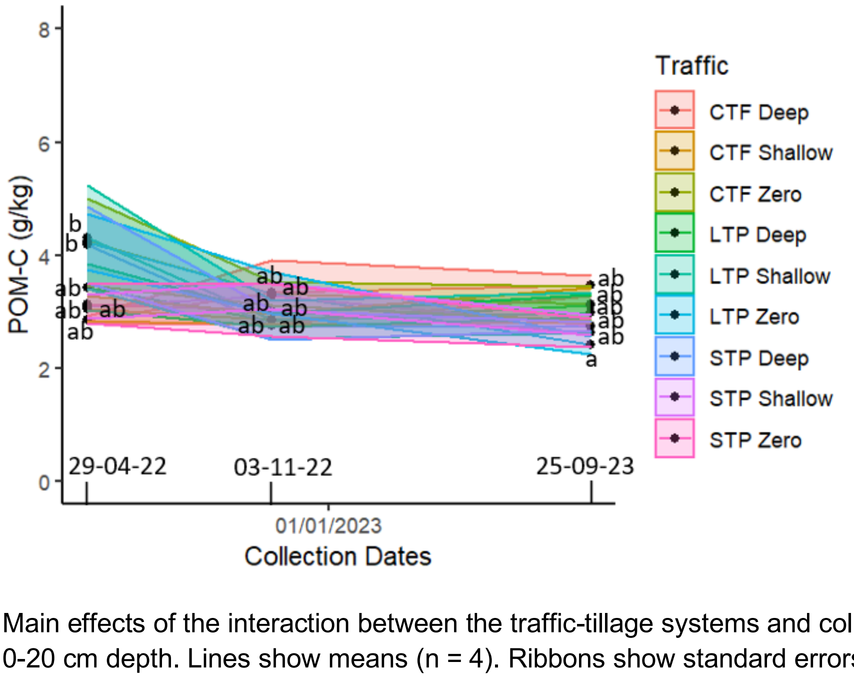


Figure 6. 13 – Main effects of the interaction between the traffic-tillage systems and collection dates on POM-C at 10-20 cm depth. Lines show means (n = 4). Ribbons show standard errors. Letters indicate significant differences based on (p < 0.05).

C. POM-C AT 20-30 CM DEPTH

The overall effect of traffic (p = 0.68) and tillage (p = 0.15) were not statistically significant. Only the effect of the interaction between traffic and tillage (p < 0.001) was significant (Fig. 6.14). The average POM-C at 20-30 cm was 2.11 g/kg.

Within the traffic and tillage interaction: CTF Zero (2.50 g/kg, CV = 17.28%) had significantly more POM-C than STP Deep (1.87 g/kg, CV = 19.36%) and LTP Zero (1.78 g/kg, CV = 21.43%). And LTP Shallow (2.32 g/kg, CV = 19.14%) had significantly more POM-C than LTP Zero (1.78 g/kg, CV = 21.43%) (Fig. 6.14).

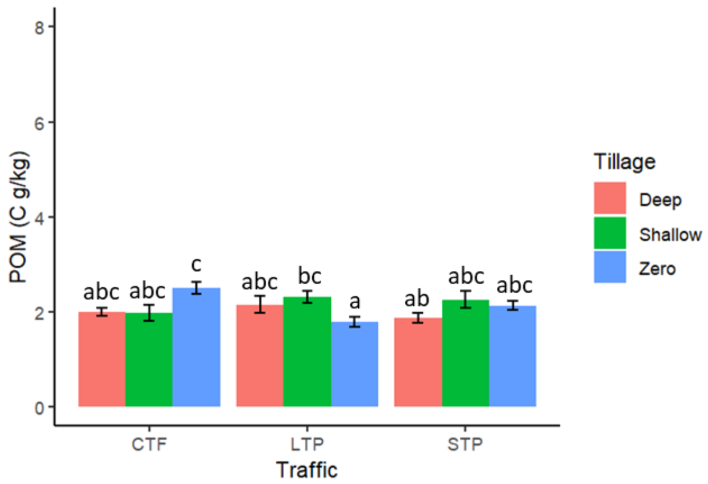


Figure 6. 14 – Effects of the interaction between the traffic and tillage systems on POM-C at 20-30 cm. Data from 2022-2023. Columns show means (n = 12). Bars show standard errors. Letters indicate significant differences based on (p < 0.05).

C.1. POM-C AT 20-30 CM OVER TIME

The main effects of traffic and tillage over time: the main effect of traffic (p = 0.73), tillage (p = 0.21), the interaction between traffic and collection (p = 0.31), and the interaction between tillage and collection were not significant (p = 0.43) (Fig. 6.15). Only the main effect of collection (p < 0.001) was statistically significant.

Within the collection dates, the collection on 03/11/2022 (1.89 g/kg, CV = 23.01%) had significantly less POM-C than the collection on 29/04/2022 (2.29 g/kg, CV = 21.44%) and 25/09/2023 (2.14 g/kg, CV = 21.31%).

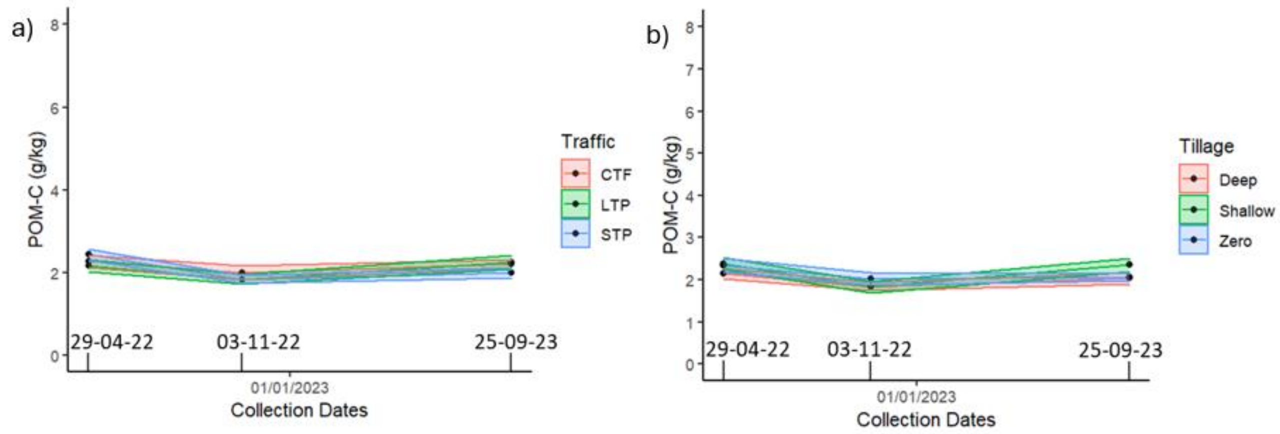


Figure 6. 15 – **a)** Main effects of the interaction between the traffic systems and collection dates on POM-C at 20-30 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p = 0.31). **b)** Main effects of the interaction between the tillage systems and collection dates at 20-30 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection was not significant (p = 0.43).

The effects of traffic-tillage over time: at 20-30 cm, the main effect of traffic-tillage (p < 0.001), collection (p < 0.001) and the interaction between traffic-tillage and collection (p = 0.047) were all significant (Fig. 6.16).

Within the collection dates, the results were the same as above, within the effects of traffic over time.

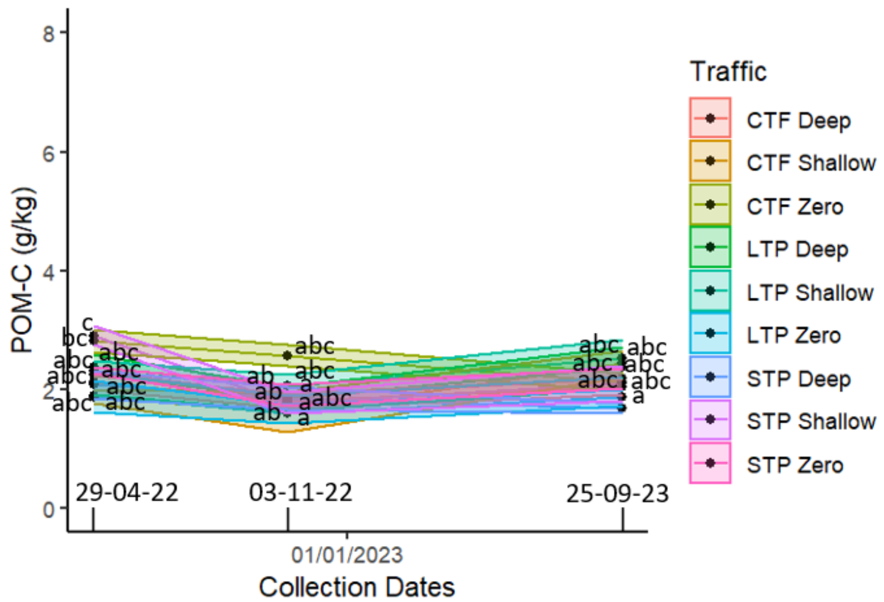


Figure 6. 16 – Main effects of the interaction between the traffic-tillage systems and collection dates on POM-C at 20-30 cm depth. Lines show means (n = 4). Ribbons show standard errors. Letters indicate significant differences based on (p < 0.05).

D. POM-C AT 0-30 CM

The overall effects of traffic ($p < 0.001$), tillage ($p = 0.004$) and depth ($p < 0.001$) were all statistically significant. And the interaction effects between traffic and tillage ($p < 0.001$), traffic and depth ($p < 0.001$) and tillage and depth ($p = 0.03$) were also significant. However, the interaction effect of traffic-tillage and depth ($p = 0.37$) was not significant.

Within the overall traffic systems at 0-30 cm, the permanent crop bed of CTF (3.84 g/kg, CV = 20.39%) had significantly higher POM-C content than LTP (3.45 g/kg, CV = 25.97%) and STP (3.30 g/kg, CV = 21.19%) systems. Therefore, CTF systems stored 13.94% more POM-C than LTP and STP systems (Fig. 6.17).

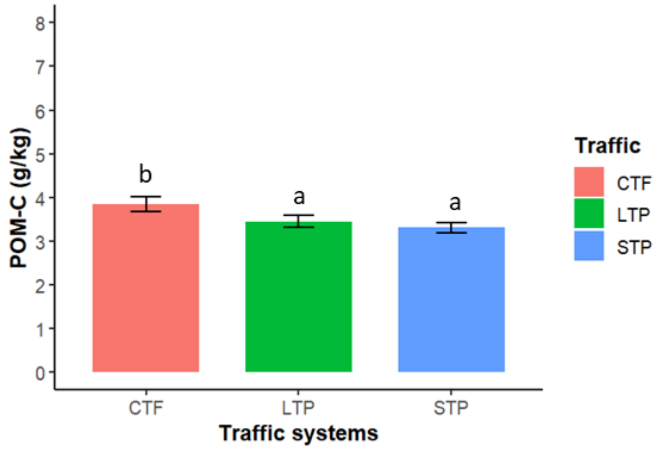


Figure 6. 17 – Effect of the different traffic systems on POM-C at 0-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 108). Bars show standard errors.

Within the tillage systems at 0-30 cm, Zero (3.65 g/kg, CV = 25.31%) and Shallow (3.58 g/kg, CV = 23.55%) tillage systems had significantly higher POM-C than Deep (3.36 g/kg, CV = 20.19%) tillage systems. Zero and Shallow tillage systems stored 7.44% more POM-C than Deep tillage systems (Fig. 6.18).

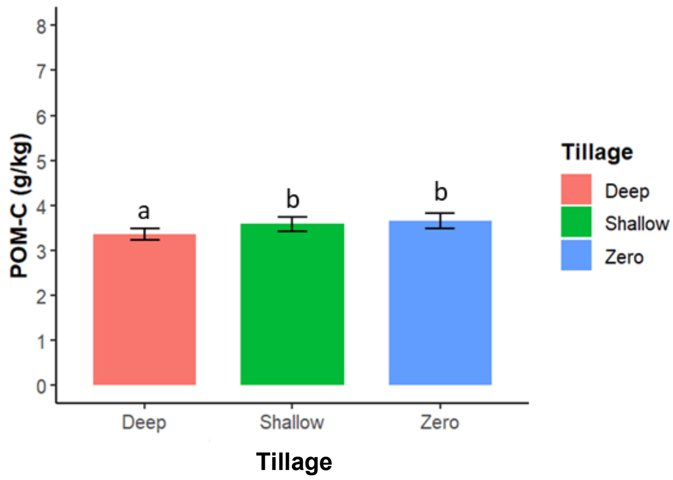


Figure 6. 18 – Effect of the different tillage systems on POM-C at 0-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means ($n = 108$). Bars show standard errors.

Within the different soil depth layers, POM-C was significantly greater at 0-10 cm depth (5.28 g/kg, CV = 21.28%) than at 10-20 cm depth (3.20 g/kg, CV = 25.96%) which was, in turn, significantly greater than at 20-30 cm depth (2.11 g/kg, CV = 23.35%) (Fig. 6.19).

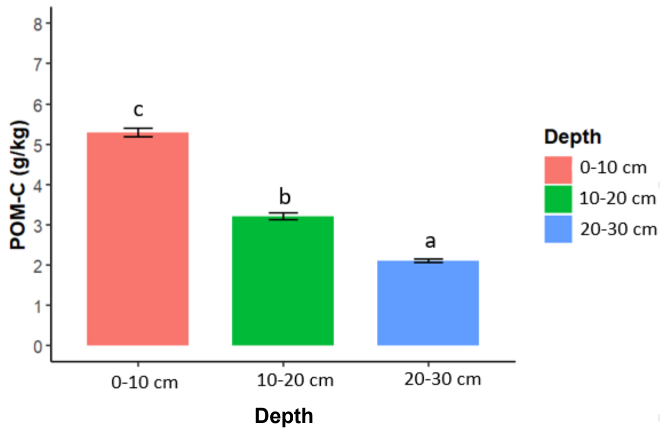


Figure 6. 19 – Effect of the different Depth layers on POM-C at 0-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means ($n = 108$). Bars show standard errors.

Within the traffic and tillage interaction at 0-30 cm, CTF Zero tillage (4.31 g/kg, CV = 47.56%) was significantly higher than the rest of the treatment combinations (avg. 3.43 g/kg, CV = 42.56%). Therefore, CTF Zero tillage stored 25.65% more POM-C than the rest of the treatment combinations (Fig. 6.20).

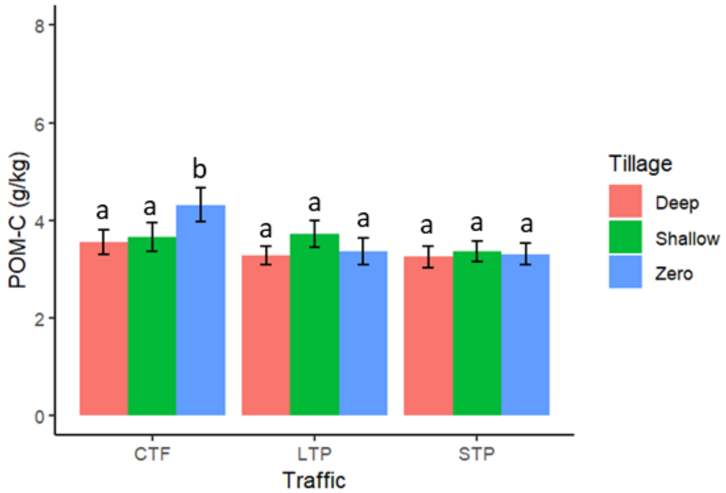


Figure 6. 20 – Main effects of the interaction between traffic and tillage systems on POM-C at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 36). Bars show standard errors.

Within the traffic and depth interaction at 0-30 cm, CTF at 0-10 cm (6.10 g/kg, CV = 15.06%) was significantly higher than the rest of the traffic systems and depth layers combinations. LTP (4.99 g/kg, CV = 23.32%) and STP (4.76 g/kg, CV = 16.36%) at 0-10 cm were significantly higher than the rest of the traffic systems and (10-20, 20-30 cm) depth layers. CTF (3.26 g/kg, CV = 23.27%), LTP (3.28 g/kg, CV = 29.74%) and STP (3.07 g/kg, CV = 24.35%) at 10-20 cm were significantly higher than all the traffic systems at 20-30 cm (Fig. 6.21). Therefore, 0-10 cm stored 39.39% more POM-C than the 10-20 cm depth layer. And 10-20 cm stored 34.06% more POM-C than the 20-30 cm depth layer (Fig. 6.21).

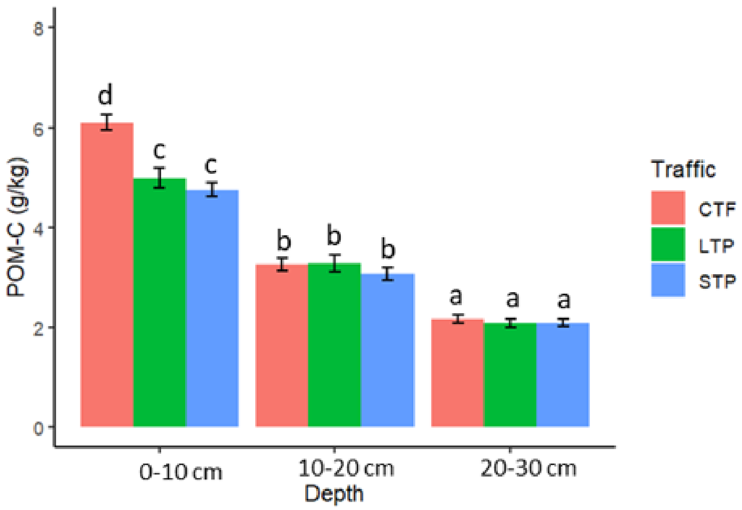


Figure 6. 21 – Main effects of the interaction between traffic systems and depth on POM-C at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 36). Bars show standard errors.

Within the tillage and depth interaction at 0-30 cm, Zero (5.55 g/kg, CV = 25.15%) and Shallow (5.41 g/kg, CV = 19.48%) tillage systems at 0-10 cm were significantly higher than the rest of the Tillage systems and Depth combinations. Deep (4.88 g/kg, CV = 15.10%) tillage at 0-10 cm was significantly higher than the rest of the tillage systems and depth combinations (at 10-20 and 20-30 cm). Zero (3.27 g/kg, CV = 28.54%), Shallow (3.15 g/kg, CV = 25.72%) and Deep (3.19 g/kg, CV=23.83%) tillage systems at 10-20 cm were significantly higher than the tillage systems at 20-30 cm (Fig. 6.22).

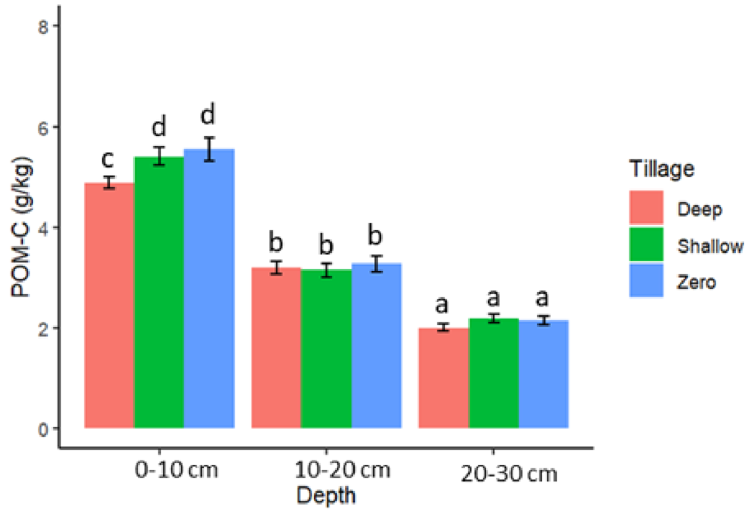


Figure 6. 22 – Main effects of the interaction between tillage systems and depth on POM-C at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

E. MAOM-C AT 0-10 CM DEPTH

The overall effect of traffic (p = 0.017) and the interaction between traffic and tillage (p = 0.013) were statistically significant. But the main effect of tillage (p = 0.06) was only borderline non-significant. At 0-10 cm, the average MAOM-C was 15.65 g/kg.

Within the traffic systems, the permanent crop bed of CTF (15.97 g/kg, CV (%) = 8.72%) had significantly more MAOM-C than STP (15.40 g/kg, CV (%) = 9.50%) traffic systems (Fig. 6.23).

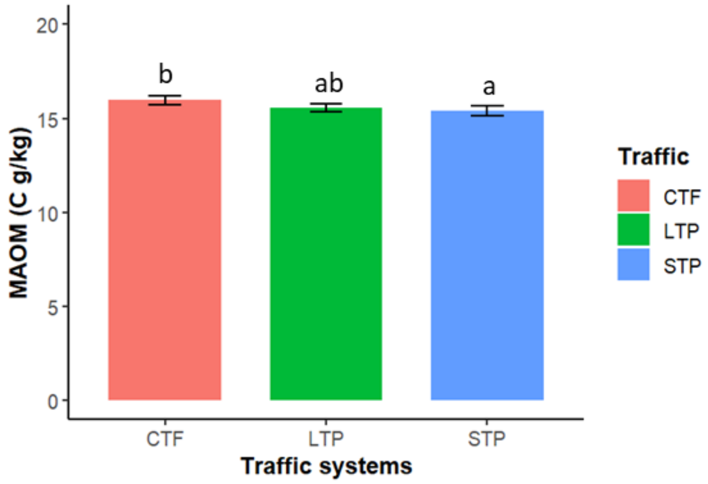


Figure 6. 23 – Main effects of the different traffic systems on MAOM-C at 0-10 cm. Data from 2022-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 12). Bars show standard errors.

Within the traffic and tillage interaction: CTF Zero (16.44 g/kg, CV (%) = 10.66%) tillage had significantly more MAOM-C compared to LTP Zero (15.16 g/kg, CV (%) = 9.62%) and STP Zero (15.04 g/kg, CV (%) = 10.62%) tillage systems (Fig. 6.24).

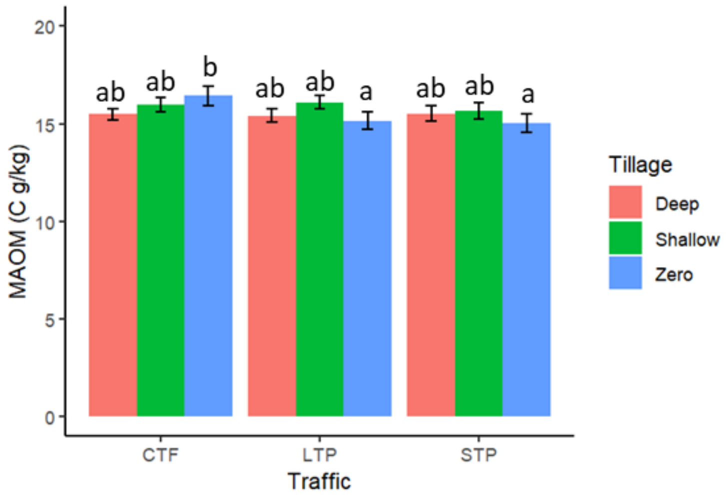


Figure 6. 24 – Effects of the interaction between the traffic and tillage systems on MAOM-C at 0-10 cm. Data from 2022-2023. Columns show means (n = 12). Bars show standard errors. Letters indicate significant differences based on ($p < 0.05$).

E.1 MAOM-C AT 0-10 CM OVER TIME

The effects of traffic over time: the main effect of traffic (*p* = 0.02) and collection (*p* < 0.001) were statistically significant. But the effect of the interaction between traffic and collection dates (*p* = 0.46) was not significant (Fig. 6. 25).

Within the traffic systems, the results were the same as the main traffic systems above (Fig. 6.23).

Within the collection dates, the collection on 29/04/2022 (14.46 g/kg, CV = 6.88%) had significantly less MAOM-C than the collection on 03/11/2022 (16.20 g/kg, CV = 7.30%) and 25/09/2023 (16.26 g/kg, CV = 7.06%).

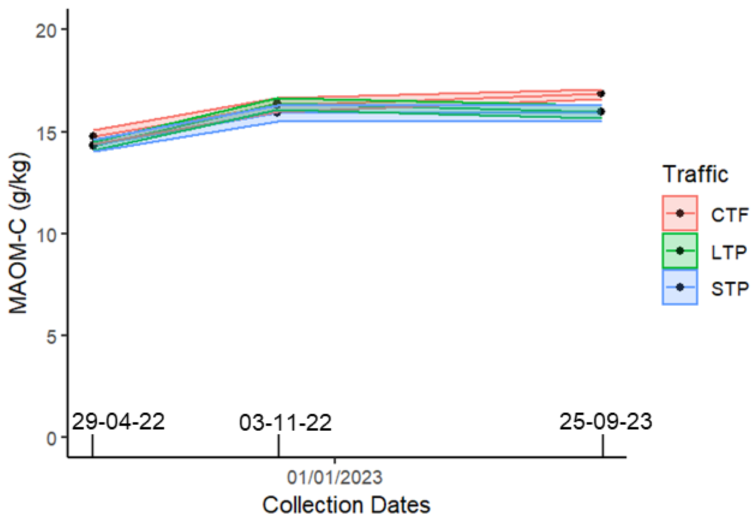


Figure 6. 25 – Main effects of the interaction between the traffic systems and collection dates on MAOM-C at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (*p* = 0.46).

The effects of tillage over time: at 0-10 cm, the overall effect of tillage (*p* = 0.10) and the interaction between tillage and collection (*p* = 0.80) were not significant. Only the main effect of collection (*p* < 0.001) was statistically significant (Fig. 6.26).

Within the collection dates, the results were the same as above within the main effects of Traffic over time.

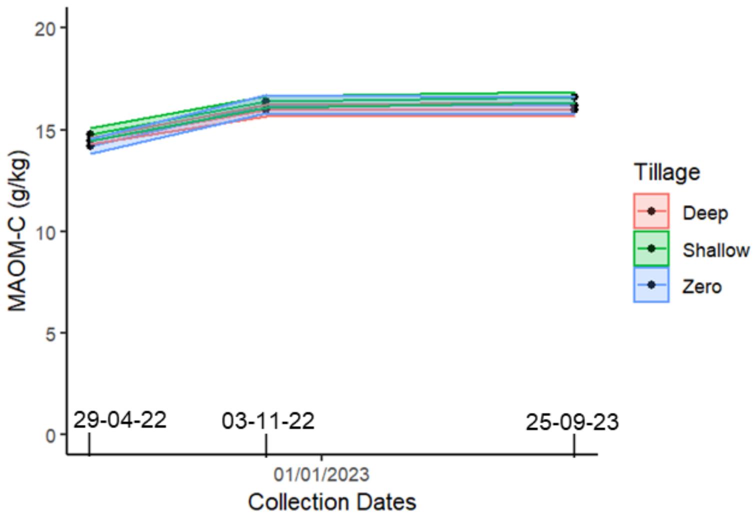


Figure 6. 26 – Main effects of the interaction between the tillage systems and collection dates on MAOM-C at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (*p* = 0.80).

The effects of the traffic-tillage interaction over time: at 0-10 cm, the main effect traffic-tillage (*p* = 0.003) and the main effect of collection (*p* < 0.001) were significant. However, the effect of the interaction between traffic-tillage and collection (*p* = 0.90) was not significant (Fig. 6.27).

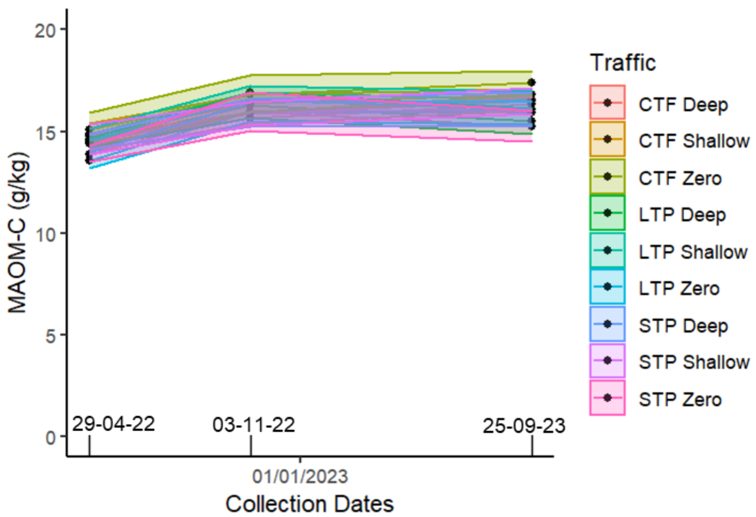


Figure 6. 27 – Main effects of the interaction between the traffic-tillage systems and Collection dates on MAOM-C at 0-10 cm depth. Lines show means (n = 4). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p=0.90).

F. MAOM-C AT 10-20 CM DEPTH

The main effect of traffic (*p* = 0.58), tillage (*p* = 0.56), and the interaction between traffic and tillage (*p* = 0.82) were all not significant (Fig. 6. 28). At 10-20 cm, the average MAOM-C was 13.89 g/kg.

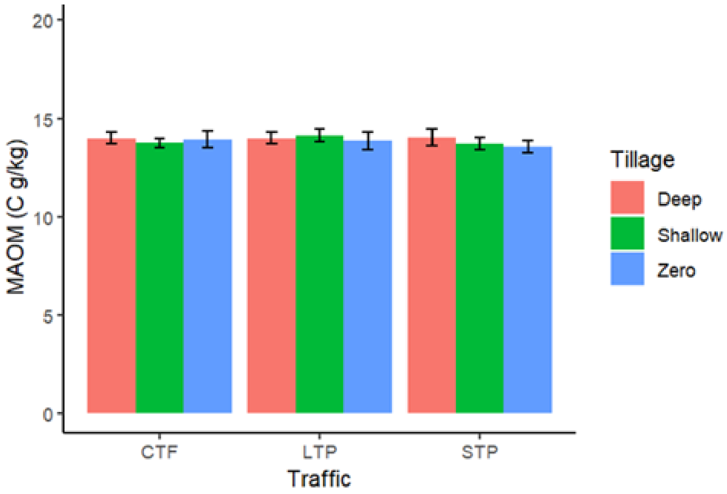


Figure 6. 28 – Effects of the interaction between the traffic and tillage systems on MAOM-C at 10-20 cm. Data from 2022-2023. Columns show means (n = 12). Bars show standard errors. Letters indicate significant differences based on ($p < 0.05$).

F.1. MAOM-C AT 10-20 CM OVER TIME

The effects of traffic over time: the main effect of traffic (*p* = 0.57) and the interaction between traffic and collection dates (*p* = 0.33) were not significant. Only the main effect of collection was statistically significant ($p < 0.001$) (Fig. 6.29).

Within the collection dates, the collection on 03/11/2022 (14.41 g/kg, CV = 7.58%) had significantly more MAOM-C than the collection on 29/04/2022 (13.39 g/kg, CV = 8.44%) and 25/09/2023 (13.85 g/kg, CV = 7.51%).

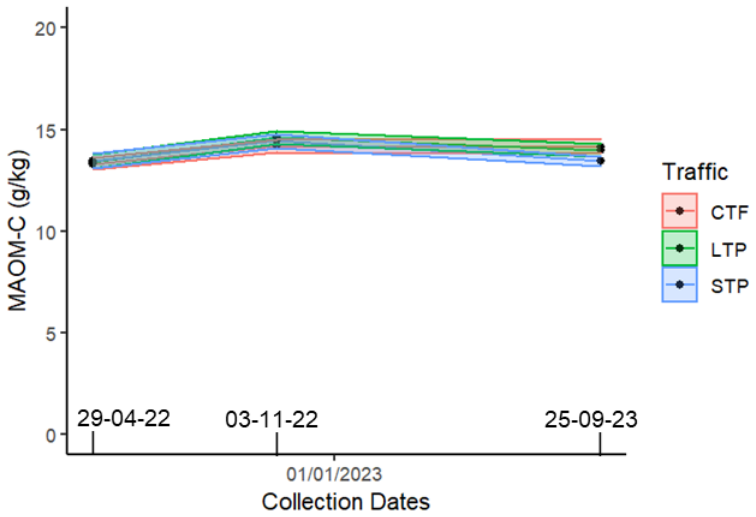


Figure 6. 29 – Main effects of the interaction between the traffic systems and collection dates on MAOM-C at 10-20 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p = 0.33).

The effects of tillage over time: at 10-20 cm, the main effect of tillage (p = 0.53) was not significant. But the main effect of collection (p < 0.001) and the interaction between tillage and collection (p = 0.05) were statistically significant (Fig. 6.30).

Within the collection dates, the results were the same as above within the main effects of traffic over time.

Within the interaction between tillage and collection dates, CTF on 03/11/2022 (14.46 g/kg, CV = 8.68%) had significantly higher MAOM-C compared to Deep and Shallow tillage on 29/04/2022 and Zero tillage on 25/09/2023 (avg. 13.28 g/kg, CV = 8.49%). And Shallow tillage on 03/11/2022 (14.42 g/kg, CV = 5.77%) had significantly higher MAOM-C than Zero tillage on 25/09/2023 (13.27 g/kg, CV = 9.62%) (Fig. 6.30).

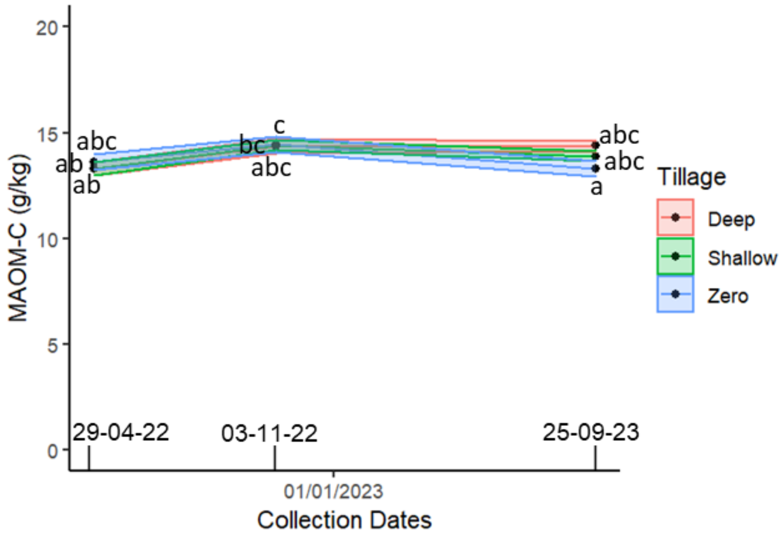


Figure 6. 30 – Main effects of the interaction between the tillage systems and collection dates on MAOM-C at 10-20 cm depth. Lines show means (n = 12). Ribbons show standard errors. Letters indicate significant differences based on (p < 0.05).

The effects of the traffic-tillage interaction over time: at 10-20 cm, the main effect of traffic-tillage systems (p = 0.87) and the interaction between the traffic-tillage systems and collection dates (p = 0.54) were not significant (Fig. 6.31). Only the main effect of collection (p < 0.001) was statistically significant.

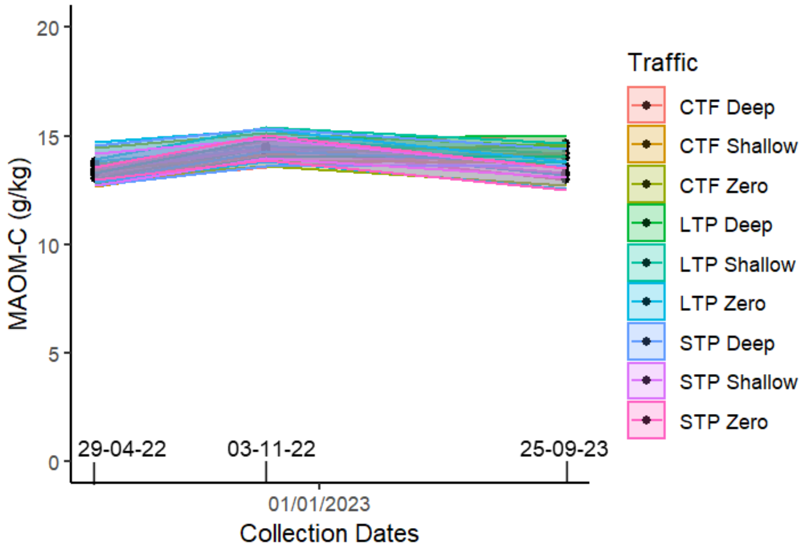


Figure 6. 31 – Main effects of the interaction between the traffic-tillage systems and collection dates on MAOM-C at 10-20 cm depth. Lines show means (n = 4). Ribbons show standard errors. Letters indicate significant differences based on (p < 0.05).

G. MAOM-C AT 20-30 CM DEPTH

The overall effect of traffic (*p* = 0.06) and tillage (*p* = 0.07) were borderline non-significant. And the interaction between traffic and tillage systems was not significant (*p* = 0.14) (Fig. 6. 32). At 20-30 cm, the average MAOM-C was 11.57 g/kg.

Although not significant, CTF Zero tillage (12.63 g/kg, CV = 9.87%) stored 10.4% more MAOM-C than the rest of the treatment combinations (11.43 g/kg, CV = 10.96%).

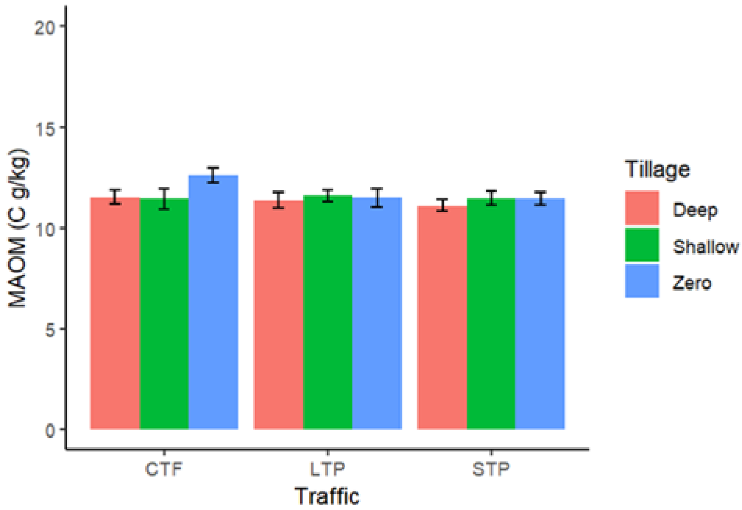


Figure 6. 32 – Effects of the interaction between the traffic and tillage systems on MAOM-C at 20-30 cm. Data from 2022-2023. Columns show means (n = 12). Bars show standard errors. The interaction between traffic systems and collection was not significant (*p* = 0.14).

G.1. MAOM-C AT 20-30 CM OVER TIME

The effects of traffic over time: the main effect of traffic (*p* = 0.08) was borderline not significant, and the interaction between traffic and collection dates (*p* = 0.59) was not significant. Only the main effect of collection was significant (p < 0.001) (Fig. 6.33).

Within the collection dates, the collection on 29/04/2022 (10.63 g/kg, CV = 11.42%) had significantly less MAOM-C than the collection on 03/11/2022 (11.85 g/kg, CV = 9.29%) and 25/09/2023 (12.22 g/kg, CV = 7.56%).

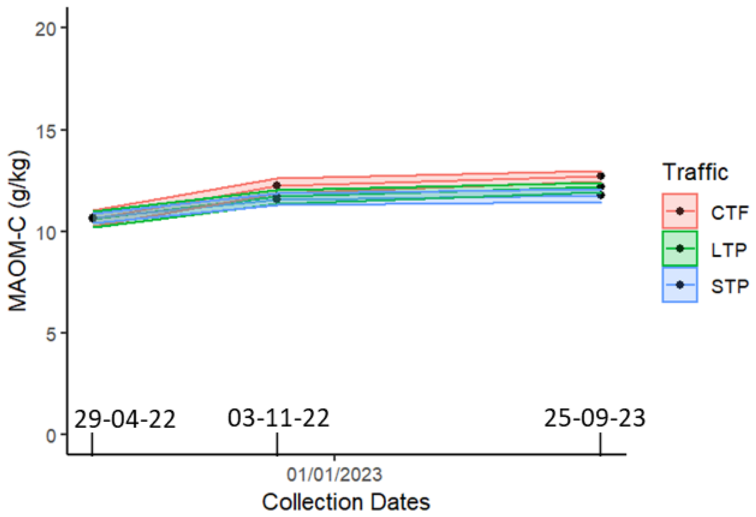


Figure 6. 33 – The effects of the interaction between the traffic systems and collection dates on MAOM-C at 20-30 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p = 0.59).

The effects of tillage over time: at 20-30 cm, the main effect of tillage (p = 0.08) was borderline non-significant. And the interaction between tillage and collection (p = 0.41) was not significant (Fig. 6. 34). Only the main effect of collection (p < 0.001) was statistically significant.

Within the collection dates, the results were the same as above regarding the main effects of traffic over time.

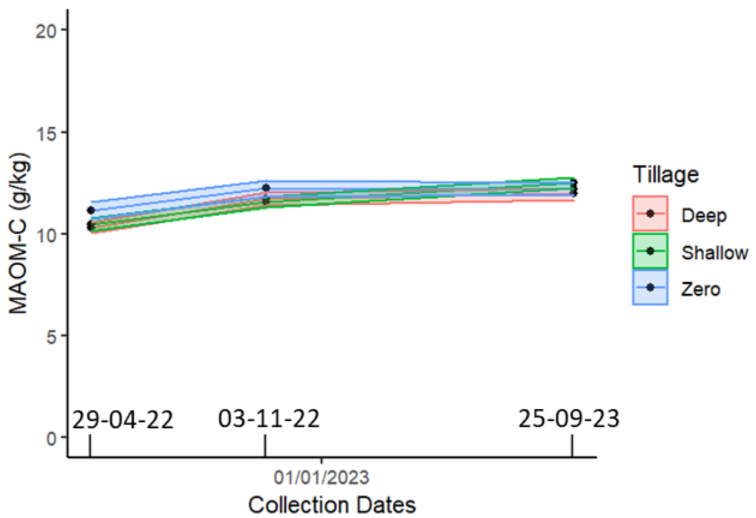


Figure 6. 34 – Main effects of the interaction between the tillage systems and collection dates on MAOM-C at 20-30 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between traffic systems and collection was not significant (p = 0.41).

The effects of the traffic-tillage interaction over time: at 0-10 cm, the main effect of traffic-tillage ($p = 0.02$) and collection ($p < 0.001$) were statistically significant. But the interaction effect between traffic-tillage and collection ($p = 0.20$) was not significant (Fig. 6. 35).

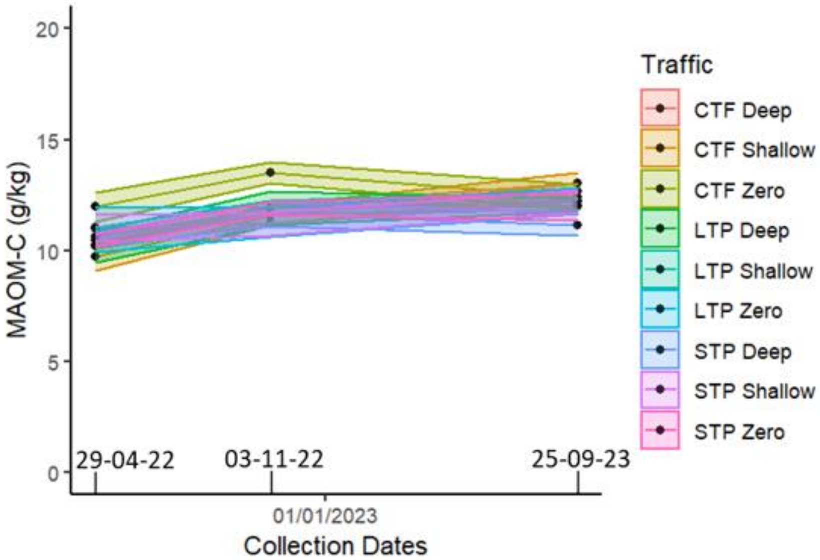


Figure 6. 35 – Main effects of the interaction between the traffic-tillage systems and collection dates on MAOM-C at 20-30 cm depth. Lines show means (n = 4). Ribbons show standard errors. The interaction between traffic systems and collection was not significant ($p = 0.20$).

H. MAOM-C AT 0-30 CM

The main effects of traffic ($p = 0.009$) and depth ($p < 0.001$) were significant. The interaction effects between traffic and tillage ($p = 0.007$) and tillage and depth ($p = 0.05$) were also significant. But the main effect of tillage ($p = 0.45$), the interaction between traffic and depth ($p = 0.41$) and the interaction between traffic, tillage and depth ($p = 0.78$) were not significant.

Within the main traffic systems at 0-30 cm, CTF (13.91 g/kg, CV = 15.36%) had significantly higher POM-C content than STP (13.50 g/kg, CV = 15.39%). Therefore, CTF systems had 2.94% more SOM than STP systems (Fig. 6. 36).

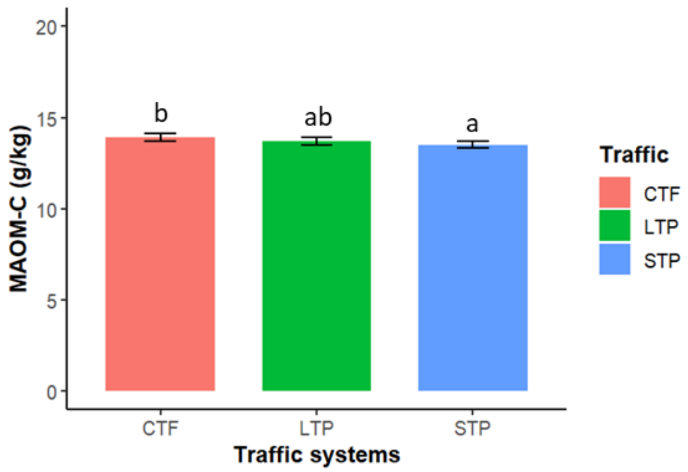


Figure 6. 36 – Effect of the different traffic systems on MAOM-C at 0-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 108). Bars show standard errors.

Within the different soil depth layers, MAOM-C was significantly greater at 0-10 cm depth (15.64 g/kg, CV=8.94%) than at 10-20 cm depth (13.89 g/kg, CV = 8.31%) which was, in turn, significantly greater than at 20-30 cm depth (11.57 g/kg, CV = 11.14%) (Fig. 6.37). Therefore, 0-10 cm stored 12.59% more MAOM-C than 10-20 cm depth layer. And 10-20 cm stored 20.05% more MAOM-C than 20-30 cm depth layer.

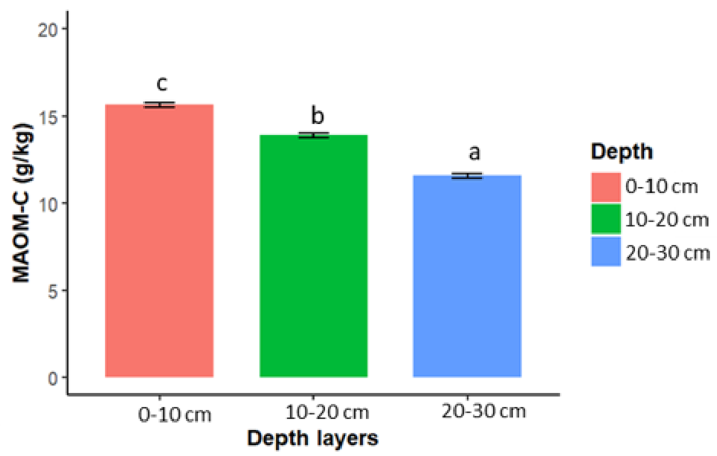


Figure 6. 37 – Effect of the different depth layers on MAOM-C at 0-30 cm depth. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 108). Bars show standard errors.

Within the traffic and tillage interaction at 0-30 cm, CTF Zero tillage (14.32 g/kg, CV = 15.15%) stored significantly more (5.83% more) MAOM-C content than LTP Deep (13.60 g/kg, CV = 15.31%), LTP Zero (13.50 g/kg, CV = 15.62%) and STP (Deep, Shallow and Zero) tillage (avg. 13.50 g/kg, CV = 15.49%) (Fig. 6. 38).

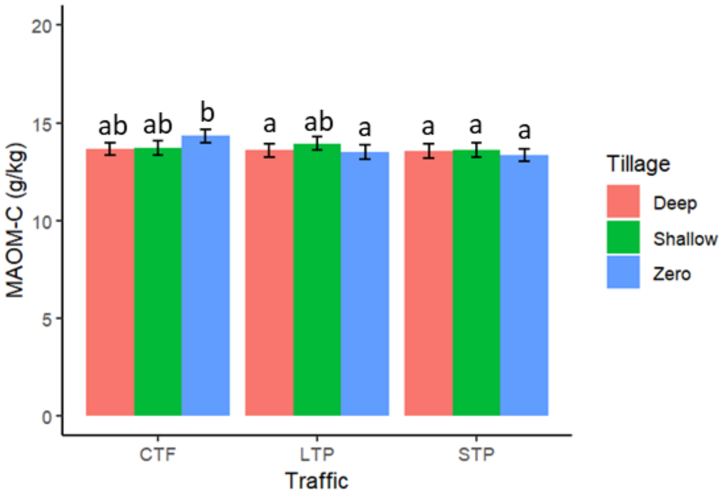


Figure 6. 38 – Main effects of the interaction between traffic and tillage systems on MAOM-C at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

Within the tillage and depth interaction at 0-30 cm, at 0-10 cm (Deep, Shallow and Zero) tillage systems (avg. 15.64 g/kg, CV = 8.83%) had significantly higher MAOM-C content than the rest of the tillage systems at 10-20 and 20-30 cm. The tillage systems (Deep, Shallow and Zero) at 10-20 cm (avg. 13.89 g/kg, CV = 8.31%) had significantly higher MAOM-C content than the tillage systems at 20-30 cm (avg. 11.57 g/kg, CV = 11.06%) (Fig. 6. 39).

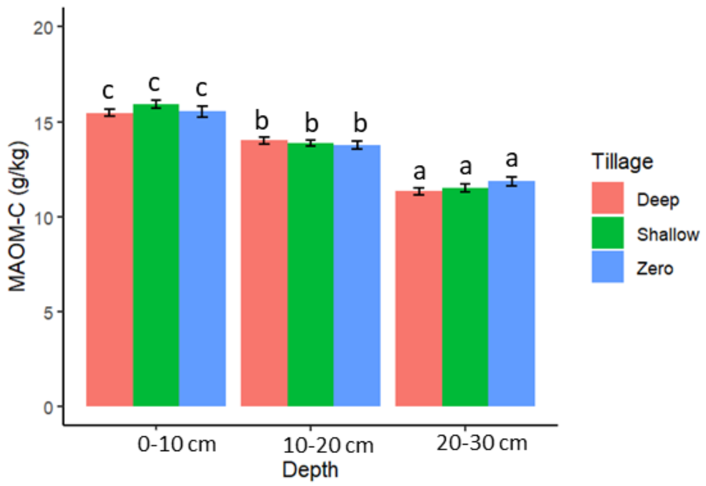


Figure 6. 39 – Main effects of the interaction between traffic systems and depth on MAOM-C (%) at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

6.4.2. EFFECTS OF TRAFFIC AND TILLAGE ON C/N RATIO OF SOM FRACTIONS

6.4.2.1. POM C/N RATIO (0-30 CM)

The C/N ratio of the POM fraction at 0-30 cm, including 3 soil sample collections, was 18.86, CV = 104.16%. The overall effect of depth (p = 0.01) was statistically significant, with 10-20 cm depth having a significantly higher POM C/N ratio (24.06, CV = 111.08%) than 20-30 cm (POM C/N ratio = 10.45, CV = 102.54%), while 0-10 cm (POM C/N ratio 22.06, CV = 98.85%) was not statistically different from the other two soil depths (Fig. 6. 40) (traffic p = 0.30, tillage p = 0.36, traffic-tillage p = 0.41, traffic-depth p = 0.23, tillage-depth p = 0.16 and traffic-tillage-depth p = 0.09).

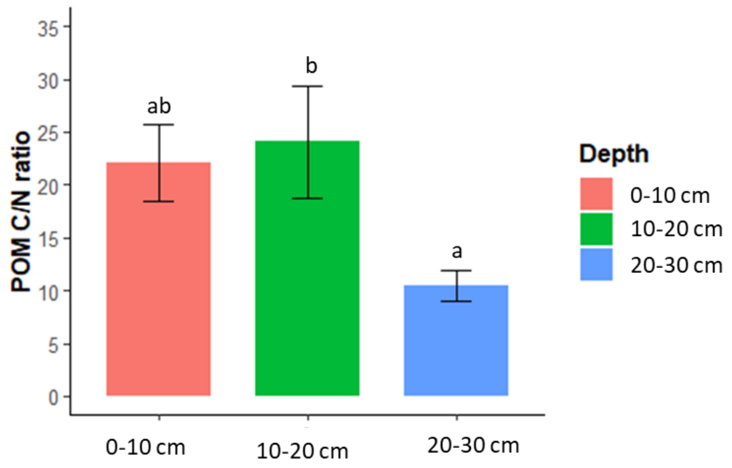


Figure 6. 40 – Main effect of depth on the POM C/N ratio at 0-30 cm (Data from 2022-2023). Letters indicate significant differences based on (p < 0.05). Columns show means (n = 108). Bars show standard errors.

POM C/N ratio on 0-30 cm over time showed that the main effect of collection (p < 0.001), depth and collection (p = 0.001) and the interaction between traffic, tillage, depth and collection date (p = 0.01) were statistically significant meaning that the POM C/N ratio was not constant over time. The collection on 29/04/2022 had a significantly higher POM C/N ratio (POM C/N ratio 33.23, CV = 144.76%) than the collection on 03/11/2022 (POM C/N ratio 10.75, CV = 15.67%) and the collection on 29/09/2023 (POM C/N ratio 12.59, CV = 15.07%) (Fig. 6. 41).

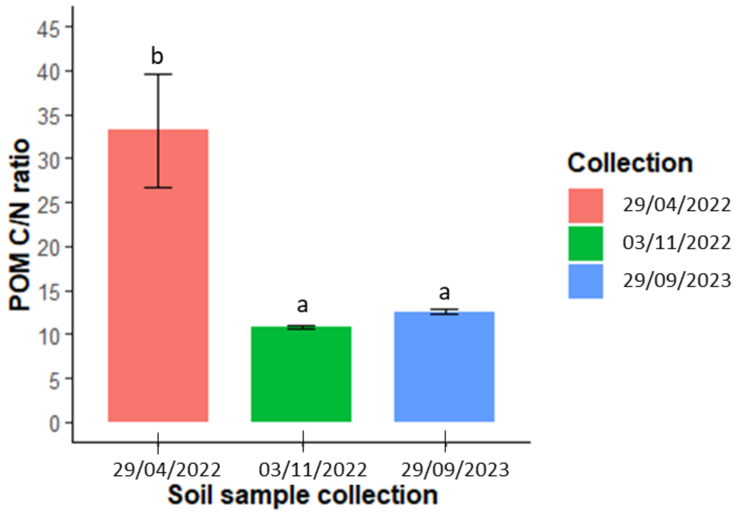


Figure 6. 41 – Main effect of collection on the POM C/N ratio at 0-30 cm (Data from 2022-2023). Letters indicate significant differences based on (p < 0.05). Columns show means (n = 108). Bars show standard errors.

Within collection and depth, the collection on 29/04/2022 at 0-10 (41.42, CV = 77.34%) and 10-20 cm (49.82, CV = 106.41%) had significantly higher POM C/N ratio than the rest of the collection and depths combinations (Fig. 6. 42).

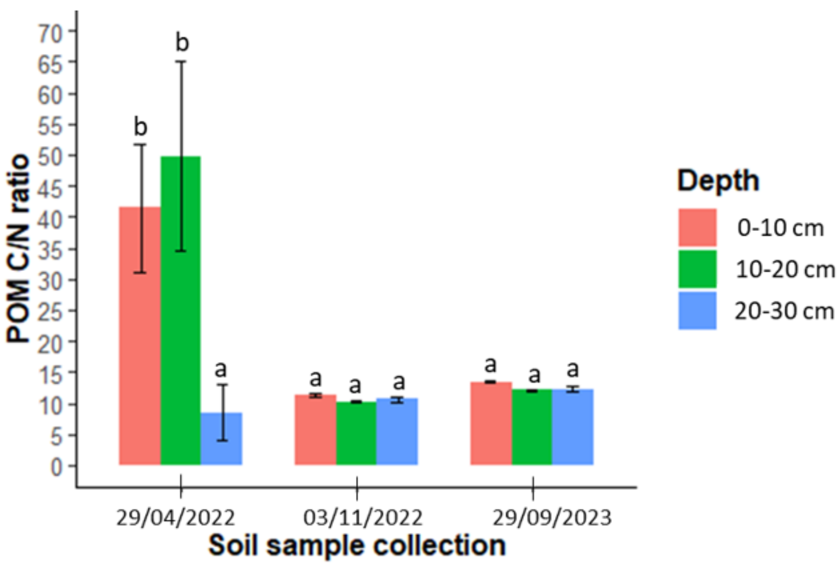


Figure 6. 42 – Main effect of the interaction between collection date and depth on the POM C/N ratio at 0-30 cm (Data from 2022-2023). Letters indicate significant differences based on (p < 0.05). Columns show means (n = 12). Bars show standard errors.

Within the interaction between traffic, tillage, depth and collection the statistical analysis revealed that CTF Shallow on 10-20 cm and collection on 29/04/2022 was significantly higher than all of the traffic, tillage, depth and collection combinations, except for LTP Deep on 0-10 cm and collection on 29/04/2022.

6.4.2.2. MAOM C/N RATIO (0-30 CM)

The C/N ratio of the MAOM fraction on 0-30 cm, including 3 soil sample collections, was 8.42, CV = 9.36%. The statistical analysis revealed that only the main effect of depth (p = 0.03) was statistically significant, with 0-10 cm depth layer having a significantly higher MAOM C/N ratio (8.46, CV = 8.76%) than 10-20 cm (MAOM C/N ratio = 8.35, CV = 9.57%), while 20-30 cm (POM C/N ratio 8.44, CV= 9.75%) was not statistically different from the other two soil depths (Fig. 6. 43). (Traffic p = 0.40, tillage p = 0.20, traffic-tillage p = 0.10, traffic-depth p = 0.39, tillage-depth p = 0.36 and traffic-tillage-depth p = 0.36).

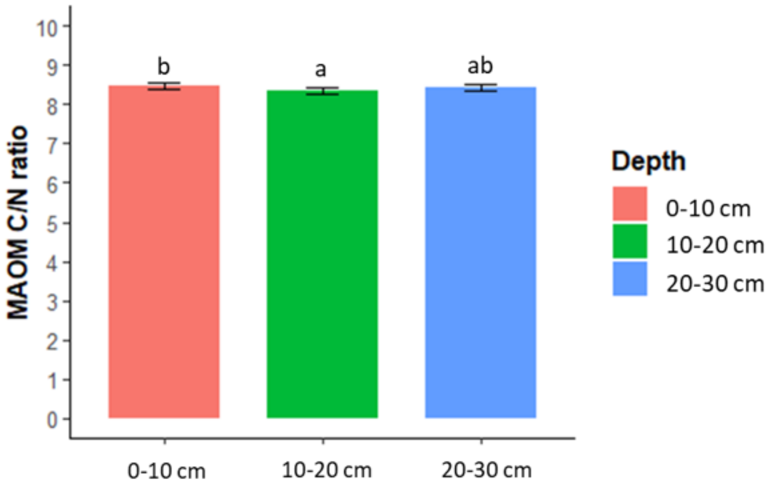


Figure 6. 43 – Main effect of depth on the MAOM C/N ratio at 0-30 cm (Data from 2022-2023). Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 108). Bars show standard errors.

MAOM C/N ratio on 0-30 cm over time showed that only the main effect of collection ($p < 0.001$) was statistically significant. The interaction between traffic, tillage, depth and collection date was not statistically significant (p = 0.94), meaning that the MAOM C/N ratio was constant over time. The collection on 29/09/2023 had a significantly higher MAOM C/N ratio (9.33, CV = 3.54%) than the collection on 03/11/2022 (MAOM C/N ratio 8.16, CV = 3.88%), which in turn had significantly higher than collection 29/04/2022 (MAOM C/N ratio 7.75, CV = 6.12%) (Fig. 6. 44).

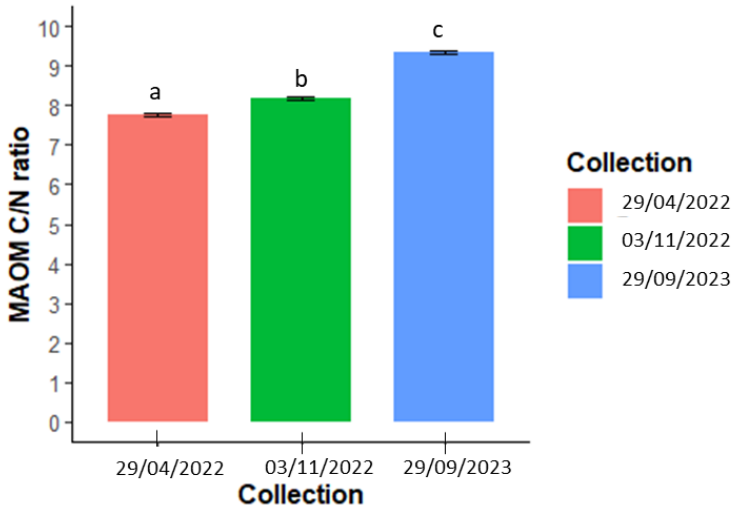


Figure 6. 44 – Main effect of collection on the MAOM C/N ratio at 0-30 cm (Data from 2022-2023). Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 108). Bars show standard errors.

6.5. DISCUSSION

6.5.1. SOM FRACTIONS

SOC dynamics in cropland are affected by the biotic (living organisms and plants) and abiotic (climate, topography, vegetation, and edaphic) factors, as well as the different management practices imposed, which constantly interact with each other. Because the SOM fractions (POM and MAOM) have different formation, stability and turnover rates and behaviours (Luo, Rossel and Shi, 2020; Sampson *et al*., 2020), they can serve as a sensitive indicator for assessing the effects of the different traffic and tillage management practices.

While relative C change does not directly measure the soil carbon sequestration, it provides a comparative assessment of how the different traffic and tillage systems and their interaction affect SOC across the different SOM fractions and soil depths.

SOM inputs and environmental conditions remained constant across treatments; therefore, observed differences in POM and MAOM carbon content can be directly attributed to the different traffic and tillage management systems.

The results showed that POM-C and MAOM-C concentrations decreased with depth under all systems. These results are consistent with other studies (e.g., Li *et al.,* 2016; Tautges et al., 2019). In this study, the majority of SOC was recovered in the MAOM fraction (approx.

13.70 g/kg) with considerably less recovery in the POM fraction (approx. 3.53 g/kg) at 0-30 cm. On average MAOM-C accounted for 80.20% and POM-C accounted for 19.78% of the total SOC at 0-30 cm. This trend has been similarly observed in other arable soils across Europe and global continental assessments, indicating averages of MAOM-C accounting for 71-86% of the total SOC (Salonen *et al.,* 2024; Sokol *et al.,* 2022*;* Lugato *et al.,* 2021).

The vertical distribution of POM-C and MAOM-C showed a depth-related gradient. POM-C was predominantly concentrated in the uppermost soil layer (0-10 cm), with a marked decrease at greater depths. Conversely, MAOM-C exhibited a more consistent distribution across the soil profile (Table 6.1).

Table 6. 1. Depth-dependent distribution of the total POM-C and MAOM-C within the 0-30 cm soil profile.

	POM-C	**MAOM-C**
0-10 cm	51.30%	38.78%
10-20 cm	30.20%	33.78%
20-30 cm	19.90%	28.14%

6.5.1.A. POM-C AT 0-10 CM

At 0-10 cm the main effect of traffic, tillage and the interaction between traffic and tillage systems were all significant. Within the traffic systems, CTF stored significantly more POM-C (25.25% more) than LTP and STP systems. CTF systems had 14% less soil BD than LTP and STP systems, indicating that soil compaction significantly reduced POM-C content at 0-10 cm.

Within the tillage systems, Zero and Shallow tillage systems stored more POM-C (12.29% more) than Deep tillage systems, indicating that reduced tillage significantly increased POM-C content at 0-10 cm. Deep tillage systems redistribute the SOM deeper in the soil profile, enhancing their decomposition by increasing the soil-SOM contact, the oxygen supply, and breaking of soil aggregates (Six *et al*., 2000), while Zero and Shallow tillage systems accumulate most of the residues on the top 0-10 cm. This is because Shallow tillage systems only go down to 10 cm, and the Zero tillage systems used in this site often use discs (~5 cm) to break the crop residues left on the surface. These results agree with Mikha *et al*. (2013) and Mikha and Marake (2023), who reported reduced POM-C concentrations in the upper layers of mouldboard plough systems when compared to Zero tillage systems. Their results showed a higher difference between the POM-C content of Zero tillage and mouldboard plough systems than the results of this study. This difference could be because our deep tillage systems (not being inversion) will have a smaller soil mixing effect when compared to the mouldboard plough systems.

Within the interaction between the traffic and tillage systems, CTF with Zero tillage stored significantly higher POM-C content (38.7% more) than the rest of the treatment combinations, except CTF Shallow tillage. CTF Shallow tillage stored significantly higher POM-C content (27.4% more) than STP (Zero, Shallow and Deep) and LTP Deep. The results also showed that the lowest POM-C content was stored in LTP Deep tillage and STP (Zero, Shallow and Deep) tillage systems. These results showed the additional benefits in POM-C content at 0-10 cm by combining CTF systems with reduced tillage systems (Zero and Shallow).

POM-C is made up mostly of fresh and partly decomposed plant litter and is considered the more dynamic and labile fraction (Mayer *et al.*, 2022; Schiedung *et al*., 2017). When it is not occluded within aggregates, it can be easily decomposed (Lutzo *et al.,* 2006). However, if it is occluded within aggregates that are not disturbed by soil compaction or tillage, they can be relatively stable, in some forest ecosystems the residence time can reach a hundred years (Angst *et al*., 2023). In fact, in agricultural soils, the SOC loss historically occurred predominantly from the POM-C fraction (Lugato *et al.,* 2021).

Much of the focus on arable soils has been on increasing the MAOM fraction because it accounts for the bigger proportion of the total SOC. However, the POM fraction on arable soils can also play a very important role. POM mineralisation promotes soil fertility, aggregate stability, soil health and biodiversity (Chenu *et al*., 2019), improving agricultural productivity. The POM fraction also has a bigger expansion potential than the MAOM fraction, which can be constrained by priming effects and mineral surface saturation.

Increasing POM concentrations also promote MAOM formation (Cortufo *et al*., 2013) because increased POM supports higher levels of microbial biomass, much of which eventually becomes microbial necromass, a main component of MAOM (Lavallee *et al*., 2020).

6.5.1.A.1 POM-C AT 0-10 CM OVER TIME

The effects of traffic over time: the main effect of traffic ($p < 0.001$), collection ($p < 0.001$) and the interaction between traffic and collection ($p < 0.001$) were all significant.

Within the traffic systems, the results were the same as the main as described above where CTF had significantly higher POM-C than LTP and STP systems.

Within the collection dates, the soil sample collection on 29/04/2022 (after the winter cover crop) had significantly higher POM-C content (16.4% more) than the following two soil sample collections (03/11/2022 after Millet crop, and 29/09/2023 after spring oats). This could be due to the combined effect of the previous crop biomass residue (winter barley) plus the winter cover crop biomass residue, increasing the POM-C fraction for all traffic and tillage treatments. This aligns with the higher C/N ratio (48.31) of POM 0-10 cm on 29/04/2022, compared to the following two soil sample collections: 03/11/2022 C/N ratio = 12.53 and 29/09/23 C/N ratio = 14.69. The higher C/N ratio of POM (0-10 cm) can lead to N immobilisation as microbes require additional nitrogen and will look for inorganic nitrogen in the soil to break down the carbon-rich material (Averill and Waring, 2018).

Within the interaction between traffic systems and collection dates, CTF systems on all the collection dates and LTP systems on 29/04/2022 had significantly higher POM-C than the rest of the traffic systems and collection dates combinations, except STP systems on 29/04/2022 (Fig. 6.45. a). CTF had consistently lower soil BD than LTP and STP systems on those soil sample collections (Fig. 6.45 b), showing that traffic-induced soil compaction affected POM-C over time. However, the increased crop biomass inputs on collection 29/04/24 also played an important role, increasing both LTP and STP POM-C concentrations on collection (29/04/2022).

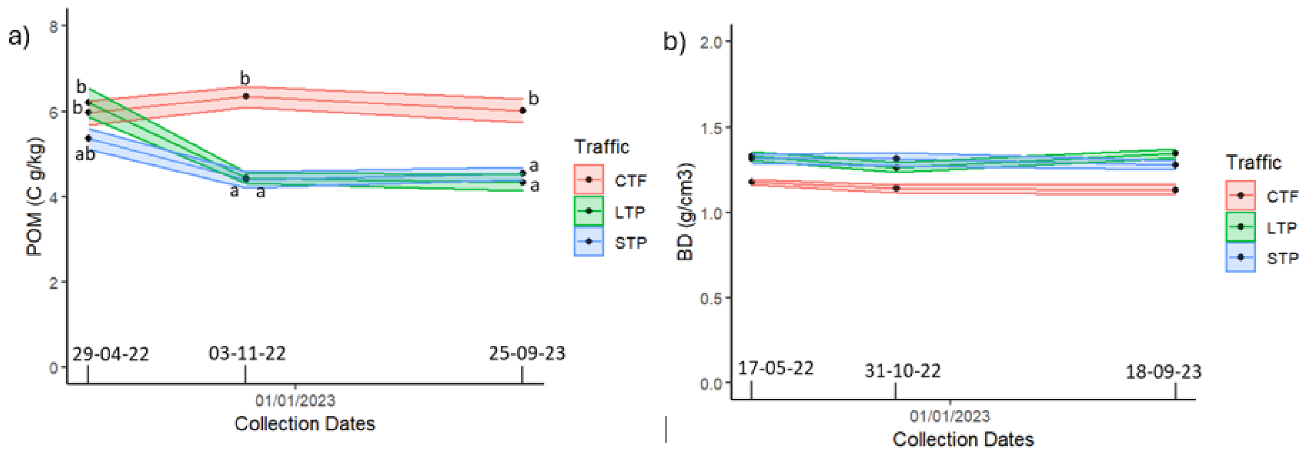


Figure 6. 45 – **a)** Main effects of the interaction between traffic systems and collections on POM-C (%) at 0-10 cm. Data from 2021-2023. Lines show means (n = 4). Ribbons show standard errors. Letters indicate significant differences based on (P<0.05). **b)** Main effects of the traffic systems on soil bulk density at 0-10 cm. Data from 2021-2023. Lines show means (n = 4). Ribbons show standard errors. The interaction between traffic systems and collection dates was not significant.

The main effects of tillage over time: at 0-10 cm the main effect of tillage (p = 0.01) and collection (p < 0.001) were significant, but the interaction between tillage and collection (p = 0.80) was not significant, indicating no temporal variation in POM-C content across the tillage systems (Fig. 6. 46 a). Correspondingly, the interaction between the soil bulk densities of the different tillage systems and collections at 0-10 cm was also not statistically significant (Figure 6. 46 b), corroborating that soil bulk density can affect POM-C dynamics.

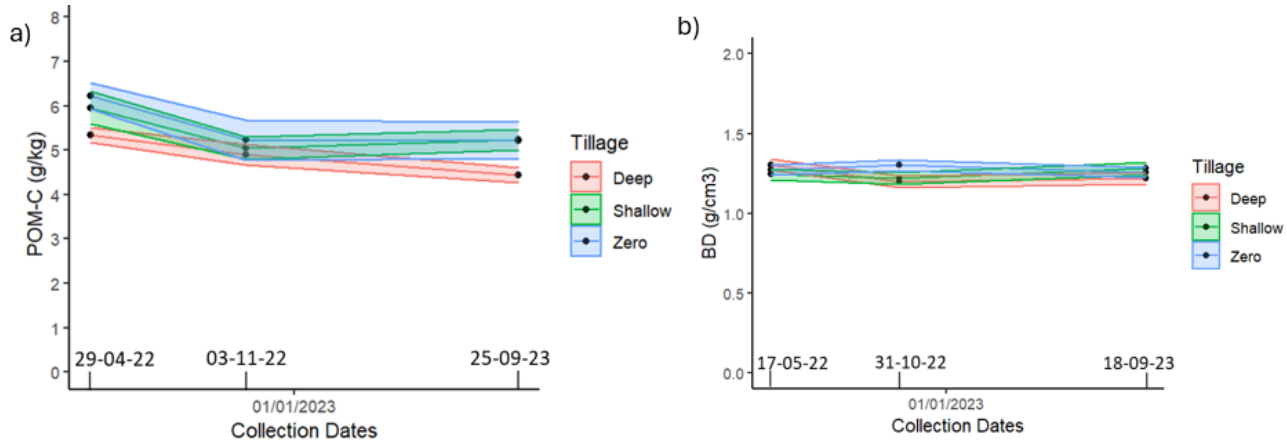


Figure 6. 46 – **a)** Main effects of the interaction between the tillage systems and collection dates at 0-10 cm depth. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection was not significant (p=0.80). **b)** Main effects of the interaction between the tillage systems and collection dates at 0-10 cm depth on soil bulk density. Lines show means (n = 12). Ribbons show standard errors. The interaction between tillage systems and collection was not significant.

However, the interaction effect of the different traffic-tillage systems and collection was significant, meaning that there was temporal variation in the POM-C content between the different Traffic-Tillage systems.

6.5.1.B. POM-C AT 10-20 CM

At this soil depth, POM-C was not significantly affected by the main effect of traffic, tillage and their interactions. However, the average coefficient of variation (CV = 22%) at 10-20 and 20-30 cm was quite high, indicating that the data was highly variable. This could be due to a higher spatial variability of SOC at these depths; increasing the sample size could improve the statistical power to detect differences between treatments, however, this was not possible within the time and practical constraints of this project.

6.5.1.B.1 POM-C AT 10-20 CM OVER TIME

The main effects of the traffic and tillage systems over time: The interaction between the traffic and tillage systems and collection dates was not significant, indicating no temporal variation in POM-C content at 10-20 cm.

The main effect of collection (p < 0.001) was significant, with the collection on 29/04/2022 having significantly higher POM-C content than the next two soil sample collections, the reason was previously described.

However, the interaction effect of the different traffic-tillage systems and collection was significant, meaning that there was temporal variation in the POM-C content between the different traffic-tillage systems.

6.5.1.C. POM-C AT 20-30 CM

Only the effect of the interaction between traffic and tillage systems ($p < 0.001$) was significant, with CTF Zero tillage storing higher POM-C concentrations than STP Deep and LTP Zero tillage systems. And with LTP Shallow tillage systems having the second highest POM-C concentrations at 20-30 cm.

These results showed that over the last 12 years, CTF with Zero tillage and LTP Shallow tillage can facilitate the translocation of POM-C down the soil profile or the formation of litter residues from the enhanced root systems and fungal networks, facilitating the creation of soil aggregates and their physical protection due to the lack of tillage.

The results of this study show that the combination of both traffic and tillage management practices can play a significant effect on POM-C concentrations across the soil profile (0-30 cm).

6.5.1.C.1 POM-C AT 20-30 CM OVER TIME

The main effects of traffic and tillage over time: at 20-30 cm only the main effect of collection dates (p = 0.001) was significant, meaning that the collection on 29/04/2022 (after winter cover crop) and 25/09/2023 (after spring oats) had as a whole significantly higher POM-C content than the collection on 03/11/2022 (after Millet crop).

The interaction effect between the traffic systems and collection, and the tillage systems and collection were both not significant, meaning that POM-C content of the different traffic and tillage systems at 10-20 cm did not change over time.

However, the interaction effect of the different traffic-tillage systems and collection was significant, meaning that there was temporal variation in the POM-C content between the different traffic-tillage systems.

6.5.1.E. MAOM-C AT 0-10 CM

MAOM-C is essential for soil carbon sequestration due to its larger contribution to bulk soil C storage and its high stability (Lavallee *et al*., 2020).

At 0-10 cm the results showed that the main effect of traffic systems was significant, with CTF systems having significantly more MAOM-C (3.7% more) than STP systems, indicating that soil compaction affected MAOM-C concentrations at this soil depth. CTF systems had a lower soil bulk density than STP systems, which means an enhanced network of pore spaces, increasing aeration and water infiltration, but also providing the space for roots and microbes to grow and thrive.

The MAOM fraction consists of soluble extracts that have either leached from plant litter or root exudates or been chemically transformed by the soil biota (Lavallee *et al*., 2020). But also, from the dead microbes themselves, called microbial necromass. They can be tightly bound to minerals or occluded within microaggregates. Their mean residence time is assumed to be from decades to centuries or even millennia, depending on the ecosystem properties (Schmidt *et al*., 2011). However, MAOM-C can show rapid turnover dynamics (Kleber *et al.,* 2021) and serve as a potential plant nutrient source (Jilling *et al.,* 2018). The main MAOM-C formation pathways can differ across diverse ecosystems, but on temperate agricultural soils, it has been estimated that around 55% of MAOM-C is considered to be derived from microbial necromass (dead microbes and fungi) (Liang *et al*., 2019), with plant-derived compounds accounting for ~45% of MAOM-C. However, in forests or perennial crops, the plant-derived compounds account for a higher percentage (Angst *et al*., 2021).

The main effect of tillage was borderline non-significant (p = 0.06), meaning that the main effect of tillage systems was not strong enough to affect MAOM-C at 0-10 cm.

Within the traffic and tillage interaction, CTF Zero tillage systems had significantly more MAOM-C (2.2% more) than LTP Zero tillage and STP Zero tillage systems. At 0-10 cm, the average coefficient of variation was low CV = 8%, indicating that the data had low variability.

6.5.1.E.1 MAOM-C AT 0-10 CM OVER TIME

The main effects of traffic and tillage and their interaction over time: only the main effect of traffic (p = 0.02), traffic-tillage (p = 0.003), and collection (p < 0.001) were significant. Meaning that the effect of the different traffic and tillage systems and their interaction on MAOM-C was consistent over time. This is consistent with literature reporting the long-term stability of MAOM-C compared with POM-C, especially on undisturbed soils where it can remain for hundreds of years.

Within the collection dates, the collection on 29/04/2022 (after the winter cover crop) had significantly less MAOM-C (12.2% less) than the collection on 03/11/2022 and 25/09/2023. This could be due to a “positive priming effect” where the enhanced microbial activity due to the increase in C inputs can accelerate the mineralisation of MAOM-C (Liang *et al.*, 2023). After two decades of cover cropping on sandy loam soil in Denmark with Atlantic climate, Liang et al., (2023) showed that low cover crop C input resulted in overall C loss due to positive priming. They concluded that a cover crop C input should exceed a certain threshold to ensure C sequestration.

6.5.1.F. MAOM-C AT 10-20 CM

After 12 years, neither the main effect of traffic, tillage nor the interaction between traffic and tillage systems significantly affected MAOM-C at this soil depth. The average coefficient of variation was low CV = 8%, indicating that the data had a low variability.

6.5.1.F.1 MAOM-C AT 10-20 CM OVER TIME

The main effects of traffic and tillage and their interaction over time: Only the main effect of collection was significant, as above (0-10 cm). Therefore, at 10-20 cm, there was no temporal variation in MAOM-C affected by the different treatments.

6.5.1.G. MAOM-C AT 20-30 CM

The main effect of traffic ($p = 0.06$) and tillage ($p = 0.07$) were borderline non-significant. And the interaction between the traffic and tillage ($p = 0.14$) systems was not significant. Although not significant, CTF Zero tillage stored 10.4% more MAOM-C than the rest of the treatment combinations, this could be due to the enhanced soil structure and network of pore spaces reaching this soil depth of CTF with Zero tillage systems.

The average coefficient of variation was low, CV = 10%, indicating that the data had a low variability.

6.5.1.G.1 MAOM-C AT 20-30 CM OVER TIME

The main effects of traffic, tillage and their interaction over time: Only the main effect of collection was significant ($p < 0.001$) (as above). The interaction effect between the different traffic and tillage systems and collection was not significant, meaning that their effect on MAOM-C was consistent over time.

6.5.1.H. POM-C AND MAOM-C AT 0-30 CM

The main effect of traffic significantly affected both POM-C and MAOM-C at 0-30 cm, with CTF systems storing 13.94% more POM-C than LTP and STP systems and 2.94% more MAOM-C than STP systems. These results showed that CTF systems had a greater positive effect on the POM-C content than the MAOM-C content. And although the MAOM-C had a larger proportion of the total SOC, the greater relative increase in POM-C was the primary driver of the total SOC gains.

The main effect of tillage significantly affected POM-C but not MAOM-C at 0-30 cm, with Zero and Shallow tillage systems storing 7.44% more POM-C than Deep tillage systems. Therefore, reduced tillage practices (Zero and Shallow) only enhanced the POM-C fraction at 0-30 cm. Tillage did not affect MAOM-C stabilisation, this is possibly a result of microbial transformations, necromass production and root exudates (the main component of MAOM-C on arable soils) were favoured in both undisturbed and tilled soil conditions, although through different mechanisms.

These results also showed that reducing traffic-induced compaction (CTF systems) had a stronger influence on POM-C content than reducing tillage intensity (Zero and Shallow Tillage systems).

The interaction effect between the traffic and tillage systems affected both POM-C and MAOM-C at 0-30 cm: CTF with Zero tillage stored 25.65% more POM-C content than the rest of the treatment combinations and 5.83% more MAOM-C than LTP (Deep and Zero) and STP (Deep, Shallow and Zero). The results also showed that the interaction effect had a greater effect on the POM fraction than the MAOM fraction. As time passes, if the same management practices continue to be implemented, the relatively larger increases in POM-C may progressively balance the C distribution between POM-C and MAOM-C, approximating the C allocation to natural soils (Lugato, *et al*., 2021).

These findings align with a recent meta-analysis by Prairie, King and Cotrufo (2023) on the effects of regenerative agricultural practices on POM-C and MAOM-C in cropland that concluded that zero tillage and cropping system intensification (which includes planting cover crops and perennial crops in rotation) increased POM-C by 19.7 and 33.3%, respectively, and MAOM-C by 8.5% and 7.1%, respectively, at 0-20 cm, but not in the subsoil (>20 cm). They also showed that combining zero tillage with integrated crop-livestock systems and cropping intensification had the highest increase in both POM-C and MAOM-C, concluding that regenerative agriculture practices are key to increasing C sequestration and promoting soil health.

The coefficient of variation of POM-C at 0-30 cm was avg. CV = 43% and MAOM-C at 0-30 cm was avg. CV = 15.41%, indicating that POM-C had a higher data variability than MAOM-C, which is also consistent with the literature.

At 0-30 cm, Depth significantly influenced organic carbon concentrations, with POM-C exhibiting greater vertical variability compared to MAOM-C. The 0-10 cm layer contained 39.39% more POM-C and 12.59% more MAOM-C than the 10-20 cm layer, while the 10-20 cm layer stored 34.06% more POM-C and 20.5% more MAOM-C than the 20-30 cm layer.

This study provides clear evidence for the ability of CTF systems to increase both POM-C and MAOM-C in topsoil (0-30 cm) compared to STP systems.

The absolute response of POM-C to the different traffic and tillage systems was bigger but also more variable than that of MAOM-C, suggesting that other factors might affect more POM-C stabilisation compared to MAOM-C (e.g. temperature, moisture, etc).

6.5.2. C/N RATIO OF POM AND MAOM

Soil N in agricultural soils has complex spatiotemporal dynamics due to many interacting factors, such as N fertilisation, soil properties, climatic conditions, and management practices (Dou *et al*., 2008). Intensified N fertilisation can contribute to a higher soil N and a decrease in C/N ratio. The C/N ratio shows the N availability, which directly affects C sequestration (Tian *et al*., 2006).

Soil organic matter fractions exhibit distinct C/N ratios: POM ranges from 10 to 40, while MAOM ranges from 8 to 13 (Lavallee, Soong and Cotrufo, 2020). These ratios reflect the relative contributions of plant-derived material (winter wheat, spring oats and winter barley biomass C/N 70-80) (USDA, 2011), microbial necromass (C/N 3-15) (Cleveland and Liptzin, 2007) and root exudates. Our results align with these studies, POM C/N ratio was 18.86 and MAOM C/N ratio was 8.42, supporting the idea that microbial contributions were greater than the plant-derived ones for MAOM C/N ratio, which is common in cropland due to the smaller root systems and annual die-offs compared to forest soils or perennial crops where plant derived organic matter could also contribute to MAOM C/N ratio > 15 (especially in wet forest (Yu *et al*., 2022). The higher POM C/N ratio reflects the contribution of crop residues (mainly straw) with a high a high C/N ratio.

There were no significant differences between treatments for both POM and MAOM C/N ratios, this could be due to all treatments having the same or very similar SOM inputs and fertilisation rates.

POM and MAOM C/N ratios were not affected by the different traffic and tillage management practices, they were only affected by the overall soil depth and the different soil sample collections. This could be due to all the soil samples being collected at the end of each cropping season and all the treatments having the same crop residue inputs and fertilisation rates. It is possible that if the samples would have been collected after a tillage event, the increase in SOM mineralisation from Deep tillage systems might have affected the C/N ratio. When looking at the C/N ratio of both fractions over time, only POM C/N ratio was significantly affected by the interaction between the traffic and tillage systems, depth and collection date, meaning that it was not constant over time, while MAOM C/N ratio remained constant over time. This agrees with other studies that say that the POM fraction is more variable in the system and MAOM is more stable (Lavallee, Soong and Cotrufo, 2020).

The collection on 29/04/2022 had a significantly higher POM C/N ratio than the rest of the soil sample collections (especially on 0-10 and 10-20 cm), meaning that there was hardly any N on this fraction and soil depths. This can be attributed to the soil sample collection (29/04/2022) that happened after a winter cover crop (composition: 80% Black Oats, 15% Vetch, 5% Phacelia) (Table 3.3 -Methodology Chapter and Fig. 6. 47), followed by the decomposition of the previous winter barley crop residue. The barley residue and cover crop had a high C/N ratio. Additionally, the cover crop was not fertilised, therefore, microbes and plants would have used the available soil N, producing a temporary N deficiency or immobilisation.

Fig. 6. 47 - Photo of winter cover crop on 02/03/2022, after being sprayed on 15/02/2022.

In fact, this soil sample collection POM-N had many outliers with N values lower than 0.01. While the MAOM C/N ratio exhibited greater stability across the different soil sample collections and contained the majority of soil organic N.

Soil microorganisms need an ideal diet with a C/N ratio of 21:1 (USDA, 2011). Therefore, organic nitrogen availability is an important factor that regulates SOC sequestration. Its limitation can have opposing effects on the POM and MAOM fractions, slowing down the decomposition of plant litter short-term, microbial biomass growth and therefore microbial necromass, resulting in less MAOM and more POM (Averill and Waring, 2018; Cotrufo et al, 2013). In fact, the collection on 29/04/2022 had the lowest MAOM-C, being significantly lower than the next soil sample collection on 03/11/2022.

However, N fertilisation over time and if applied in excess, can contribute to soil acidification (through nitrification). Soil pH has a strong effect on soil microbial activity, affecting bacterial and fungal growth and microbial community composition (Averill and Waring, 2018). In acidic soils, microbial growth will be reduced, ultimately slowing down the decomposition of SOM, and increasing POM-C relative to the increase in MAOM-C (Zhan, Chen and Ruan, 2018). Therefore, there is a delicate balance between N fertilisation, soil pH and SOM decomposition.

There is a current emphasis on increasing SOC sequestration in agricultural land (e.g. the "4 per 1000" initiative launched at the COP21 conference in Paris), however as van Groenigen *et al*. (2017) reported, to be able to increase 0.4% SOC stocks in agricultural land, it would also require an increase in the current global N-fertilizer production, concluding that the 0.4% SOC storage is unlikely to be met due to stoichiometric constraints.

6.5.3. LIMITATIONS

The absence of an initial SOC baseline data from the beginning of the field experiment prevented the determination and assessment of whether true C sequestration was observed with SOC change (three years is not enough to see a significant increase). The differences in SOC storage from the different treatments show a higher SOC storage in comparison with business as usual (STP Deep tillage systems), but not the overall C sequestration over the last 12 years. This data can act as a baseline for future studies on this long-term project.

6.6. CONCLUSIONS

The hypothesis number one, "*Traffic-induced soil compaction will store less POM-C and MAOM-C content, therefore, reduced traffic and wheel pressure will lead to depth-specific higher storage of POM-C and MAOM-C content*", was confirmed by our results at 0-30 cm, showing that the overall effect of traffic significantly affected both POM-C and MAOM-C concentrations, with the non-trafficked crop area of CTF systems storing 13.94% more POM-C and 2.94% more MAOM-C than LTP and STP systems.

The hypothesis number two, "*Tillage-induced soil disturbance increases SOM decomposition; therefore, reduced tillage will lead to depth-specific higher storage of POM-C and MAOM-C content",* was partially confirmed by our results at 0-30 cm, showing that the main effect of tillage significantly affected POM-C, but not MAOM-C concentrations. Zero and Shallow tillage systems stored 7.44% more POM-C than Deep tillage systems.

The hypothesis number three, "*The interaction between traffic and tillage systems will affect POM-C/ MAOM-C content at different depths*" was confirmed by our results at 0-30 cm, showing that the effect of the interaction between the traffic and tillage systems affected both POM-C and MAOM-C at 0-30 cm: CTF with Zero tillage stored 25.65% more POM-C content than the rest of the treatment combinations and 5.83% more MAOM-C than LTP (Deep and Zero) and STP (Deep, Shallow and Zero).

Therefore, the different traffic and tillage systems, both independently and interactively, exhibited stronger effects on the POM fraction than the MAOM fraction. The treatment effects on POM and MAOM fractions decreased with soil depth (0-10 > 10-20 > 20-30 cm), with POM showing greater accumulation potential than MAOM.

CHAPTER 7

INVESTIGATING THE EFFECTS OF TRAFFIC AND TILLAGE ON SOIL CARBON DYNAMICS THROUGH APPLICATION OF THE NATURAL ABUNDANCE ^{13}C ISOTOPE TECHNIQUE

7.1. INTRODUCTION

To better understand SOC sequestration and storage, and to accurately predict SOM decomposition and stabilisation over time, it is necessary to understand SOM's fate and residence time. Research suggests that there are two major pathways for the formation of stabilised SOC: the first pathway is driven by soil microbes, which break down the Particulate organic matter (POM) and transform it into microbial necromass and metabolites that become bound to mineral surfaces or in micro-aggregates (Liang *et al*., 2017; Cotrufo, 2019). The second pathway is driven by plant-derived compounds (e.g. root exudates, litter leaching) that bind to mineral surfaces or get protected within micro-aggregates (Liang *et al.,* 2017; Cotrufo, 2019). Both of these forms of SOM are referred to as Mineral associated organic matter (MAOM). Depending on the land use and soil management, one or another pathway can become dominant. In arable soils with seasonal crops, usually the microbial pathway is the most dominant (>50%). In temperate agricultural soils, 55.6% of the total SOC was considered microbial necromass (Liang *et al.,* 2019).

The results from previous chapters confirmed that soil carbon storage varies significantly according to the different traffic and tillage management practices imposed. The results showed that over the long-term (12 yo), the combination of CTF with Zero tillage systems and crop residue management was an effective strategy to store higher SOM, SOC stocks and POM-C and MAOM-C concentrations. It also showed that changes in POM-C were the primary driver of SOC storage. This leads to the question of how and why more plant-derived C is stored under this treatment combination. Therefore, this chapter will investigate the fate of millet's crop litter and root residues in the different SOM fractions and soil depths through the natural abundance stable isotopic approach. To do so, it will use the isotopic discrimination by applying $\delta^{13}C$ of millet (C_4-plant) compared to native SOC (C3-plant dominated) to determine the insights into soil C dynamics. This may provide insights into the mechanisms underlying these C dynamics in soils and how they are affected by the traffic and tillage treatments.

During photosynthesis C3 plants, using the Calvin cycle, exhibit stronger discrimination against the stable isotope ^{13}C compared to C4 plants, which instead photosynthesise with the Hatch-Slack pathway (Hobbie and Werner, 2004). This results in distinctly lower $\delta^{13}C$ signatures in C3 (mean δ13C value of -27‰) than in C4 plant tissue (mean $\delta^{13}C$ value of -13‰). This natural abundance differential between the millet and soil containing predominantly C3-derived SOC provides a viable isotopic tracer mechanism for monitoring the fate of added carbon within the soil matrix (Balesdent *et al*., 1987) (Fig. 7.1). The rate of loss of the C derived from the original C3 plants and the incorporation of C from the C4 plants can be calculated (Balesdent *et al*., 1987).

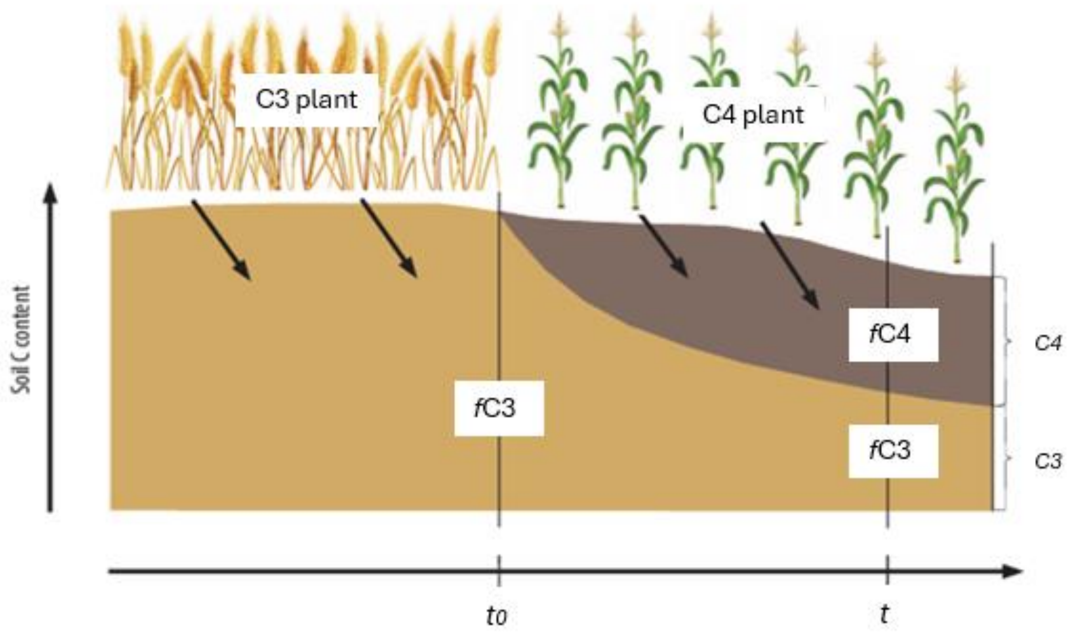


Fig. 7.1 –The replacement of SOM derived from C3 plants minus the new vegetation C4 plants. Before the change of vegetation, SOM exhibits the isotopic composition *f*C3 approximating that of the original vegetation. After the vegetation change C3 – C4 shows the proportion of C derived from C4 plants (*f*C4) (source: adapted from Zacháry *et al*., 2019, after Balesdent and Mariotti, 1996).

7.2. AIM AND HYPOTHESIS

This chapter aims to quantify the effects of alternative traffic systems and their interaction with different tillage systems on the decomposition and stabilisation of plant-derived C. To do so, it will explore the fate of millet (crop litter and root exudates) and the *f*C4 (proportion of C from millet) in the POM and MAOM fractions at different soil depths to determine how the inclusion of recent C inputs into POM and MAOM differs between treatments and soil depths.

The hypotheses for this chapter are:

1. After the incorporation of a C4 plant, both POM and MAOM $\delta^{13}C$ values will be higher compared to previous C3 plants, due to C4 plants' lower $\delta^{13}C$ values.
2. Traffic-induced soil compaction will affect the storage of *f*C4 (the proportion C derived from millet); therefore, reduced traffic and wheel pressure will lead to higher *f*C4 in the different SOM fractions and soil depths.
3. Tillage-induced soil disturbance increases SOC decomposition; therefore, reduced tillage will lead to higher *f*C4 in the SOM fractions and soil depths.
4. The interaction between traffic and tillage systems will affect *f*C4 storage into the different SOM fractions and soil depths.

7.3. METHODOLOGY

7.3.1 LABORATORY ANALYSIS

Soil samples were collected as described in Chapter 3 methodology. They were then fractioned as described in Chapter 6 to distinguish between POM and MAOM fractions (Lavalle *et al.,* 2020) at three soil depths (0-10, 10-20 and 20-30 cm). The dried soil was ball milled to a flour consistency. Bulk soil samples were then weighed (2.0 mg) in tin cups (IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany). These samples were then analysed at Consejo Superior de Investigaciones Científicas (CSIC), Spain for $^{13}C/^{12}C$ relative abundance. The abundance of $^{13}C/^{12}C$, is expressed as relative abundance ($\delta^{13}C$), based on the international Vienna Pee Dee Belemnite standard. It was measured using an isotope ratio mass spectrometry Flash Smart™ elemental analyser (Thermo Scientific, Bremen, Germany), equipped with a combustion reactor for C and N determinations. The soil samples were analysed together with appropriate calibration standards, within each batch of samples. The analytical standard deviation of $\delta^{13}C$ was typically less than ± 0.5 ‰.

The millet crop biomass was dried at 60°C overnight and ball milled to a flour consistency. Samples were analysed at Cardiff University and measured using a Thermo Flash EA 1112 series elemental analyser connected to a Conflo III and Thermo Delta V Advantage mass spectrometer. Samples are analysed in combination with three in-house standards, a lab-grade caffeine [$\delta^{13}C$=-33.30‰, $\delta^{15}N$ = -1.4‰], and two commercial collagen food supplements, [MarCol ($\delta^{13}C$= -16.20‰, $\delta^{15}N$=16.36) and MCF ($\delta^{13}C$ =-22.36‰, $\delta^{15}N$=4.26‰)]. The in-house standards were calibrated against the IAEA-CH6, IAEA-600, and IAEA-N-2 international standards. Results for δ13C were reported in the $\delta^{13}C$ notation relative to Vienna-Pee Dee Belemnite (VPDB).

7.3.1.1 ESTIMATION OF THE PROPORTION OF C FROM MILLET

To calculate the proportion of newly incorporated C4-derived C (*fC4*) into the bulk soil, each SOM fraction, and different soil depths after the millet crop, the following isotopic mixing equation of Balesdent *et al.* (1987) was used:

$$fC4 = \frac{\delta 13C\ (soil\ fraction\ C4\ soil) - \delta 13C\ (reference\ soil\ fraction\ C3\ soil)}{\delta 13C\ (C4\ millet\ biomass) - \delta 13C\ (reference\ soil\ fraction\ C3\ soil)}$$

equation 7.1

Where:

fC4 is the proportion of new C4 -derived C in the SOC fraction of interest.

$\delta^{13}C$ (soil fraction C4 soil) is the $\delta^{13}C$ of the SOC fraction of interest after millet.

$\delta^{13}C$ (reference soil fraction C3 soil) is $\delta^{13}C$ in the reference soil fraction before millet.

$\delta^{13}C$ (C4 millet biomass) is the $\delta^{13}C$ of millet crop biomass.

The soil sample collections analysed for δ^{13}C values were sampled at the end of each cropping season (Table 7.1).

Table 7. 1. Soil sample collections analysed for δ^{13}C values

soil sample collection	crops
29/04/2022	C3 crops for at least the previous 10 years (the last crop was winter barley, followed by a winter cover crop)
03/11/2022	C4 crop (millet)
25/09/2023	C3 crop (spring oats)

7.4. RESULTS

7.4.1. SOIL NATURAL ABUNDANCE δ^{13}C VALUES FOR COLLECTIONS 29/04/2022 AND 03/11/2022:

To trace the incorporation of millet-derived C into the SOM, the natural abundance δ^{13}C analysis was used. POM and MAOM had very different δ^{13}C values before and after the millet crop. POM and MAOM δ^{13}C values for the collection on 03/11/2022 (after the millet crop) were enriched compared to the collection on 29/04/2022 (before the millet crop).

The average POM and MAOM δ^{13}C values for collection 29/04/2022 (before millet crop) were -26.71± 0.15‰ and -27.73± 0.04‰, respectively, at 0-30 cm. And for the collection 03/11/2022 (after the millet crop), the average POM and MAOM *δ^{13}*C values were -25.52± 0.51‰ and -27.6± 0.04‰ respectively (at 0-30 cm) demonstrating a significant higher ^{13}C (Fig. 7.2) (POM *δ^{13}*C $p < 0.001$; MAOM *δ^{13}*C $p < 0.001$) (Table 7.2). The average *δ^{13}C* value of the millet crop biomass was -13.98‰. The POM *δ^{13}*C values of the collection after the millet (03/11/2022) were 4.45% higher than before the millet (29/04/2022). The MAOM *δ^{13}*C values were 0.43% higher than the collection before millet (29/04/2022) (Fig. 7.2).

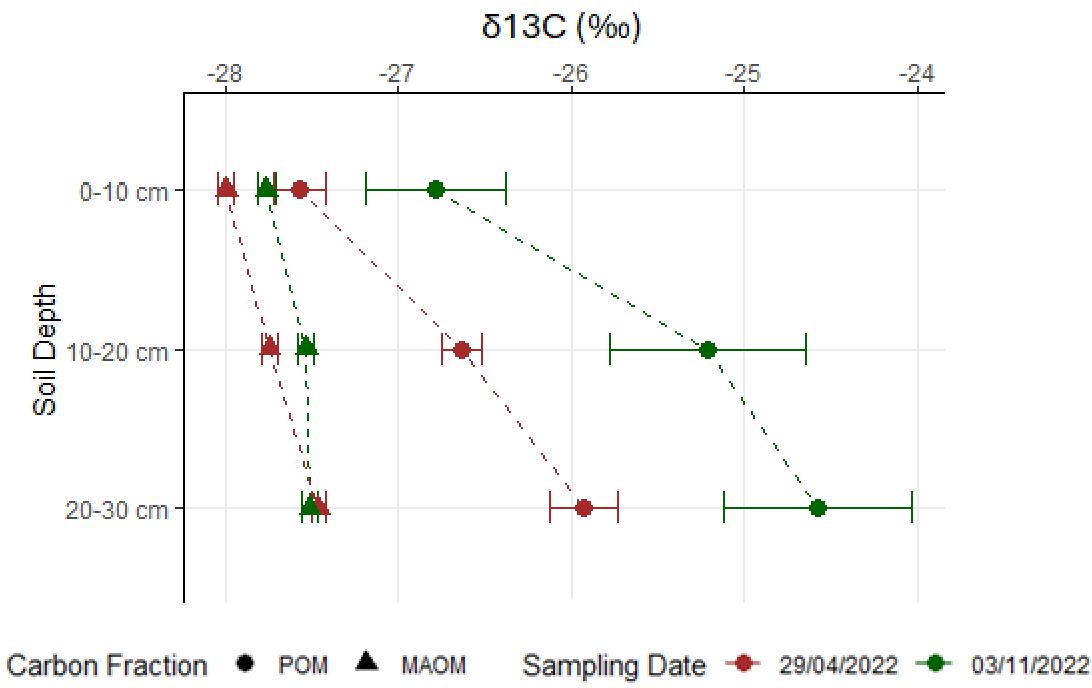


Figure 7. 2 - δ^{13}C POM and MAOM fractions values by soil depth at two soil sample collections: 29/04/2022 (before millet) and 03/11/2022 (after millet). Points show means. Bars show standard errors.

The POM *δ^{13}*C values of both collections showed a consistent, statistically significant increase with depth and followed a similar pattern ($p < 0.001$) (Fig. 7.2, Table 7.2), with increasing values from 0-10 cm, followed by 10-20 cm and 20-30 cm. The MAOM *δ^{13}*C values exhibited a small increase from 0-10 to 10-20 cm between sample collections. However, at 20-30 cm, the MAOM *δ^{13}*C values were the same in both sample collections.

Table 7. 2. Statistical significance of two soil sample collections on 29/04/2022 and 03/11/2022 (before and after millet crop) with a mixed effect model with block as a random effect and Collection and Depth as fixed effects.

p values	POM	MAOM
Collection	**< 0.001**	**< 0.001**
Depth	**< 0.001**	**< 0.001**
Collection x Depth	0.59	**< 0.001**

In both soil sample collections, the POM and MAOM exhibited more negative δ^{13}C in surface soils relative to deeper soil ($p < 0.001$, Fig. 7.2 and Table 7.2).

7.4.2. SOIL NATURAL ABUNDANCE δ13C VALUES FOR COLLECTIONS 03/11/2022 AND 25/09/2023:

Two years after the C4 crop was incorporated and with a C3 crop included (collection 25/09/2023) post-millet, the POM δ^{13}C values were significantly lower (i.e. more negative) while the MAOM δ^{13}C values were significantly higher (i.e less negative) compared to collection 03/11/2022 (millet crop) (POM δ^{13}C $p < 0.001$; MAOM δ^{13}C $p < 0.001$) (Fig. 7.3. and Table 7.3).

The average POM and MAOM δ^{13}C values for collection 25/09/2023 were -24.80± 0.42‰ and -27.9± 0.03‰ respectively (at 0-30 cm). The POM δ^{13}C values of collection 25/09/2023 (two years post millet cultivation) were 2.8% higher than collection 03/11/2022 (millet crop). Conversely, the MAOM δ^{13}C values were 1.09% lower than the collection 03/11/2022 (Fig. 7.2).

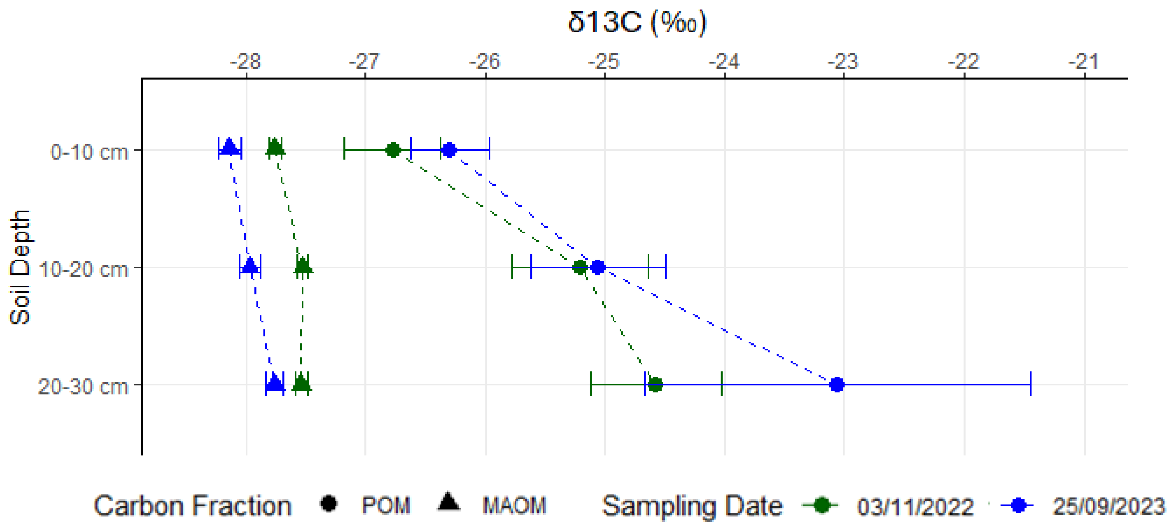


Figure 7. 3 - δ^{13}C POM and MAOM fractions values by soil depth at two soil sample collections: 03/11/2022 (after millet) and 25/09/23 (one year after millet with another C3 crop). Points show means. Bars show standard errors.

The POM $\delta^{13}C$ values of the last two soil sample collections showed a consistent increase with depth and followed a similar pattern ($p < 0.001$) (Fig. 7.2, Table 7.2), with higher $\delta^{13}C$ in the deeper layers. The MAOM $\delta^{13}C$ values of the collection on 25/09/2023 also increased with soil depth, albeit with less of a marked change with depth than the POM $\delta^{13}C$ values.

Table 7. 3. Statistical significance of two soil sample collections on 03/11/2022 and 25/09/2023 (after millet crop and one year after with a C3 crop) with a mixed effect model with block as a random effect and Collection and Depth as fixed effects.

p values	POM	MAOM
Collection	**0.007**	**< 0.001**
Depth	**< 0.001**	**< 0.001**
Collection x Depth	0.09	**0.025**

In both soil sample collections, the POM and MAOM exhibited lower $\delta^{13}C$ in surface soils relative to deeper soil ($p < 0.001$, Fig. 7.2 and Table 7.3).

7.4.3. PROPORTION OF MILLET DERIVED CARBON (*f*C4)

The (fC4) proportion of millet derived carbon is typically expressed as a value 0 to 1 (Poeplau *et al.*, 2018; Just *et al.*, 2021), although it could also be expressed as a percentage (e.g., 0.03 means 3% of the carbon is from a C4 source). In this study, an *f*C4 of 1 means all C from the sample is new millet derived C, whereas an *f*C4 of 0 means no input of millet derived C. Following the cropping millet season (collection 03/11/2022), the average *f*C4 that went in POM and MAOM at 0-30 cm was 0.13 and 0.01, respectively. Therefore, 93% of the total *f*C4 went into POM and only 7% went into MAOM.

Following the spring oats cropping season, post-millet (collection 25/09/2023), the average *f*C4 that remained in the POM or had been transformed into MAOM at 0-30 cm were 0.156 and 0.004, respectively. Therefore, by this date, 97% of the total *f*C4 remained in POM and 3% in the MAOM.

*f*C4 into POM *over time*: while the total *f*C4 at 0-30 cm increased from 0.14 to 0.16 from collection 03/11/2022 to 25/09/2023, the statistical analysis revealed that the main effect of collection ($p = 0.18$) and the interaction between depth and collection ($p = 0.08$) showed no statistically significant change over time.

*f*C4 into MAOM *over time*: the total *f*C4 at 0-30 cm decreased from 0.011 to 0.004 from collection 03/11/2022 to 25/09/2023. The statistical analysis revealed that the overall effect of collection ($p < 0.001$), depth ($p < 0.001$), and the interaction between depth and collection ($p < 0.001$) were all statistically significant, indicating that MAOM fC4 changed significantly with depth and over time.

7.4.3.1. fC4 IN THE POM FRACTION (COLLECTION 03/11/2022)

There were no statistically significant differences between the treatments in the *f*C4 in the POM fraction when analysed at the individual soil depths of 0-10, 10-20 and 20-30 cm. The average CV at 0-10 cm was CV = 152%, at 10-20 cm CV = 180% and at 20-30 cm CV = 167%, indicating high levels of variability.

However, when combined to explore the effects over 0-30 cm, the *f*C4 in POM showed a statistically significant overall effect of tillage ($p = 0.01$) (Fig. 7.4.). The overall effect of traffic ($p = 0.61$), depth ($p = 0.17$) and the interaction between traffic and tillage ($p = 0.13$), traffic and depth ($p = 0.55$), tillage and depth ($p = 0.97$) and traffic, tillage and depth ($p = 0.71$) were not statistically significant. The average *f*C4 in POM was 0.13 at 0-30 cm.

For the overall effect of tillage, deep tillage (POM *f*C4 = 0.187, CV = 149%) exhibited a significantly higher *f*C4 than Shallow tillage (POM *f*C4 = 0.078, CV = 209%) (Fig. 7.4.).

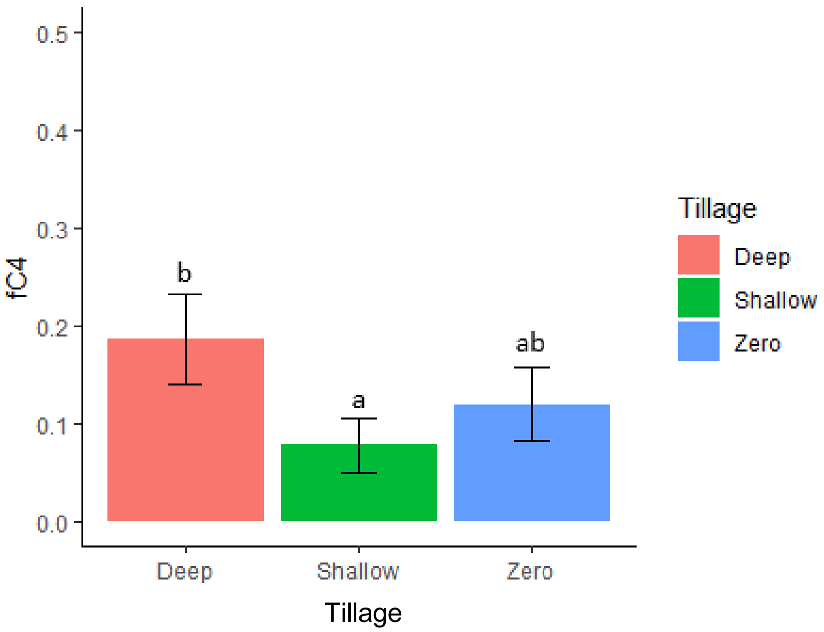


Figure 7. 4 – Main effects of the different tillage systems on the proportion of millet derived carbon (*f*C4) in the POM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 36). Bars show standard errors.

To further investigate if the *f*C4 could be affected by the dilution (Bougnères and Bier, 1983; Huang *et al.,* 2013; Zhu *et al.,* 2024) with the C content of the different treatments of the POM fraction, *f*C4 was multiplied by the C content of the POM fraction at 0-30 cm. The statistical analysis also showed that only the overall effect of tillage systems (p = 0.03) was statistically significant, with Deep tillage systems (0.52 g/kg of POM-C4, CV = 152%) having significantly higher C content than Shallow tillage (0.21 g/kg of POM-C4, CV = 207%) (Fig. 7.5).

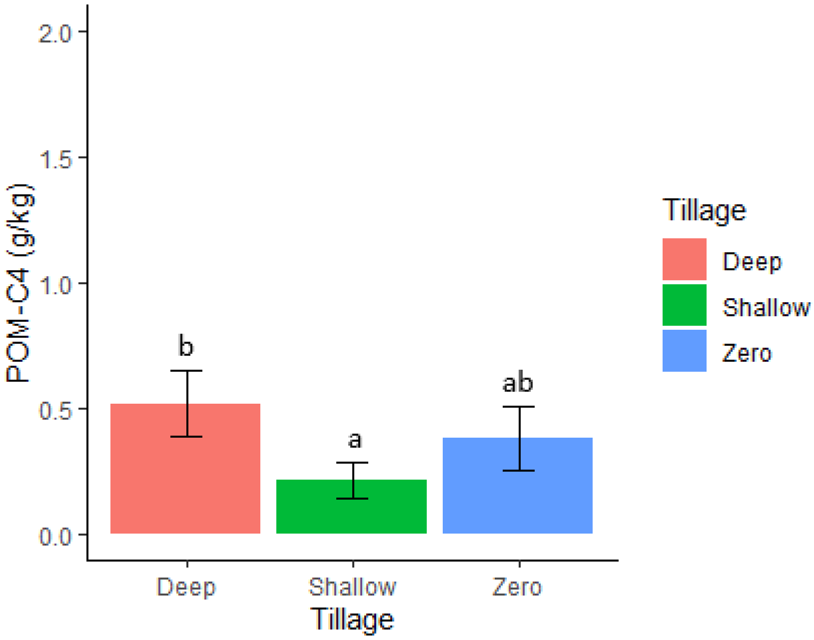


Figure 7. 5 – Main effects of the different tillage systems on the C content of millet in the POM fraction at 0-30 cm. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors.

7.4.3.2. fC4 IN THE MAOM FRACTION (COLLECTION 03/11/2022)

At 0-10 cm fC4 in MAOM was statistically significant only for the overall effect of traffic (p = 0.02) (Fig. 7.6). The overall effect of tillage (p = 0.10) and the interaction between traffic and tillage (p = 0.18) were not statistically significant.

Within the traffic systems, CTF had significantly higher *f*C4 into MAOM (*f*C4 MAOM = 0.028, CV = 72%) than STP systems (*f*C4 MAOM = 0.014, CV = 109%) at 0-10 cm.

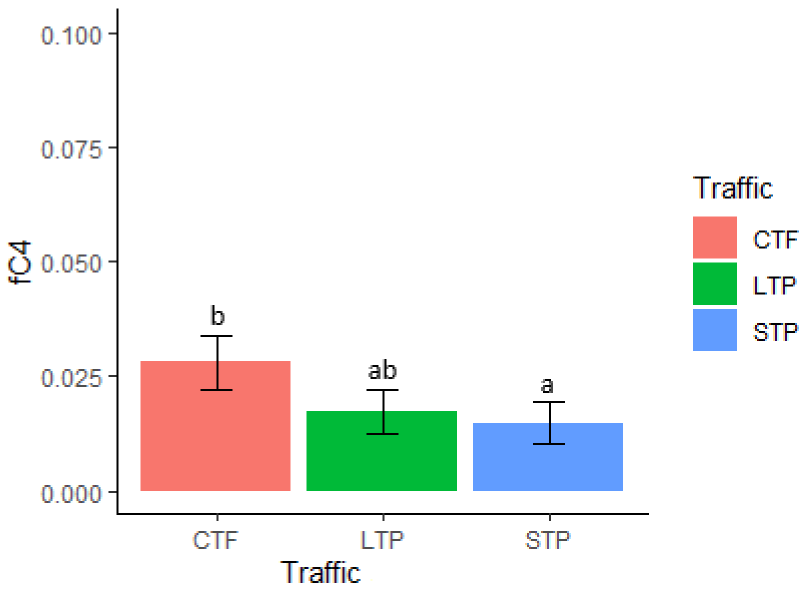


Figure 7. 6 – The overall effects of the different traffic systems on the proportion of *f*C4 (proportion of Millet derived C) in the MAOM fraction at 0-10 cm. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 36). Bars show standard errors. (Y axis has a different range than the previous graph).

At 10-20 cm there were no statistically significant differences between the treatments on the *f*C4 in MAOM (Fig. 7.7.). The average *f*C4 in MAOM at 10-20 cm was 0.0063, CV = 107%.

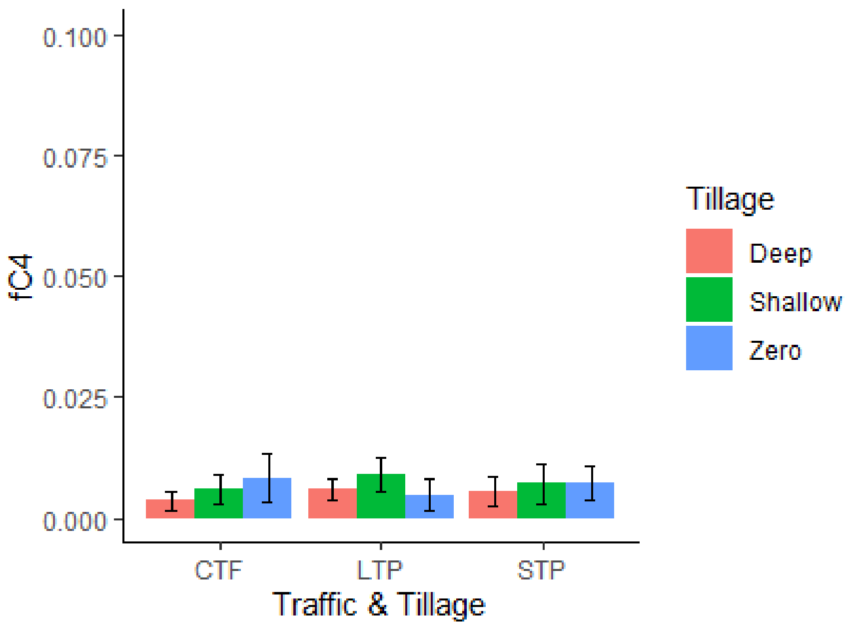


Figure 7. 7 – The interacting effects of the different traffic and tillage systems on the proportion of *f*C4 (proportion of millet derived C) in the MAOM fraction at 10-20 cm. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 4). Bars show standard errors.

At 20-30 cm, the interaction effect between the different traffic and tillage systems was statistically significant (p = 0.01) (Fig. 7.8.). The overall effect of traffic (p = 0.77) and tillage (p = 0.12) on *f*C4 were not statistically significant at this depth.

Within the traffic and tillage interaction, CTF with Zero tillage had significantly higher *f*C4 into MAOM at 20-30 cm (*f*C4 MAOM = 0.023, CV = 46%) than CTF Deep and Shallow tillage (both had *f*C4 MAOM = 0, CV = 0%) (Fig. 7.8).

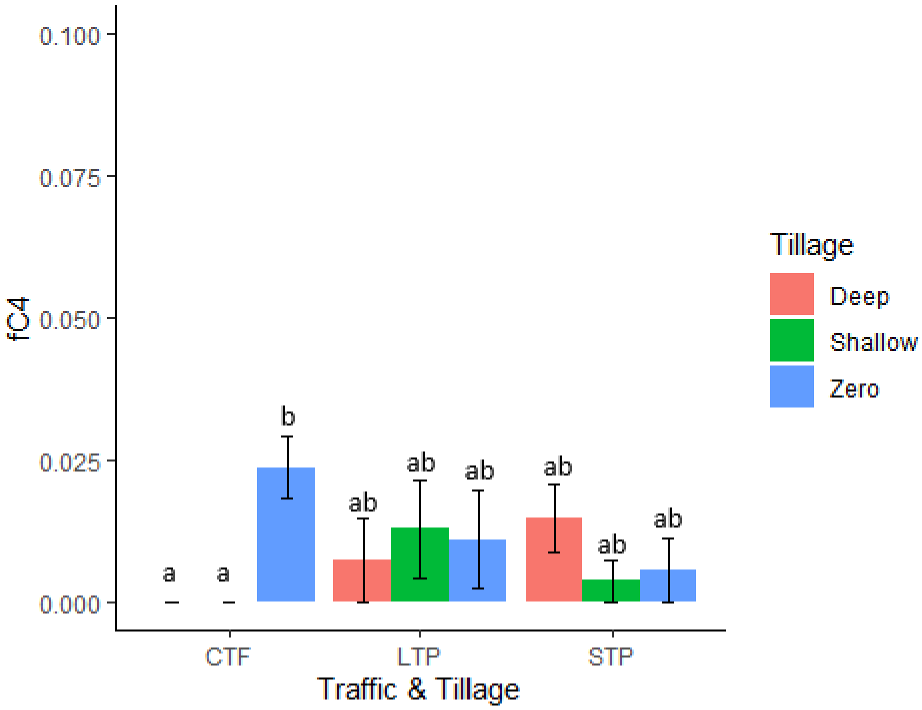


Figure 7. 8 – The interacting effects of the different traffic and tillage systems on the proportion of *f*C4 (proportion of Millet-derived C) in the MAOM fraction at 20-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 4). Bars show standard errors.

At 0-30 cm, the overall effect of depth ($p < 0.001$) and the interaction between traffic and tillage (p = 0.01), tillage and depth (p = 0.05) and traffic and depth (p = 0.05) were all statistically significant. But the overall effect of traffic (p = 0.25) and tillage (p = 0.23) independently, nor the interaction between traffic, tillage and depth (p = 0.18) were not statistically significant. The *Post hoc* analysis of the traffic and tillage interaction revealed no significant differences between treatments. The average *f*C4 in the MAOM at 0-30 cm was 0.01.

Within the different soil depths, the 0-10 cm depth layer had significantly higher *f*C4 in the MAOM (*f*C4 MAOM = 0.02, CV = 91%) than 10-20 cm (*f*C4 MAOM = 0.006, CV = 99%) and 20-30 cm (*f*C4 MAOM = 0.008, CV = 145%) (Fig. 7.9.)

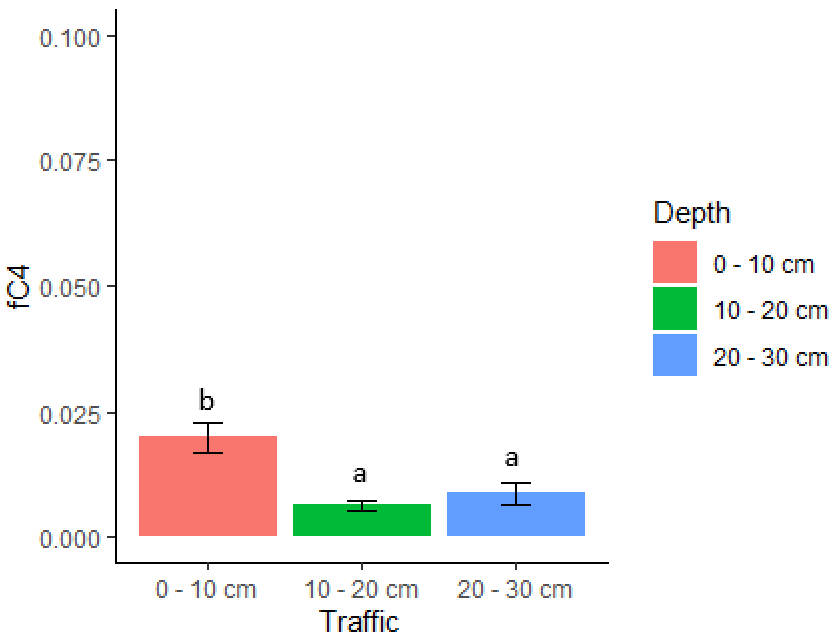


Figure 7. 9 – The overall effects of the different soil depth layers on the proportion of *f*C4 (proportion of Millet-derived C) in the MAOM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 36). Bars show standard errors.

Within the tillage and depth interaction, Shallow at 0-10 cm had significantly higher *f*C4 into MAOM (*f*C4 MAOM = 0.025, CV = 72%) than Deep, Shallow and Zero at 10-20 cm (avg. *f*C4 MAOM = 0.006, CV = 98%) and Deep and Shallow at 20-30 cm (avg. *f*C4 MAOM = 0.006, CV = 182%). Zero tillage at 0-10 cm had significantly higher *f*C4 into MAOM (*f*C4 MAOM = 0.018, CV = 96%) than Deep at 10-20 cm (*f*C4 MAOM = 0.004, CV = 94%) and Shallow at 20-30 cm (*f*C4 MAOM = 0.005, CV = 204%) (Fig. 7.10).

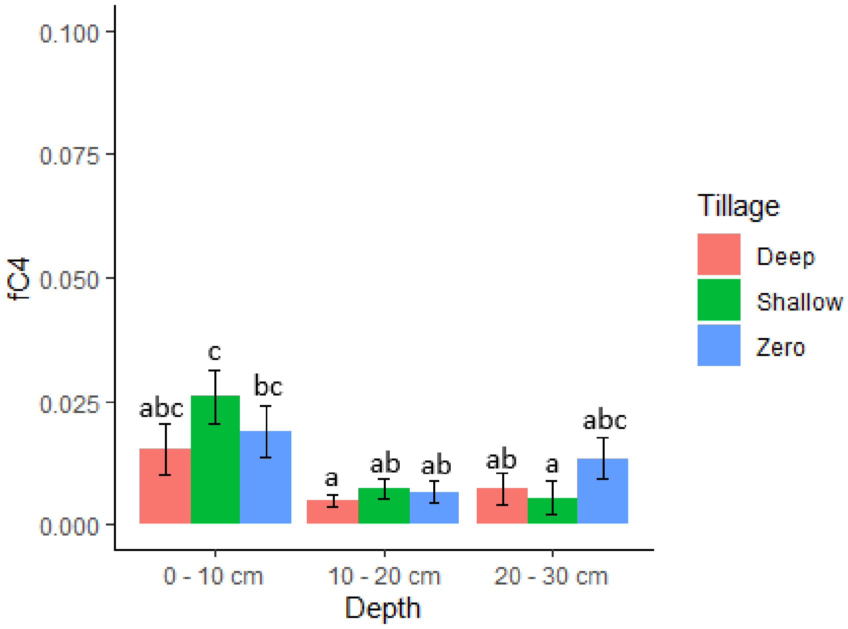


Figure 7. 10 – The effects of the interaction between tillage and soil depth on the proportion of *f*C4 (proportion of Millet-derived C) in the MAOM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 12). Bars show standard errors.

Within the traffic and depth interaction, CTF at 0-10 cm had significantly higher *f*C4 in the MAOM (*f*C4 MAOM = 0.028, CV = 72%) than all the other treatment combinations and soil depths (avg. *f*C4 MAOM = 0.008, CV = 122%), except for LTP at 0-10 cm which was not statistically significantly different from CTF (Fig. 7.11).

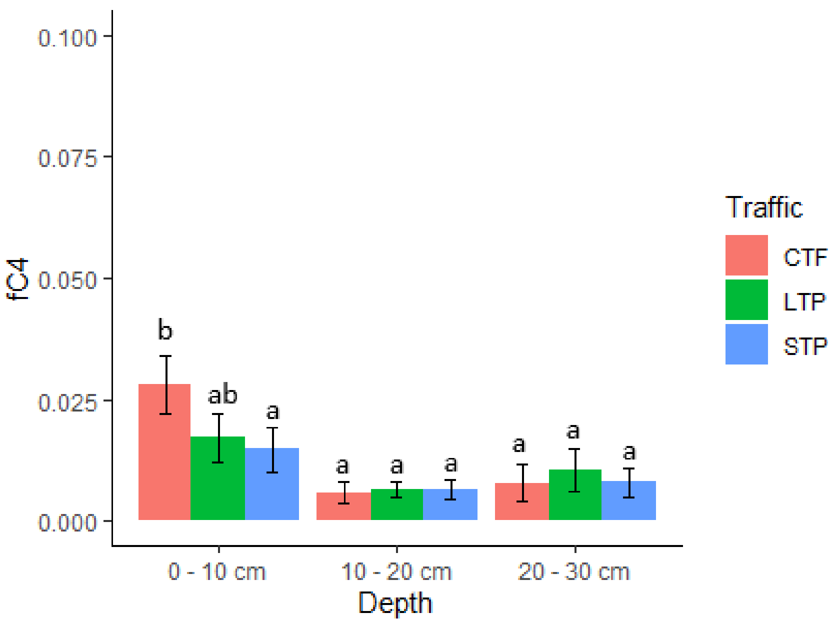


Figure 7. 11 – The effects of the interaction between traffic and soil depth on the proportion of *f*C4 (proportion of Millet-derived C) in the MAOM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 12). Bars show standard errors.

7.4.3.3. fC4 IN THE POM FRACTION (COLLECTION 25/09/2023)

There were no statistically significant differences between the treatments in the *f*C4 into the POM fraction when analysed at the individual soil depths of 0-10, 10-20 and 20-30 cm. The average CV at 0-10 cm was CV = 63%, at 10-20 cm CV = 80% and 20-30 cm CV = 113%.

However, at 0-30 cm, the *f*C4 into POM was statistically significant for the overall effect of traffic ($p = 0.04$) (Fig.7.12.) and depth ($p < 0.01$) (Fig. 7.13). The overall effect of tillage ($p = 0.76$), and the interaction between traffic and tillage ($p = 0.95$), traffic and depth ($p = 0.74$), tillage and depth ($p = 0.77$) and traffic, tillage and depth ($p = 0.53$) were not statistically significant. The average *f*C4 into POM was 0.156 at 0-30 cm.

For the overall effect of traffic, LTP systems (POM *f*C4 = 0.203, CV = 103%) exhibited a significantly higher *f*C4 than the permanent crop bed of CTF systems (POM *f*C4 = 0.143, CV = 119%) (Fig.7.12.).

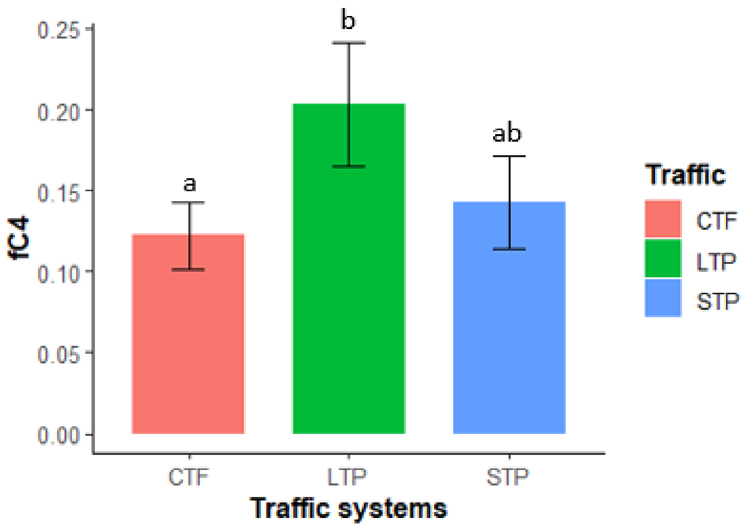


Figure 7. 12 – The overall effects of the different traffic systems on *f*C4 (proportion of millet derived C) in the POM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means ($n = 36$). Bars show standard errors.

Within the different soil depths, 20-30 cm (POM *f*C4 = 0.245, CV = 109%) exhibited a significantly higher *f*C4 than 0-10 (POM *f*C4 = 0.096, CV = 68%) and 10-20 cm (POM *f*C4 = 0.126, CV = 82%) (Fig. 7.13).

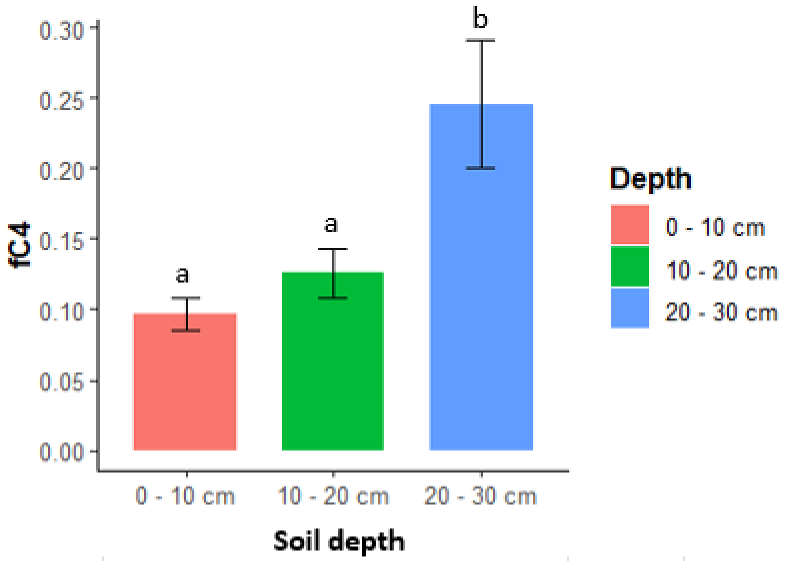


Figure 7. 13 – The overall effects of the different soil depths on *f*C4 (proportion of millet derived C) in the POM fraction at 0-30 cm. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 36). Bars show standard errors.

7.4.3.4. fC4 IN THE MAOM FRACTION (COLLECTION 25/09/23)

There were no statistically significant differences between the treatments in the *f*C4 in the MAOM fraction when analysed at the individual soil depths of 0-10, 10-20 and 20-30 cm. The average fC4 at 0-10 cm was *f*C4 MAOM = 0.006, CV = 187%, at 10-20 cm *f*C4 MAOM = 0.005, CV = 168% and 20-30 cm *f*C4 MAOM = 0.002, CV=122%. When combined to explore the *f*C4 average over the top 0-30 cm, only the interaction between traffic and tillage was significant ($p = 0.02$). But the *Pos hoc* analysis revealed no significant differences between the treatments. The average *f*C4 MAOM = 0.004 and CV = 159%.

7.5. DISCUSSION

7.5.1. SOIL NATURAL ABUNDANCE δ^{13}C VALUES

The fate of millet derived C, including from the crop biomass and root residues, into the different SOM fractions and soil depths was analysed using the isotopic differences in δ^{13}C of millet (C_4-plans, with a $\delta^{13}C$ value *-13.98* ‰) and native SOC (C3-plants, with a δ^{13}C value of -27‰) to determine the interactions between soil management and C dynamics.

By using natural abundance carbon isotope discrimination, the millet derived C could be traced. When plants absorb atmospheric CO_2 during photosynthesis, they preferentially take up the lighter carbon isotope (^{12}C) over the heavier (^{13}C). This results in plant tissues (e.g.

leaves, roots) being depleted in ^{13}C relative to soil C (Midwood *et al.,* 2021; Ehleringer *et al*., 2000). Soil C is relatively enriched in ^{13}C as, during microbial decomposition, microbes preferentially respire ^{12}C-enriched CO_2, increasing the proportion of ^{13}C of the remaining SOC, leading to it having a more negative $\delta^{13}C$ value (Camino-Serrano *et al*., 2019).

MAOM and POM exhibited distinctly different $\delta^{13}C$ values. MAOM $\delta^{13}C$ values were consistently more negative than POM, indicating that it had a higher proportion of ^{12}C than the POM. This isotopic fractionation was likely because the ^{12}C was preferentially taken up by the microbial community during the decomposition process, meaning that both microbial exudates and microbial necromass were relatively enriched with ^{12}C compared to ^{13}C (Boschker and Middelburg, 2002). The relationship between SOM $\delta^{13}C$ and respired $\delta^{13}C$ is known to be complex (Crow *et al*., 2006); it seems that the effects of microbial respiration on isotopic discrimination was weaker than that imposed on the MAOM by the decomposition processes. However, to confirm this, stable isotope analysis of the respired CO_2 would be required, which was not done as part of this study.

POM $\delta^{13}C$ values after millet cropping season (03/11/2022) exhibited higher $\delta^{13}C$ values than before millet (29/04/22). POM isotopic signatures typically reflect the $\delta^{13}C$ composition of the current vegetation (Del Galdo *et al*., 2003), which is consistent with the characteristic $\delta^{13}C$ signature of millet (C4 plant signature -13.98‰), compared to the mean C3 plant signature (-27‰).

Two years after millet was incorporated, the POM $\delta^{13}C$ values of collection 25/09/2023 were 2.8% higher than collection 03/11/2022 (millet crop). This suggests that POM from material other than the millet is being preferentially decomposed. That means that the proportion of the POM that is derived from millet has increased over time as the other material is decomposed. This is confirmed by de Almeida (2022), who reported that oats decompose faster than millet. Although it should be noted that other studies, such as Koukoura (1998), found no change in decomposition rates between C3 and C4 plants, and Wynn and Bird (2007) reported that C4 plants decompose faster than C3 plants, suggesting that this process is variable, likely dependent on soil and climate conditions.

However, the MAOM $\delta^{13}C$ values were 1.09% lower than the collection on 03/11/2022. This could be due to the MAOM adsorption onto mineral surfaces, reducing their availability for decomposition, therefore exhibiting lower MAOM$\delta^{13}C$ values.

POM and MAOM $\delta^{13}C$ values increased with soil depth for all collections. This is likely due to both fractions becoming older with depth, increasing the microbial processing of SOM that leads to a gradually higher $\delta^{13}C$ in older SOC. The POM fraction composition also becomes smaller as it is slowly decomposed into fragments that are more chemically resistant to decomposition (Del Galdo *et al.,* 2003). For collection 03/11/2022, MAOM ^{13}C values did not increase with depth at 20-30 cm, which could be due millet's shallow root system not reaching down to this soil depth, meaning no exudates with the C4 plant signature would have been exuded into the soil.

7.5.2. PROPORTION OF MILLET DERIVED CARBON (*f*C4)

The average *f*C4 at 0-30 cm in collection 03/11/2022 and 25/09/2023 was 0.14 and 0.16, respectively. These numbers indicate a very small contribution of carbon from the millet to the total SOM pools. This is in line with Poeplau *et al*. (2018), who reported that the *f*C4 for three agricultural temperate fields after 22, 36 and 22 years under a C4 crop after a change from C3, were 0.54, 0.39 and 0.3, respectively, demonstrating the long timeframes required for *f*C4 to become substantial.

Of the total *f*C4, more than 90% went into the POM fraction and less than 10% went into the MAOM fraction. This, again, is in line with previous results presented in this thesis, which indicated that the majority of C inputs into the system come into the POM fraction. The higher C/N ratio in the POM fraction reflects the major contributions from plant litter, while the lower C/N ratio of the MAOM fraction reflects more microbial contributions.

7.5.2.1. fC4 IN THE POM FRACTION (collection 03/11/2022)

The *f*C4 (proportion of millet derived C) into the POM fraction at each soil depth interval (of 0-10, 10-20 and 20-30 cm) revealed no statistically significant differences between the treatments. This was possibly due to the relatively small increase after only one C4 crop-year and the very high data variability (i.e. 0-10 cm CV = 108%, at 10-20 cm CV = 688% and 20-30 cm CV = 131%). This means that if there were any treatment effects, they could well have been masked by the high variability.

However, when the *f*C4 was analysed as an average across the top 0-30 cm, Deep tillage systems had a significantly higher *f*C4 than Shallow tillage systems. To investigate whether this was an artefact caused by a reduced dilution effect, whereby the C4 derived C was moving into a small pool of C3 derived C (Bougnères and Bier, 1983; Huang *et al.,* 2013; Zhu *et al.,* 2024), the *f*C4 was multiplied by the C content of the POM fraction to normalise. When done, the results remained consistent; Deep tillage systems had significantly higher POM-C4 (0.52 g/kg, CV = 152%) compared to Shallow tillage (0.21 g/kg, CV = 207%). These unexpected results contradict Hypothesis 3, which stated that "*reduced tillage will* *lead to higher fC4 in the SOM fractions and soil depths*". This result cannot be fully explained within the current study, suggesting the presence of additional factors or mechanisms not accounted for in our initial hypothesis. Further work characterising the microbial and faunal communities, using approaches such as metagenomics and eDNA analysis under the different treatments, may provide insights into this mechanism.

7.5.2.2. fC4 IN THE MAOM FRACTION (collection 03/11/2022)

At 0-10 cm, the *f*C4 in the MAOM fraction was statistically significant only for the overall effect of traffic, with CTF systems having significantly higher *f*C4 in MAOM than STP systems. This could be explained by the lower bulk density and improved soil structure of the non-trafficked crop area of CTF systems, facilitating better root production and biological activity. This was observed by Kaczorowska-Dolowy (2022) at the same site, who found that the permanent crop bed of CTF systems had significantly higher root production, springtail population and soil fauna feeding activity.

At 10-20 cm, there were no statistically significant differences between the treatments for the *f*C4 into MAOM, which agrees with the SOM/ SOC results (Chapter 4. Fig. 4.7/ Appendix A.5.2. Fig. 5.7). However, at 20-30 cm, the interaction between traffic and tillage systems was statistically significant, with CTF with Zero tillage (*f*C4 MAOM = 0.023) having a significantly higher *f*C4 compared to CTF Shallow and Deep (both had *f*C4 MAOM = 0). These results are also consistent with the results for SOM and SOC concentrations, where at 20-30 cm, CTF with Zero tillage had significantly higher SOM/ SOC compared to CTF Deep and STP Deep. This could be due to the lack of soil disturbance by traffic and tillage (in CTF Zero) over the last 12 years, which has improved soil structure down the soil profile, facilitating biological processes such as fungal and microbial activity, root exudates, earthworm and other microfauna activity.

At 0-30 cm, only the overall effect of depth and the interaction between tillage and depth and traffic and depth were statistically significant. Within the different soil depths, the 0-10 cm depth layer had significantly higher *f*C4 in the MAOM fraction than those at 10-20 cm and 20-30 cm. This is also consistent with the significantly higher SOM concentration and MAOM-C found at 0-10 cm compared to 10-20 cm and 20-30 cm. The higher SOM concentration at 0-10 cm promotes greater biological activity because it provides more nutrients and better habitat conditions for soil organisms.

Within the tillage and depth interaction, Shallow at 0-10 cm had significantly higher *f*C4 MAOM, compared to the other tillage and depth combinations. This could also be explained by the slightly higher SOM concentration of Shallow (0-10 cm) (4.69%) than Zero (4.67%) and Deep (4.58%) at 0-10 cm on 03/11/2022.

Within the traffic and depth interaction, the permanent crop bed of CTF at 0-10 cm had significantly higher *f*C4 in the MAOM (*f*C4 MAOM = 0.028, CV = 72%) than all the other treatment combinations and soil depth combinations. This also coincides with the higher SOM concentration of the permanent crop bed of CTF (4.9%) at 0-10 cm on 03/11/2022 compared to the other traffic treatment and depth combinations.

7.5.2.3. fC4 IN THE POM FRACTION (collection 25/09/2023)

Following the cropping season after the millet crop, in which spring oats were grown, the results of the *f*C4 in the POM fraction were not significantly different to the previous collection (i.e. directly after the millet crop). There were no statistically significant differences between the treatments in the *f*C4 in the POM fraction when analysed at the individual soil depths of 0-10, 10-20 and 20-30 cm. However, when combined to explore the combined effects over 0-30 cm, the *f*C4 in POM showed a statistically significant overall effect of traffic ($p = 0.04$) and depth ($p < 0.01$), instead of tillage as was observed in the previous collection. For the overall effect of traffic*,* LTP systems had significantly higher POM *f*C4 than the permanent crop bed of CTF systems. This may have been due to a higher preference for decomposition of C3 plants, which may have been more effective in LTP systems compared to CTF systems, leaving a higher proportion of millet derived C over time following the faster decomposition of the oats as discussed previously.

Within the different soil depths, 20-30 cm POM *f*C4 exhibited a significantly higher *f*C4 than POM *f*C4 at 0-10cm and 10-20 cm depth. This suggests that at 20-30 cm depth, POM decomposition had a preference for oat derived C compared to millet derived C. This may have been due to conditions being less favourable for the microbes at this soil depth, and therefore they preferentially decomposed the most labile C (i.e. oat derived C; de Almeida, 2022). But further work would be needed to investigate this hypothesis.

7.5.2.4. fC4 IN THE MAOM FRACTION (collection 25/09/2023)

The results of the *f*C4 in the MAOM fraction showed that there were no statistically significant differences between the treatments when analysed at individual soil depths and 0-30 cm. However, the average *f*C4 was very small (avg. 0.004) and the variability was very high (avg. CV = 159%) at 0-30 cm, making the detection of small treatment effects very difficult. As *f*C4 in the MAOM is reliant on a combination of both root exudates, which were likely low due to the low millet biomass, as well as conversion of the millet derived POM into MAOM, it is recommended that longer term studies be used, ideally with multiple seasons of C4 plants being grown, in order to better understand the dynamics of C into MAOM under different tillage and traffic management systems.

7.5.2.5. fC4 IN POM AND MAOM OVER TIME

The δ^{13}C values and fC4 in the POM fraction did not change significantly over the timeframe of this study. However, the fC4 in the MAOM fraction exhibited significant effects over time for collection, depth, and the interaction between depth and collection; the fC4 in the MAOM fraction decreased a year after the C4 cropping season following the C3 crop. The *f*C4 results differ from those reported in other studies across different cropping systems and soil/climatic conditions. This means caution must be applied when extrapolating findings across systems, as soil carbon dynamics are known to be context-dependent (Schmidt *et al*., 2011).

7.5.3. LIMITATIONS AND FURTHER RESEARCH

The millet crop was planted late due to agronomic issues (the first crop failed to establish), which meant that less crop biomass and root exudates went into the soil than otherwise might have been expected.

This study was conducted after only one year of a C4 plant incorporation and one year thereafter, in a single pedoclimatic context. The results presented here show that the effect of the different traffic and tillage management systems on *f*C4 POM and MAOM were small. Due to the high levels of heterogeneity and data variability inherent in most field-scale soil experiments, longer timeframe or very high replication studies are required to better understand the impacts of soil condition on soil C dynamics under different tillage and traffic treatments. Further research would also be needed across diverse soil/climate systems with the same crop rotation and soil management, to validate and expand upon these findings.

Furthermore, implementing a sustained transition of C4 vegetation for a minimum five-year period would enable assessment of longer-term C4 stabilisation processes, potentially having a stronger *f*C4 storage. Additionally, the inclusion of a C4 crop such as maize would also be recommended because of its bigger crop biomass and root system. This crop was not possible in this study because of the lack of equipment to harvest maize at the scale of the plots.

Further research could also be conducted using Stable Isotope Probing (SIP) enrichment studies using 13C-labelled plants to trace carbon flow into microbial communities. Analysis of labelled DNA and phospholipid fatty acids (PLFA) would provide valuable insights into microbial community dynamics across the experimental treatments.

7.6. CONCLUSIONS

Hypothesis number one, "*After the incorporation of a C4 plant, both POM and MAOM $\delta^{13}C$ values will be enriched compared to previous C3 plants, due to C4 plants' lower $\delta^{13}C$ values*", was confirmed. The difference in natural abundance ^{13}C after the C4 cropping season (collection 03/11/2022) confirmed that the C from the millet was incorporated into both the POM and MAOM fractions, as indicated by the change in the $\delta^{13}C$ values.

Hypothesis number two "*Traffic-induced soil compaction will affect the storage of fC4; therefore, reduced traffic and wheel pressure will lead to higher fC4 in the different SOM fractions and soil depths*", was partially supported by the data in this study. For collection 03/11/2025, the overall effect of traffic did not affect *f*C4 in POM, but it significantly affected *f*C4 in MAOM at 0-10 cm, where CTF had significantly higher *f*C4 than STP. For collection 25/09/2023, the overall effect of traffic only affected POM at 0-30 cm, with LTP systems having significantly higher *f*C4 than CTF systems. MAOM was not significantly affected by the overall effect of traffic at any soil depth.

Hypothesis number three "*Tillage-induced soil disturbance increases SOC decomposition; therefore, reduced tillage will lead to higher fC4 in the SOM fractions and soil depths*", was partially supported. For collection 03/11/2022, the overall effect of tillage only significantly affected *f*C4 in POM at 0-30 cm, where Deep tillage had significantly higher *f*C4 than Shallow tillage. The *f*C4 in MAOM was not significantly affected by tillage at any soil depth. For collection 25/09/2023, the overall effect of tillage did not significantly affect either *f*C4 in POM or MAOM at any soil depth.

Hypothesis number four, "*The interaction between traffic and tillage systems will affect fC4 storage into the different SOM fractions and soil depths*, was partially supported. For collection 03/11/2022, the interaction effect between traffic and tillage did not significantly affect *f*C4 in POM at any soil depth, but it significantly affected *f*C4 in MAOM at 20-30 cm, where CTF with Zero tillage had significantly higher *f*C4 than CTF with Shallow and CTF with Deep tillage. For collection 25/09/2023, the interaction effect of traffic and tillage did not significantly affect either *f*C4 in POM or MAOM at any soil depth.

The average *f*C4 that went into POM was 0.13, and MAOM was 0.01 down through the top 0-30 cm of the soil profile. Therefore, 93% of the total *f*C4 went into POM and only 7% went into MAOM (collection 03/11/2022). These findings corroborate previous observations, indicating that after a cropping season, most of the C entering the system comes from crop residues that go into the POM fraction. Therefore, the primary mechanism of carbon storage observed within our system was driven by changes in POM. This suggests that soil management practices that increase the amount of POM going into a soil system will be favourable in terms of maximising SOM concentrations and SOC stocks. These include the CTF and Zero tillage systems discussed here, as well as maximising straw return to the soil at harvest and incorporating cover crops for use as green manures. Incorporating these practices likely maximises both soil carbon storage and soil health by building SOM concentrations over time.

CHAPTER 8

THE EFFECT OF TRAFFIC AND TILLAGE MANAGEMENT SYSTEMS ON CROP GROWTH AND YIELD

8.1. INTRODUCTION

In the UK cereals are mainly grown in conventional or intensive management practices with random traffic of heavy agricultural machinery, causing soil compaction, which can negatively impact crop yields, crop establishment, soil physical properties, infiltration rates and tillage forces (Godwin et al., 2022). CTF systems and the use of low tyre pressure have the potential to address these issues, however, the current understanding of the long-term interactions between different traffic management systems and tillage practices on crop yield is still limited.

Long-term experimental data from this site demonstrated that CTF systems with a 30% trafficked area increased grain yield by 4% compared to STP systems. If recalculated to a 15% trafficked area, more commonly achieved by farmers, an additional 3% yield can be added. The traffic-tillage interaction analysis revealed that LTP systems combined with deep tillage increased grain yield by 4% compared to STP systems. The tillage-time interaction analysis revealed temporal yield improvements under zero tillage, with significantly higher yields compared to deep tillage in years 7 and 8 (Kaczorowska-Dolowy, 2022).

This chapter extends the analysis of crop growth and yields through crop seasons 9 to 12 of the long-term traffic and tillage project at Harper Adams University.

8.2. AIM AND HYPOTHESIS

This chapter aims to quantify the effects of alternative traffic systems (CTF, LTP and STP) and their interaction with different tillage systems (Deep, Shallow and Zero) on crop growth and yield, including plant establishment, hand-harvested data (e.g. crop yield components) and crop yields in a long-term field experiment over the last four years (2021-2024).

The hypotheses for this chapter are:

1. CTF systems (30% trafficked area) and the reduced wheeled pressure of LTP systems positively affect crop establishment, crop growth and yields.
2. Reduced tillage (Shallow and Zero tillage) positively affects crop growth and yields compared to Deep tillage.
3. The interaction between traffic and tillage systems can positively affect crop growth and yields.

8.3. METHODOLOGY

For the different crops and varieties, drilling date, fertiliser and spraying information, and weather information, refer to Chapter 3. Methodology. 3.7. Crops and varieties and 3.8. Weather data.

8.3.1 PLANT EMERGENCE

Winter barley cv. Belfry (Hordeum vulgare L.) was drilled on 17 October 2020, and the plant emergence count was performed on 27 October 2020. The number of plants was counted for a sample length of 1 m in each of 22 rows in total for each plot. The count was performed around 2 meters from the first tramline. A stick 1 m in length was used to determine the width of the transect. The crop was at the “seedling growth” stage of GS10-GS13 (first to three leaves unfolded on the main shoot).

Following this crop, a winter cover crop was drilled on 10 August 2021. However, no subsequent plant count was conducted, as this crop was meant to be killed by the first winter frost. The cover crop had a poor establishment, dominated by volunteers from the previous crop.

White millet (*Panicum miliaceum)* was initially drilled on 25 May 2022, but due to poor crop emergence and high weed pressure, it was redrilled again on 8 July 2022. The plant emergence count was performed on 24-26 August 2022. The same methodology as the previous crop was used. The row sample length was 1 meter (Fig.8.1a). The crop exhibited considerable variability in size and density across the field. Plants in block four were generally bigger in size (growth stage I, growth stages varied between three to five leaves and panicles (Khairwal *et al.,* 2007). (Fig. 8.1. c).

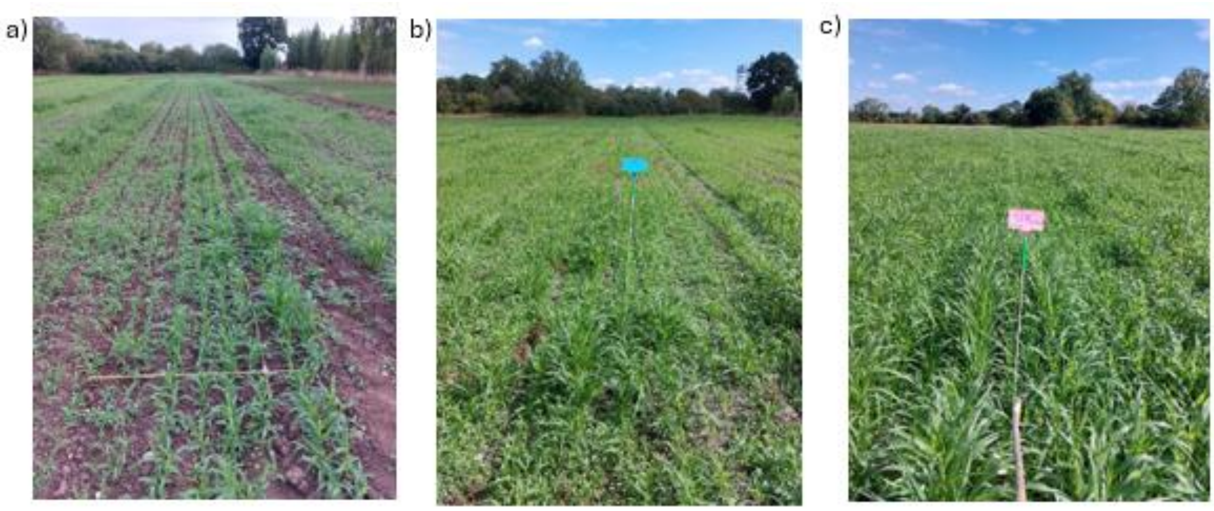


Figure 8. 1 – Millet establishment count on 26 August 2022. **a)** A measuring stick is used to count the number of plants in each row up to 1 m in length, Plot 1: STP Deep. **b)** Plot 6: STP Zero (Block 1) and **c)** Plot 23: STP Shallow (Block 4) show the different growth stages.

Spring oats cv. Isabel (*Avena sativa*) was drilled on 8 March 2023 and the plant emergence count was conducted on 2-3 April 2023, following the same procedure as the previous year. The plant height was 5-7 cm, growth stage: GS 11 (first leave unfolded, ligule visible) (Hutton, 2019). The crop emergence appeared uniform, although some bare patches were observed on the permanent wheelways in STP Zero tillage plots. Fig. 8.2 a) and b)).

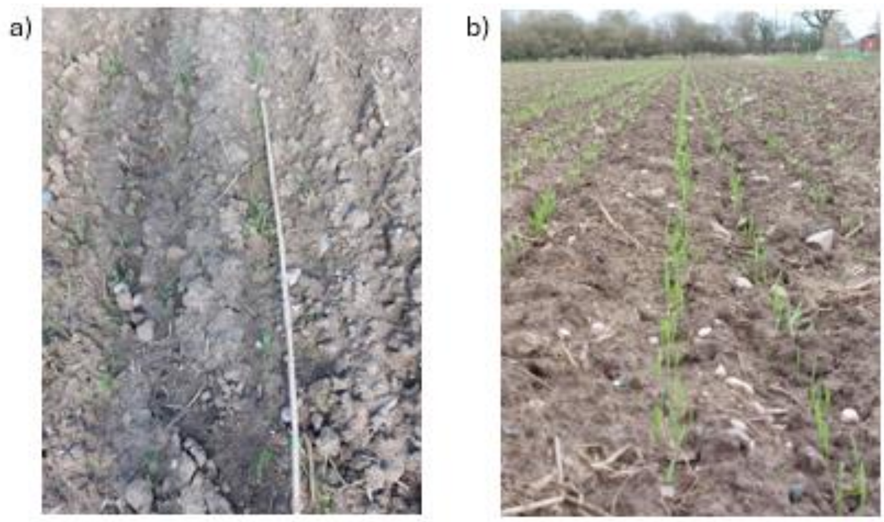


Figure 8. 2 – Oat establishment count on 2 April 2023. Photos **a)** and **b)** document the spring oats plant count using a 1-meter stick in plot 1: STP Deep tillage.

Winter wheat cv. KWS Extase (*Triticum aestivum* L.) was drilled on 17 October 2023 and the plant emergence count was performed on 28 November 2023. The number of plants was counted for every row, but this year the row sample length was 0.75 meters. Growth stage GS10-GS13 (1 – 3 leaves unfolded on the main shoot) (AHDB, 2023).

8.3.2 EMERGENCE PERCENTAGE

Plant emergence percentage was determined using the target seed rate, following equation 8.1:

*Percentage Emergence (%) = [(Total plant count/ m^2)/ (Target Seeds/ m^2)]*100*

Equation 8.1

8.3.3 HAND HARVEST CROP COMPONENTS

The grain was threshed and weighed for each sample and treatment, and the moisture content was measured so that yield could be adjusted to 15% moisture content.

Harvest Index (HI) was determined from equation 8.2:

HI (%) = Dry weight of grain ÷ Dry weight of whole plant (grain, straw, chaff) × 100

Equation 8.2

The thousand grain weight (TGW) was calculated using equation 8.3:

TGW (g) = (Weight of dried grain sample (g) ÷ Number of grains in sample) x 1000

Equation 8.3

The grain weight determination varied by crop species: winter barley used a "seed count" mobile application; spring oats employed an automated seed counter for 100-seed samples; winter wheat was calculated by manually counting 200-seeds. All samples were subsequently weighed.

8.3.4 CROP YIELDS

The data on crop yields were collated from two independent sources, detailed in the following subsections:

1. The yield data from the hand harvest.
2. The yield data from the combine harvester.

8.3.4.1 HAND HARVESTED YIELD

A hand harvest was conducted for each growing season, with methodological variations between years to better account for environmental differences.

The hand harvest for winter barley was conducted on 14-15 July 2021. The harvesting methodology replicated the previous year's protocol used by Kaczorowska-Dolowy (2022). Crop biomass was cut at ground level for a 0.5 m transect width. Sampling encompassed two distinct plot zones: four rows from the permanent wheel ways (rows 4, 5, 18, 19) and four rows from the centre of the plots (rows 10, 11, 12, 13). Samples were harvested approximately 2 m from the initial tramline. A total of 288 samples were individually processed, with straw and ears segregated and weighed. The ear count was recorded, followed by mechanical threshing using a F. Walter and H. Wintersteiger KG laboratory thresher. Grain mass and moisture content were subsequently determined. Grain moisture content was measured by the oven-dry method in the lab. Grain weights were adjusted to 15% moisture content using equation 8.4:

Grain weight at 15% moisture content (g) = [((100- moisture content (%))/85) x grain weight (g)]

Equation 8.4

The hand harvest for millet was conducted from 29 September to 7 October 2022. Given the crop's developmental stage—growth stage III, grain filling (Khairwal *et al.,* 2007)— due to delayed planting and the limitations preventing the combine harvesting, an alternative and enhanced hand harvest sampling methodology was implemented. The protocol involved triplicate sampling of each plot, with each sample, collected approximately 2 meters from each of the three tramlines (Fig. 8.3). Crop biomass was harvested by cutting a 1 m (length) × 4 m (wide) area at ground level across all experimental plots (Fig.8.4 a) and b)). Fresh biomass was immediately weighed in the field, and a representative subsample was transported to the laboratory for gravimetric moisture determination via oven desiccation.

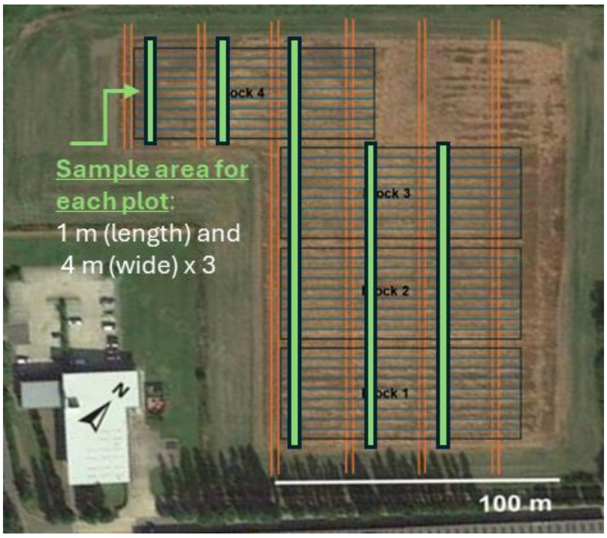


Figure 8. 3 - Indicates the triplicate sampling (in green) of millet from each plot, collected approximately 2 meters from the first three tramlines.

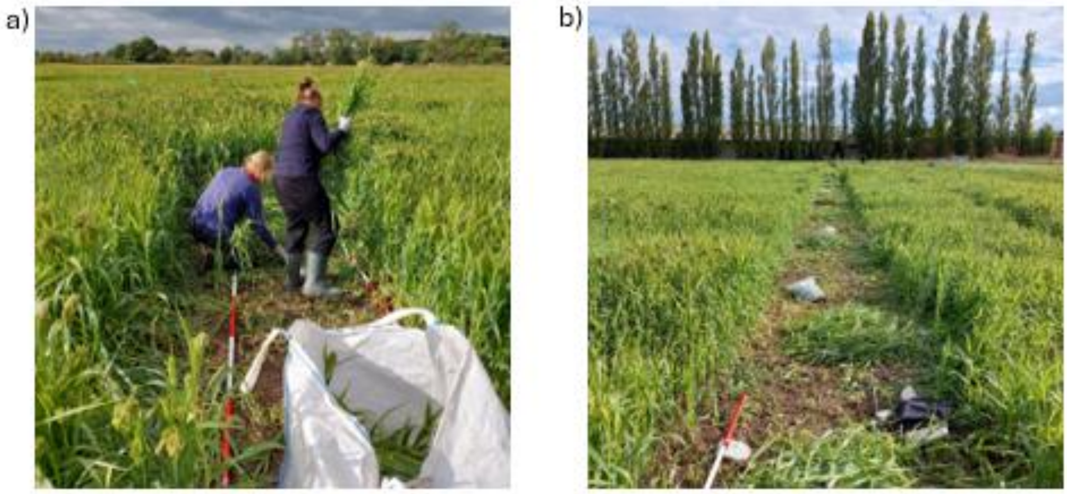


Figure 8. 4 - **a)** and **b)** Millet being harvested at ground level across the whole plot width at approximately 2 m distance from the first tramline. Sample size: 4 m (wide) and 1 m (length).

To quantify biomass variations in Millet between wheeled and un-wheeled areas in CTF systems, additional sampling was conducted on 12 October 2022. Sampling encompassed two distinct plot zones: four rows from the permanent wheel ways (rows 4, 5, 18, 19) and four rows from the centre of the plots (rows 10, 11, 12, 13). Triplicate samples were collected 2 meters from each tramline. The whole sample was transported to the laboratory and processed via oven desiccation.

The hand harvest for spring oats was conducted on 9 August 2023. Given the variability of wheeled and un-trafficked areas of the plot centres for each treatment, the hand-harvesting protocol was again modified to achieve a more representative sampling of non-trafficked plot areas. Sampling encompassed the two distinct plot zones as before: four rows from the permanent wheel ways (rows 4, 5, 6, 7) and four rows from the un-wheeled area of the plot, which changed for each different treatment as specified below:

- CTF (Deep, Shallow and Zero tillage) systems: un-wheeled rows 10, 11, 12, 13.
- LTP (Deep, Shallow and Zero tillage) systems: un-wheeled rows 12,13, 14, 15.
- STP Deep tillage systems: un-wheeled rows 2, 3 and again 2, 3 sampled further along the plot.
- STP Shallow tillage systems: un-wheeled rows 2, 3, 12, 13.
- STP Zero tillage systems: un-wheeled rows 2, 3, 10, 11.

Crop biomass was harvested at ground level using a 0.5 m transect width. Samples were aggregated into permanent wheel way rows and un-wheeled rows categories for each plot. A total of 72 samples were individually processed, with straw and ears segregated and weighed. Ear count was recorded, followed by mechanical threshing. Grain mass and moisture content were subsequently determined. Heads were threshed as previously, and grain moisture was measured using a Dickey John Grain Analysis Computer (GAC) 2500-UGMA. The grain weights were adjusted to 15% moisture content. For this crop a Farm-Tec Count-a-matic was used to count 100 grains to calculate TGW.

The hand harvest for winter wheat was conducted on 07 August 2024. The hand-harvesting protocol was again modified to accommodate labour and time restrictions and achieve a representative sampling. Crop biomass was cut at ground level using a 2 m transect width. Sampling encompassed the two distinct plot zones as before: with 2 rows sampled for the permanent wheel way (rows 18,19) and 2 rows from the centre of the plots (rows 11,12).

The hand-harvested yield was calculated for every row at 15% moisture content. For each plot, the average yield between the permanent wheel way and the centre rows was determined for all crops, except spring oats, where the traffic area percentage of each plot was incorporated into the yield calculations.

For the yield analysis of the CTF systems, comparing the permanent wheel way yield with the un-trafficked yield for the three tillage systems, the yield data at 15% moisture content was calculated for each of the above areas.

7.3.4.2 COMBINE HARVESTED YIELD

Please refer to Chapter 3: Methodology for more information on 3.6 Combine harvest operations and 3.7 Crops and varieties. Due to the first millet crop failing and the delayed planting of the second, it was not possible to do a combine harvest of the millet crop; this was replaced by an enhanced hand harvest.

The combine harvester yields were estimated for CTF systems with a traffic area of 15% (Y15%) using the hand harvested data and the following equations (8.5) and (8.6) (Godwin *et al*., 2022):

$$Y0 = Y30\%\ Ynt / (0{\cdot}7Ynt + 0{\cdot}3Ytl) \quad \textbf{Equation 8.5}$$

$$Y15\% = 0{\cdot}85Y0 + 0{\cdot}15Y0\ (Ytl / Ynt) \quad \textbf{Equation 8.6}$$

Where:

Y0 = Estimated combine harvester yield for the untrafficked area (Mg ha^{-1}),

Y30% = Combine harvester yield for CTF30% (Mg ha^{-1}),

Ynt = Hand harvested yield for the untrafficked area (Mg ha^{-1}),

Ytl = Hand harvested yield for the traffic area (Mg ha^{-1}) 0·7; 0·3; 0·85 and 0·15 = % areas expressed as a proportion·

8.4. RESULTS

8.4.1 PLANT EMERGENCE AND CROP EMERGENCE PERCENTAGE

The mean number of plants m^{-2} per treatment is represented in Table 8.1 for all the different crops.

For winter barley only the main effect of tillage was statistically significant ($p = 0.01$), with Zero tillage having higher plants/ m^2 (11.7% more) than Deep tillage systems.

For Millet only the main effect of tillage was also statistically significant ($p = 0.02$), but in this case Shallow tillage had higher plants/ m^2 (36.5% more) than Deep tillage systems.

For spring oats the main effect of tillage ($p < 0.001$) and the interaction between the traffic and tillage ($p = 0.05$) systems were both statistically significant. Shallow and Deep tillage had higher plants/ m^2 (27.7% more) than Zero tillage systems. Within the interaction, LTP Shallow had higher plants/ m^2 than STP Zero and LTP Zero. And CTF Shallow, LTP Deep, STP Deep had higher plants/ m^2 than STP Zero (Table 8.1)

Table 8. 1. Total number of plants/m^2 for winter barley (Belfry), millet (White), spring oats (Isabel) and winter wheat (Extase) for the three Traffic (CTF with 30% trafficked soil, LTP and STP) systems and three Tillage systems (Deep = 25 cm, Shallow= 10 cm and Zero= no tillage) for the crops in 2020-24 (winter barley, millet, spring oats and winter wheat). Significant differences between means are represented by different letters.

Plant establishment	**Winter barley (2020-21)**	**Millet (2022)**	**Spring Oats (2023)**	**Winter wheat (2023-24)**
Treatments	plants/ m^2	plants/ m^2	plants/ m^2	plants/ m^2
CTF	140.52	466.21	201.73	104.87 b
LTP	145.72	453.20	207.56	103.19 ab
STP	138.13	494.15	189.04	85.73 a
Deep	132.18 a	384.45 a	213.23 b	88.43
Shallow	144.61 ab	524.95 b	216.73 b	104.39
Zero	147.58 b	504.15 ab	168.37 a	100.96
CTF Deep	133.20	373.30	199.31 abc	101.88
CTF Shallow	139.40	555.05	216.12 bc	110.45
CTF Zero	148.95	470.27	189.75 abc	102.27
LTP Deep	135.72	388.92	215.69 bc	83.22
LTP Shallow	155.42	437.69	234.50 c	113.41
LTP Zero	146.02	532.99	172.50 ab	112.93
STP Deep	127.61	391.13	224.69 bc	80.17
STP Shallow	138.99	582.11	199.56 abc	89.32
STP Zero	147.79	509.21	142.87 a	87.70
p Traffic	0.33	0.72	0.17	**0.03**
p Tillage	**0.01**	**0.02**	**< 0.001**	0.11
p Traffic and Tillage	0.55	0.58	**0.05**	0.55
CV (%)	7.19	19.20	19.40	19.73
SEM Traffic	3.65	36.38	6.85	5.38
SEM Tillage	3.65	36.38	6.85	5.38
SEM T&T	6.32	63.01	11.87	9.32

The crop emergence percentage is represented in Table 8. 2 for all the different crops.

Table 8. 2. Crop emergence percentage was calculated using the TGW and seed rate.

Crops	Crop emergence (%)
Winter barley (2020-21)	56.6
Millet (2022)	94.2
Spring Oats (2023)	47.5
Winter wheat (2023-24)	24.5

8.4.2 HAND HARVEST YIELD COMPONENTS

Yield component analysis was based on hand harvested samples for all crops except millet, which failed to reach maturity due to delayed planting.

For winter barley, the ears/m^2 count revealed that only the main effect of tillage ($p = 0.006$) was statistically significant, with Zero and Shallow having higher ears/m^2 (17.2% more) than Deep tillage systems. The TGW showed that the main effect of traffic ($p = 0.001$), tillage ($p < 0.001$), and the interaction between traffic and tillage ($p = 0.01$) systems were all statistically significant. CTF and STP had higher TGW than LTP systems. Zero and Shallow tillage had higher TGW than Deep tillage systems. Between the interaction, CTF Shallow had higher TGW than LTP (Shallow and Deep) and CTF Deep. And STP Shallow had higher TGW than CTF Deep and LTP Deep (Table 8.3). The rest of the metrics analysed showed no statistically significant differences between treatments.

For spring oats none of the hand harvest yield components studied were statistically significant (Table 8.3).

For winter wheat the grains/ear count revealed that the main effect of tillage was borderline statistically significant ($p = 0.05$); however, the *Post hoc* analysis did not show any significant differences between the treatments. For the TGW the main effect of tillage was also statistically significant ($p < 0.001$), with Zero tillage having a higher TGW (13.8% more) than Shallow and Deep tillage systems (Table 8.3). The rest of the metrics analysed showed no statistically significant differences between treatments.

Table 8. 3. Crop components analysis of winter barley (2020-21), spring oats (2023), and winter wheat (2023-24) across three Traffic (CTF, LTP and STP) systems and three Tillage systems (Deep = 25 cm, Shallow= 10 cm and Zero= no tillage). Metrics analysed: ears/m^2, grains/ear, thousand-grain weight (TGW) and harvest index (HI). Millet was excluded (only dry biomass was analysed). Significant differences between means were represented by different letters.

Hand Harvest	winter barley				spring oats				winter wheat			
Treatments	**ears m^{-2}**	**grains/ ears**	**TGW**	**Harvest Index**	**panicles m^{-2}**	**grains/ panicle**	**TGW**	**Harvest Index**	**ears m^{-2}**	**grains/ ears**	**TGW**	**Harvest Index**
CTF	332.71	38.05	47.74 b	0.56	301.1	68.36	37.39	0.48	158.56	41.60	48.883	0.50
LTP	319.99	40.07	46.61 a	0.55	274.56	71.13	37.33	0.49	159.14	44.37	49.692	0.54
STP	335.70	39.43	48.17 b	0.56	274	72.45	37.17	0.5	145.9	42.40	48.823	0.51
Deep	295.53 a	39.60	46.55 a	0.55	277.38	73.63	37.35	0.48	146.21	41.34	46.74 a	0.50
Shallow	337.58 b	39.48	48.19 b	0.56	295.06	69.33	37.06	0.51	153.46	41.96	49.12 a	0.52
Zero	355.29 b	38.47	47.79 b	0.56	277.22	68.97	37.49	0.48	163.92	45.07	51.55 b	0.53
CTF Deep	305.76	37.53	45.91 a	0.54	300.9	71.27	37.34	0.46	155.88	41.33	46.188	0.50
CTF Shallow	335.33	38.37	49.36 c	0.59	319.16	63.55	37.24	0.50	154.94	38.38	49.9	0.47
CTF Zero	357.04	38.25	47.97 abc	0.57	283.23	70.26	37.6	0.48	164.86	45.09	50.563	0.53
LTP Deep	273.95	39.36	45.86 a	0.55	274.14	76.68	37.22	0.51	149.14	43.30	48.131	0.51
LTP Shallow	324.85	41.03	46.49 ab	0.55	279.79	70.47	37.37	0.50	161.18	44.30	49.044	0.58
LTP Zero	361.15	39.81	47.51 abc	0.56	269.76	66.23	37.4	0.52	167.1	45.51	51.9	0.54
STP Deep	306.89	41.91	47.91 abc	0.56	257.11	72.95	37.48	0.48	133.61	39.39	45.888	0.50
STP Shallow	352.54	39.02	48.72 bc	0.56	286.23	73.97	36.56	0.46	144.27	43.20	48.406	0.51
STP Zero	347.68	37.36	47.91 abc	0.55	278.67	70.42	37.46	0.49	159.81	44.62	52.175	0.53
Mean	329.47	39.18	47.53	0.55	283.22	70.64	37.29	0.49	154.53	42.79	49.13	0.52
$p_{Traffic}$	0.3	0.15	**0.001**	0.62	0.12	0.49	0.73	0.58	0.71	0.22	0.63	0.10
$p_{Tillage}$	**0.006**	0.58	**< 0.001**	0.51	0.38	0.34	0.34	**0.07**	0.62	0.05	**< 0.001**	0.37
$p_{Traffic\ and\ Tillage}$	0.87	0.22	**0.01**	0.49	0.8	0.54	0.6	0.37	0.99	0.42	0.6	0.26
CV (%)	16.28	3.98	2.52	2.44	7.88	8.52	0.77	4.41	6.97	6.20	4.79	5.61
SEM Traffic	32.17	2.20	0.87	0.96	10.32	2.45	0.21	0.96	18.32	1.63	1.01	0.02
SEM Tillage	32.17	2.20	0.87	0.96	10.32	2.45	0.21	0.96	18.32	1.63	1.01	0.02
SEM T&T	55.73	3.82	1.51	1.67	17.88	4.25	0.36	1.66	31.74	2.83	1.74	0.04

8.4.3 CROP YIELDS

8.4.3.1 HAND HARVESTED YIELD DATA 2021-2024

Hand harvested grain yield was quantified for winter barley, spring oats, and winter wheat, while millet yield was expressed as dry biomass.

The effect of tillage was significant only for winter barley (p = 0.004), with Zero tillage (6.62 Mg/ha) and Shallow tillage (6.39 Mg/ha) systems having significantly higher yields than Deep tillage (5.49 Mg/ha) systems. The main effect of traffic and the interaction between the traffic and tillage systems were not significant for the other crops studied (Table 8.4).

Table 8. 4- Average hand harvested grain yield for winter barley, spring oats and winter wheat, and dry biomass for millet and depending on the three Traffic (CTF, LTP and STP) systems and three Tillage systems (Deep = 25 cm, Shallow= 10 cm and Zero= no tillage) for the crops in 2020-24. Significant differences between means were represented by different letters.

Hand-harvested yield	**Winter barley (2020-21)**	**Millet (2022)**	**Spring Oats (2023)**	**Winter wheat (2023-24)**
Treatments	Mg/ha	Mg/ha	Mg/ha	Mg/ha
CTF	6.02	4.24	7.63	6.57
LTP	6.03	4.13	7.23	7.17
STP	6.45	4.30	7.31	6.31
Deep	5.49 a	4.28	7.55	5.80
Shallow	6.39 b	4.26	7.51	6.57
Zero	6.62 b	4.14	7.1	7.69
CTF Deep	5.16	4.32	7.92	6.00
CTF Shallow	6.28	4.51	7.6	6.14
CTF Zero	6.61	3.90	7.36	7.57
LTP Deep	5.00	4.01	7.76	6.27
LTP Shallow	6.21	3.97	7.32	7.36
LTP Zero	6.86	4.39	6.6	7.87
STP Deep	6.31	4.49	6.97	5.13
STP Shallow	6.68	4.29	7.6	6.20
STP Zero	6.38	4.12	7.34	7.62
Mean	6.17	4.22	7.39	6.68
$p_{Traffic}$	0.38	0.65	0.52	0.58
$p_{Tillage}$	**0.004**	0.73	0.41	**0.08**
$p_{Traffic\ and\ Tillage}$	33	0.21	0.51	0.96
CV (%)	17.69	20.44	8.74	14.23
SEM Traffic	0.72	0.14	0.26	0.84
SEM Tillage	0.72	0.14	0.26	0.84
SEM T&T	1.25	0.23	0.45	1.45

8.4.3.1.1 HAND HARVESTED YIELD ANALYSIS FROM CTF PLOTS (UNTRAFFICKED VERSUS PERMANENT WHEELWAY)

This data compares the yield from 4 permanent wheel way rows and 4 untrafficked rows.

Winter barley (2020-21):

The main effect of tillage (p = 0.01), traffic (p = 0.0003) and the interaction between the tillage and traffic (p = 0.009) were all significant (Fig. 8.5). Between the different traffic areas and tillage treatments, CTF Zero untrafficked (8 Mg/ha) had a significantly higher yield (3 Mg/ha more) more than all the other treatments and traffic areas, except for CTF Shallow untrafficked (7.2 Mg/ha). The avg. CV = 36.1%. Between the different CTF tillage treatments, Zero tillage (6.6 Mg/ha) had significantly higher yield than Deep tillage (5.2 Mg/ha). Between the different trafficked areas, the untrafficked area (6.8 Mg/ha) had a significantly higher yield than the trafficked area (5.3 Mg/ha) of the CTF plots.

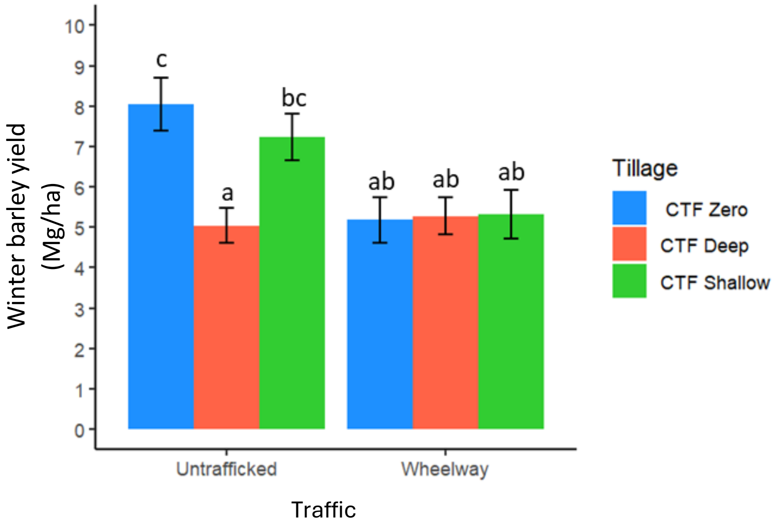


Fig. 8.5. Hand-harvested yield of winter barley (2020-2021) for the CTF plots with different tillage treatments and trafficked areas (permanent wheel way versus untrafficked). Hand harvest transects per plot: 0.5 m x 4 rows (permanent wheel way) x 4 rows (centre).

Millet (2022):

The main effect of tillage (*p* = 0.76), traffic (*p* = 0.27), and the interaction between tillage and traffic (*p* = 0.09) were not significant (avg. CV = 31.2%) (Fig. 8.6).

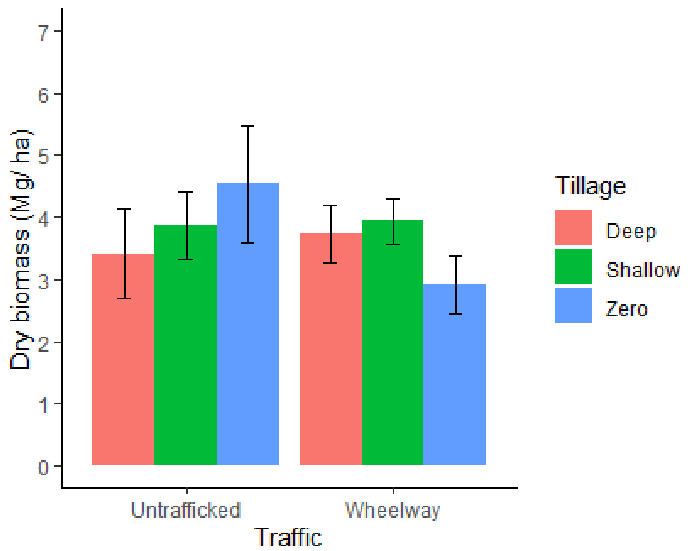


Fig. 8.6. Hand-harvested dry biomass of millet (2022) for the CTF plots with different tillage treatments and trafficked areas (permanent wheel way versus untrafficked). Hand harvest transect per plot: 1 m (length) x 4 m (wide) x 3 times.

Spring oats (2023):

The main effect of tillage (p = 0.80), traffic (p = 0.17), and the interaction between tillage and traffic (p = 0.95) were not significant (avg. CV = 21.44%) (Fig. 8.7).

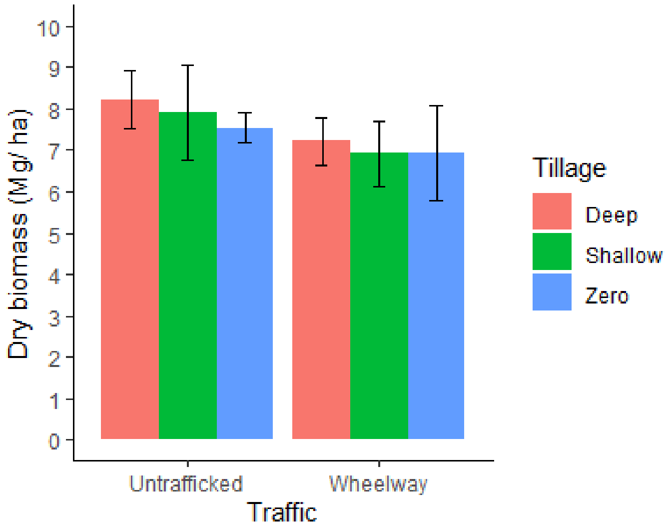


Fig. 8.7. Hand harvested yield of spring oats (2023) for the CTF plots with different tillage treatments and trafficked areas (permanent wheel way versus untrafficked). Hand harvest transect per plot: 0.5 m x 4 rows (permanent wheel way) x 4 rows (untrafficked).

Winter wheat (2024):

The main effect of tillage (p = 0.09) was not significant, but the main effect of traffic (p < 0.001) and the interaction between tillage and traffic (p = 0.04) were significant (avg. CV = 27.88%) (Fig. 8.8).

Between the different trafficked areas, the untrafficked area (8.7 Mg/ha) had significantly higher yield than the trafficked area (4.4 Mg/ha) of the CTF plots.

For the interaction effect, the untrafficked area of Zero CTF treatments (8.7 Mg/ha) was significantly higher than the trafficked area of CTF Deep and Shallow (avg. 3.37 Mg/ha) tillage treatments.

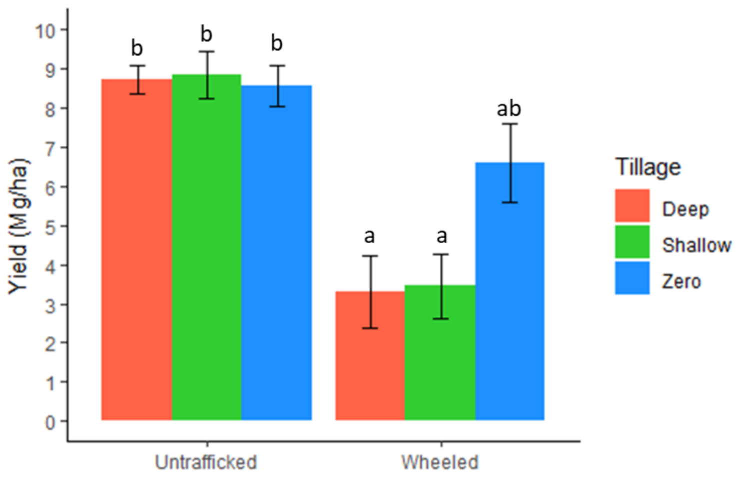


Fig. 8.8. Hand-harvested yield of winter wheat (2024) for the CTF plots with different tillage treatments and trafficked areas (permanent wheel way versus untrafficked). Hand harvest transect: 2 m x 2 rows (centre) x 2 rows (permanent wheel way)

<u>Calculated hand harvested yield increase for CTF systems with a 15% trafficked area (versus of 30% (Table 8. 5)</u>:

Table 8. 5 - Calculated hand harvested yield increase for different crops in CTF systems with a 15% trafficked area, instead of a 30% trafficked area.

crops	Yield increased percentage for 15% trafficked area in CTF systems
Winter barley	3.54%
Millet	1.60%
Spring oats	1.70%
Winter wheat	8.60%
avg.	3.86%

8.4.3.2 COMBINE HARVESTED YIELD DATA 2021-2024

The results for the combine harvested yield data analysis include CTF systems with a 30% trafficked area.

The yield data results for the winter barley showed no significant differences for the main effect of traffic (p = 0.85), tillage (p = 0.10) or the interaction between traffic and tillage systems (p = 0.44) (Table 8.6).

The yield data for the spring oat crop showed that the main effect of traffic systems ($p <$ 0.001) and the interaction between traffic and tillage systems were both significant (p =

0.034). Within the traffic systems, CTF (7.78 Mg/ha) and LTP (7.84 Mg/ha) had significantly higher yields compared to STP (7.31 Mg/ha) systems. Within the interaction between traffic and tillage systems, LTP Deep (8.10 Mg/ha) tillage systems had significantly higher yields than STP Deep and Shallow (7.09 Mg/ha and 7.34 Mg/ha, respectively) tillage systems. LTP Deep and Shallow (8.10 Mg/ha and 7.89 Mg/ha, respectively) and CTF Deep and Shallow (7.84 Mg/ha and 7.99 Mg/ha, respectively) had significantly higher yields than STP Deep (7.09 Mg/ha) tillage systems (Table 8.6).

The yield data for the winter wheat crop only showed a significant difference in the main effect of traffic (p = 0.002), with CTF (6.92 Mg/ha) and LTP (6.93 Mg/ha) having significantly higher yields than STP (6.34 Mg/ha) systems (Table 8. 6).

Table 8. 6- Average yield (Mg/ha) from combine harvester depending on the three Traffic (CTF with 30% trafficked soil, LTP and STP) systems and three Tillage systems (Deep = 25 cm, Shallow= 10 cm and Zero= no tillage) for the crops in 2020-24 (winter barley, spring oats and winter wheat). Millet was excluded due to crop maturity delays beyond October, preventing the combine harvesting. Significant differences between means are represented by different letters.

Combined harvested yield	**Winter barley (2020-21)**	**Spring oats (2023)**	**Winter wheat (2023-24)**
Treatments	Mg/ha	Mg/ha	Mg/ha
CTF	7.57	7.78 b	6.92 b
LTP	7.72	7.84 b	6.93 b
STP	7.54	7.31 a	6.34 a
Deep	7.23	7.68	6.66
Shallow	7.99	7.74	6.69
Zero	7.60	7.51	6.83
CTF Deep	6.98	7.84 bc	6.96
CTF Shallow	8.31	7.99 bc	6.66
CTF Zero	7.42	7.52 abc	7.12
LTP Deep	7.76	8.1 c	6.83
LTP Shallow	7.61	7.89 bc	7.14
LTP Zero	7.77	7.54 abc	6.81
STP Deep	6.94	7.09 a	6.19
STP Shallow	8.05	7.34 ab	6.27
STP Zero	7.62	7.48 abc	6.55
Mean	7.61	7.64	6.72
$p_{Traffic}$	0.85	**< 0.001**	**0.002**
$p_{Tillage}$	0.1	0.18	0.59
$p_{Traffic\ and\ Tillage}$	0.44	**0.034**	0.37
CV (%)	8.56	4.14	6.31
SEM Traffic	0.24	0.09	0.12
SEM Tillage	0.24	0.09	0.12
SEM T&T	0.42	0.15	0.21

Calculated combined harvested yield increase for CTF systems with a 15% trafficked area (versus 30%), using hand harvested data (Table 8. 7).

Table 8. 7 – Calculated combined harvested yield increase for different crops for CTF systems with a 15% trafficked area (versus 30% trafficked area), using the hand harvested data.

	Combine harvested yield			
Crops	CTF 30%	CTF 15%	Yield increase (Mg ha^{-1})	Yield increase (%)
Winter Barley (2020-21)	7.57	7.83	0.26	3.5
Spring Oats (2023)	7.78	7.91	0.13	1.7
Winter Wheat (2023-24)	6.92	7.52	0.59	8.6

8.5. DISCUSSION

Crop responses to the different traffic and tillage systems varied by species and interannually due to meteorological conditions.

Winter barley cv. Belfry is a hybrid six-row variety with a small grain size, high tillering capacity and high yield potential. The recommended sowing period in the UK is from 20th September to 31st October (Agrii, 2025). Seeds were sown on 17th October 2020 and plant establishment was assessed on 27th October at growth stage GS10-13. During the 10-day establishment period, the mean daily temperature was 10°C with a mean precipitation of 1.2mm day^{-1} (cumulative: 12.8 mm). The previous 10 days only had a total precipitation of 8.4 mm. The results showed that only the main effect of tillage affected the plant establishment with Zero tillage increasing plant establishment by 11.6% compared to Deep tillage (CV = 9.2%). The overall crop emergence rate was 56.6% (TGW = 49.5, seed rate: 124 kg/ha, 250 plants/m^2). The low crop establishment could be due to the wet conditions experienced before and after drilling.

The previous winter barley crop in 2019-20 also experienced a very poor crop emergence percentage (30% on average) due to a very wet winter, especially October and November 2019 (Kaczorowska, 2020).

Hand harvested yield data (15th July 2021) similarly showed tillage as the only statistically significant factor, with Zero tillage treatments yielding 26.2% higher than Deep tillage treatments. However, the combined harvested yield showed no statistically significant differences between treatments. This may be explained by the occurrence of a weed called soft brome *Bromus hordeaceus* (Fig. 7.a) and that due to heavy rain at the time of harvest, parts of the crop were lodged (Fig. 7 b and c). The later effect could reduce the harvested grain yield by making combine harvesting more difficult. However, the mean combined harvested yield (7.6 Mg/ha) was marginally higher than the UK national averages of 7.2-7.4 Mg/ha (MAGB, 2022) and higher than the average hand-harvested yield (6.2 Mg/ha).

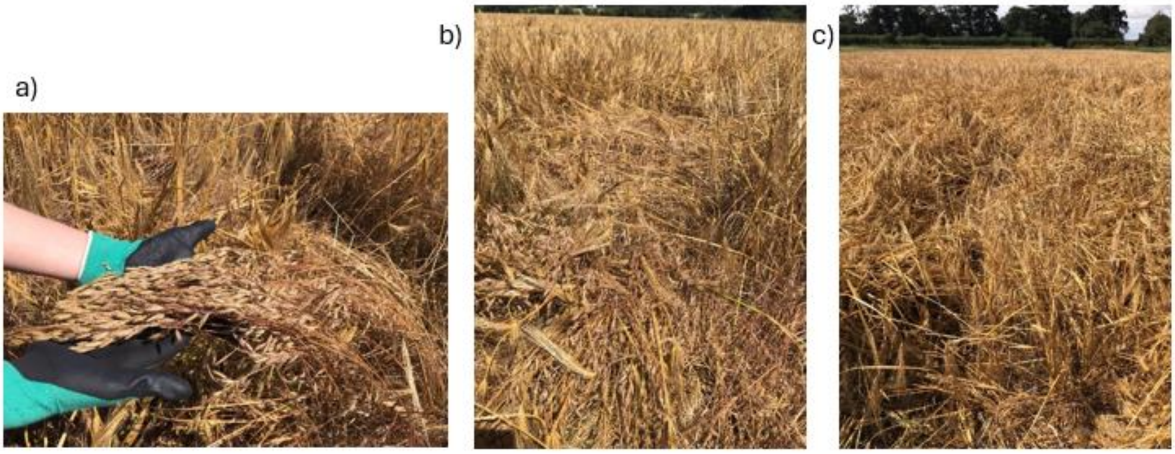


Fig. 8. 9 a) Brome, b) and c) Winter barley crop lodging in August 2021.

The spring monthly precipitation in 2021 was 25.8, 12.4, and 104.6 mm for March, April, and May, respectively. The average rainfall for these months from the same weather station during 2000-2020 showed that the average precipitation in March, April and May was 44.4, 52.9 and 52.7 mm. Therefore, March and April had lower-than-average precipitation, while May doubled the mean rainfall. The average temperature for March, April and May 2021 was similar to the average for the period 2000-2020.

The hand-harvested crop components showed that ears/m^2 were also affected by tillage, with Zero tillage having significantly higher number than Deep tillage. Considering that this is a high tillering variety, the average number of 333.2 ears/m^2 was lower than the benchmark of 774 ears/m^2 for a yield of 8.8 Mg/ha at 15% (AHBD, 2023). This could be due to the lower crop establishment and drier spring months (March and April).

Grains/ear and harvest index (HI) showed no significant difference between treatments. The average of 39.2 grains/ear, was higher than the benchmark of 25 grains/ear for two row barley (AHBD, 2023) which is to be expected because Belfry is a six-row variety. TGW was affected by the main effect of traffic, tillage and the interaction. With CTF and STP having a higher significantly higher TGW than LTP systems. Zero and Shallow tillage systems also had significantly higher TGW than Deep tillage systems. Within the interaction, CTF Shallow tillage had the highest TGW. This could be due to the drier months in spring that might have affected water availability differently on the different treatments.

White millet is typically sown between mid-April and late May in Europe. Initial sowing (25/05/2022) resulted in poor crop establishment and high weed pressure, therefore it was resown on 08/07/2022. The emergence phase coincided with a two-week heat wave with zero precipitation and elevated temperatures (Fig. 3.9. Methodology Chapter). The precipitation in the seven days pre-planting was minimal (0.6 mm) and 0 mm in the following 13 days. Seeds that were planted at sufficient depth to access soil moisture emerged a few days after planting, however about half the seeds emerged 3-4 weeks later following rain at the end of the month, creating two different growth stages. However, plant establishment assessment (26/08/2022) revealed a 94.2% emergence (TGW = 6, seed rate: 30 kg/ha, 500 plants/m^2). There were no significant differences between treatments, possibly because the data was quite variable (CV = 19.20%). The high crop emergence could be due to the high adaptation rate of this plant to dry and hot weather conditions (Millborn, 2024). The hand harvested dry biomass also revealed no significant differences between treatments (CV = 20.44%). Due to late planting, it was not possible to perform a combine harvest, the crop was still in seed production stage in November 2022.

Spring oats cv. Isabel were drilled on 8th March 2023, during snowfall conditions, with a little snow cover persisting for 1-2 days. It then rained every day, except for 2 days, until the end of the month. The total precipitation for March was 96.2 mm, which exceeded the 2000-2020 historical average of 44.4 mm by 116%. Plant establishment was assessed on 3 April 2023. The crop emergence was 47.5% (TGW = 38, seed rate: 160 kg/ha, 420 plants/m^2). The reduced crop emergence can be attributed to excessive soil moisture conditions, which can limit soil aeration and reduce seed germination (Hutton, 2019). Both the main effect of tillage and the interaction between the traffic and tillage systems were statistically significant, affecting the plant establishment. Deep and Shallow tillage had higher plants/m^2 than Zero tillage systems. This may be explained because oats are particularly sensitive to waterlogging, and the looser soil structure from the recently tilled soil might have created better drainage and warmer soil temperatures around the seeds compared to zero till soils. LTP Shallow tillage had significantly higher plants/m^2 than LTP Zero and STP Zero. CTF Shallow, LTP Deep and STP Deep had higher plants/m^2 than STP Zero. This crop experienced a below average precipitation in May, with a total of 19.4 mm, compared to the 2000-20 historical average of 52.00 mm, representing a 62.7% reduction in rainfall. The average hand harvested yield (7.39 Mg/ha) was similar to the combined harvested yield (7.64 Mg/ha). While hand-harvested yield showed no significant differences among treatments, combine-harvested yield analysis revealed significant effects for both Traffic (main effect) and the traffic and tillage interaction. Within the main traffic treatments, CTF and LTP systems yielded 6.83% higher than STP systems. Among the treatment combinations, LTP Deep tillage produced the highest yield, followed by LTP Shallow tillage and CTF Shallow and Deep tillage systems.

Analysis of the crop components showed no significant differences among treatments. The observed panicle density of 283.2 panicles/m² was 23.5% below the reference value of 370 panicles/m² for a 7 Mg/ha yield (15% moisture content) reported by Hutton, (2019). Conversely, the grain number per panicle (70.6) exceeded the reference value of 44 grains per panicle. The increased grain count per panicle could be due to oats having the potential to compensate for low plant density, by increasing tillering or developing more grains per panicle (Hutton, 2019).

Winter wheat cv. KWS Extase was drilled on 17 October 2023. This month recorded one of the wettest months with a total of 160 mm compared to the previous 20-year average of 66.67 mm (140% increase). The plant establishment assessment on 28 November 2023, revealed a 24.5% crop emergence. The low emergence rate can be attributed to soil waterlogging conditions that limit the oxygen in the soil and inhibit seed emergence. Only the main effect of traffic showed significant differences, with CTF systems having higher plant density (plants m^{-2}) than STP systems. This likely results from CTF preserving soil structure and porosity in most of the field, enabling better water infiltration compared to STP systems that have increased soil compaction and consequent waterlogging when the precipitation is high.

The total precipitation for March and May 2024 was 89.6 and 88.2mm, respectively, exceeding the 2000-2020 historical average of 44.4 and 52.7 mm. However, June was drier than the average with a total of 40.6 mm (historical average 66.45 mm) (Fig. 3.11 Methodology chapter). The hand-harvested yield showed no significant treatment effects. Among crop components, only TGW was significantly affected by Tillage, with higher values in Zero tillage compared to Deep tillage systems. For combine harvested yield, only the main Traffic treatments showed significant effects, with CTF and LTP systems yielding 9.23% higher than STP systems. These results align with the higher plant density observed in CTF systems, and the drier June might have potentially also favoured CTF systems' yield advantage. However, the very wet conditions during drilling might have also favoured LTP systems compared to STP systems.

During the past two years in the UK, increased precipitation variability was observed during autumn 2023 and 2024 (Fig. 3.10 -Chapter 3), potentially attributable to climate change. The enhanced soil structure maintained under CTF systems provides greater resilience to these extreme weather events through improved physical properties, which can lead to higher yield potential.

The hand harvested yield analysis from CTF plots comparing the permanent wheel way and the untrafficked area of the plots showed significantly higher yields in the untrafficked area compared to the permanent wheel way only for winter barley ($p < 0.001$) and winter wheat ($p < 0.001$). For winter wheat, the yield decrease caused by wheeling was greater in both tillage treatments, showing that Zero tilled soil was better able to carry the traffic. No significant yield differences were observed between those areas for spring-sown crops millet and oats, possibly due to soil recovery from the harvested-induced compaction during the winter months.

Calculating CTF system yields for 15% trafficked area resulted in mean yield increases of 3.86% compared to the CTF 30% across crops and years, with variation among individual crop-years (i.e. 3.5%, 1.7% and 8.6% for winter barley, spring oats and winter wheat, respectively).

Summary of combine harvested yield:

No significant treatment effects were observed in winter barley and millet yields, potentially due to the high weed interference for winter barley and the different growth stages for millet. However, traffic treatments significantly affected spring oat and winter wheat yields, with CTF and LTP systems having higher yields than STP systems. These findings partially align with previous eight-year data showing a 4% mean yield advantage in CTF versus STP systems (Kazaroswki-Dolowy, 2022). The yield benefit of LTP systems for the last two years, compared to STP systems, was likely amplified by excessive precipitation during planting periods (March 2023 and October 2023 for spring oats and winter wheat, respectively). If CTF with a 15% trafficked area is applied, an estimated mean yield increase of 3.9% is suggested compared to the observed CTF 30% trafficked yields.

Spring oat yields showed significant traffic and tillage interaction effects. LTP Deep yielded significantly higher than both STP Deep and STP Shallow systems. Similarly, CTF Shallow, CTF Deep, and LTP Shallow outperformed STP Deep systems. These yield advantages coincided with a below-average May 2023 precipitation (19.4 mm versus 52.7 mm 20-year mean). The enhanced yield performance may have resulted from improved soil moisture conditions in CTF and LTP systems, relative to STP Deep soils, which are susceptible to re-compaction following deep loosening (Galambošová *et al.*, 2017).

Consistent with these findings, Rataj *et al*., (2022) reported CTF system advantages during dry years, on the contrary, in wetter years, STP systems had slightly better results. Winter barley crop experienced the wettest month in May 2021 (Fig. 3.11-methodology chapter), which could have contributed to the absence of significant treatment effects on yield.

8.5.1. LIMITATIONS

The Zero tillage system may have been constrained by equipment limitations and agronomic constraints. The Zero tillage system may be subject to more disturbance than expected because the drilled used was not able to elevate the crop residues and deposit the seeds inside. Consequently, discs (~5 cm) were used to break the crop residues and enhance the tilth. Furthermore, the Zero tillage system may sometimes be at a disadvantage in comparative system effects studies due to the delay in establishing the Zero tillage crop until optimal conditions for the conventional tillage system were available, to enable simultaneous planting of all crops. This may result in a delay in the optimal growth benefit that Zero tillage system could have achieved in comparison to the conventional tillage system.

Due to temporal and labour constraints, the hand harvest procedure resulted in a limited number of samples being collected, which might have affected the statistical power and the results of the study. The manual harvesting methods used at this experimental site are very laborious and time-consuming. The university has a lack of automatic equipment and it often malfunctions, having to do all manually by the researcher. These limitations can be addressed by improving the technology available, including analytical equipment and field instrumentation.

8.6. CONCLUSIONS

The initial hypothesis that "*CTF systems (30% trafficked area) and the reduced wheeled pressure of LTP systems positively affect crop establishment, crop components and crop yields*" was partially supported by the results of this study. CTF (30% trafficked area) systems and LTP systems had significantly higher crop establishment and combine harvested yield for winter wheat and spring oats compared to STP systems. However, they showed no significant effects for winter barley and millet. These differential responses suggest that the timing of establishment and meteorological conditions might affect the crop response to traffic-induced compaction. Calculating for a more commercially desirable 15% traffic area for CTF systems results in an estimated mean yield increase of 3.9% compared to the observed CTF 30% trafficked yields.

The second hypothesis, “*Reduced tillage (Shallow and Zero tillage) positively affects crop growth and yields compared to Deep tillage*”, was partially supported. Tillage effects on crop establishment varied by species: Zero tillage significantly increased winter wheat establishment compared to Deep tillage. Shallow tillage significantly improved millet establishment relative to Deep tillage. While both Deep and Shallow tillage showed significantly higher plant establishment for spring oats compared to Zero tillage. For the hand harvested yield, Zero and Shallow tillage significantly improved winter barley’s yield, compared to Deep tillage. However, combine harvested yields showed no significant differences between the tillage systems. While Zero tillage systems achieved comparable yields to Deep tillage systems, they provide additional economic advantages (reducing labour hours and fuel consumption) and environmental benefits (reducing soil erosion and enhancing water infiltration).

The third hypothesis, “*The interaction between traffic and tillage systems can positively affect crop growth and yields*”, was also partially supported. Significant traffic and tillage interaction effects for crop establishment and combine harvested grain yield were observed only in spring oats (2023), coinciding with below-average precipitation in May 2023. For crop establishment, LTP Shallow had the highest observed plants per m^2 and STP Zero the lowest. For the combine harvested yield, LTP Deep had the highest observed yield and STP Deep had the lowest.

CHAPTER 9

DISCUSSION

9.1. SOM AND CARBON STOCKS

9.1.1. EFFECTS OF THE INTERACTION BETWEEN TRAFFIC AND TILLAGE SYSTEMS

SOM plays a crucial role in arable land to support soil health, crop productivity, food security and environmental quality (Lal, 2016). Intensive agricultural practices have progressively depleted SOM over the last few decades, compromising soil health and threatening long-term food security, as well as the provision of other ecosystem services. Generally, higher SOM levels indicate higher soil fertility, structure and resilience. Conservation agriculture practices—including cover cropping, reduced tillage, and organic amendments—mitigate SOM degradation. Despite extensive research on SOM management, the impact of different agricultural traffic systems and the interaction with different tillage systems on SOM dynamics remains underexplored. Investigating agricultural traffic's effects for a range of tillage depths on SOC storage and crop productivity is critical for developing sustainable land management in the UK.

This study revealed that the combination of CTF and Zero tillage exhibited 6.4% higher SOM concentrations at the 0-30 cm depth interval compared with other treatment combinations. Jointly using CTF and Zero tillage resulted in the storage of 5 Mg ha^{-1} more SOC stocks than all other traffic and tillage treatment combinations. This was explained by the additional POM-C (~26% more) and MAOM-C (~6% more) recorded at 0-30 cm under CTF and Zero tillage. The POM-C fraction was the primary driver of total SOC storage. These results agree with other studies such as Samsom *et al*. (2020), who reported that reduced tillage and residue retention favoured POM-C in arable soils and were particularly efficient in increasing surface SOC irrespective of soil texture in Canada (over 7 years of field experiment).

The combination of CTF with Zero tillage systems provides the best soil structure for the soil organisms and crop roots. Crop roots provide more carbon sources than above-ground crop residues (Kättere *et al*., 2011). On top of that, zero tillage systems support better fungal based communities, such as arbuscular mycorrhizal fungi and earthworms, which can substantially contribute to C sequestration in the soil (Six *et al.,* 2006). The fungal networks can also help plants access nutrients and water more efficiently, maintaining soil aggregates and pore networks, which allow better water infiltration and root penetration.

However, when CTF was combined with Shallow or Deep tillage, the beneficial effects on C storage of not compacting the soil and preserving the soil structure were negated, because tillage disrupts the soil structure. Similarly, Zero tillage preserves soil structure, but when combined with STP or LTP systems that compact the soil, this beneficial effect on C storage was lost. This highlights the importance of examining the interactive effects between the different traffic and tillage systems on SOC storage, rather than their individual effects as previously done in the literature. Farmers and policymakers should carefully consider these interactions, as inappropriate combinations can reduce C storage potential.

Changes in the distribution of C in the SOM fractions critically influence SOC sequestration and long-term C stability. There has been a focus on trying to increase SOC by increasing MAOM-C, due to its longer residence time (Sokol, *et al.,* 2022). However, MAOM-C formation is influenced greatly by soil texture, aluminium and iron oxides and pH, with soils with higher clay and silt content being able to store higher amounts (Geordiou *et al*., 2022; Salonen *et al.,* 2024). In contrast, POM-C, a precursor of MAOM-C, can continue accumulating even when MAOM is saturated, increasing the total SOC storage (Angst *et al*., 2023). Therefore, management practices that promote and protect POM should be adopted.

Evidence from the current study has shown that to maximise SOC storage, the combination of both traffic and tillage management practices that promote minimal soil disturbance is required. Therefore, by incorporating CTF with Zero tillage, UK farmers could gain carbon storage but at the same time reduce their labour hours and fuel consumption. However, the SOC storage rate and capacity will be determined by the initial carbon content, soil type, carbon inputs into the system and the time it has been with those management changes. Eventually, the system will tend to reach an equilibrium between the carbon inputs and outputs after 20-100 years (Johnston, Poulton and Coleman, 2009). Therefore, not only is there a need to keep the SOM inputs constant, but also, over time, there would be a need to increase them once the system has reached an equilibrium. Furthermore, most of the carbon which accumulated in our study was in the POM fraction, which is more labile and vulnerable to decomposition after a management practice change such as tillage. Therefore, the accumulated carbon over the years could easily be lost. This is why managing arable soils to sequester carbon has some limitations, as most of the sequestered carbon is in a more labile form with a shorter residence time. It is likely that farmers would need to change their management practices due to economic or environmental factors. And most carbon credits in the UK only last for 10 years, which doesn’t help much as a long-term climate change mitigation strategy.

Despite the slow but growing adoption of CTF systems globally, previous research has not investigated the different carbon pools, stabilisation mechanisms, and overall carbon budget of SOM under CTF systems. Nor the long-term interacting effects of different traffic and tillage systems on SOM and crop productivity. Therefore, these findings provide novel insights into the carbon cycling processes unique to these systems, thereby advancing the scientific understanding and identifying farming practices that improve carbon sequestration and food security.

The combination of CTF and Zero tillage systems with other conservation or regenerative agricultural practices such as retaining crop residue, cover cropping (covering the soil during fallow periods), crop rotation and diversification, including organic amendments (such as compost, manure or biochar), or agroforestry (integrating trees or shrubs) can all help to increase carbon inputs and improve soil structure, fertility and water retention. All of these additions will also help reduce soil erosion, which is also an important source of carbon loss in arable soils. 90% of conventionally managed arable soils globally are thinning due to erosion (Evans *et al*., 2020).

It is also important to note that the soil sampling strategy designed to represent the majority of CTF systems represents only the C concentration of the non-trafficked crop bed. The trafficked areas (30% in our plots, or 15% in more realistic farm scenarios) were not accounted for and may exhibit different C concentrations due to soil compaction effects.

9.1.2. THE OVERALL EFFECT OF ALL TRAFFIC SYSTEMS

This study demonstrated that the overall effect of STP and LTP systems led to a significantly higher soil BD at 0-30 cm and lower SOM concentrations compared with the non-trafficked crop area of CTF systems. These results contradict previous research that studied the influence of soil compaction on carbon mineralisation rate. Neve and Hofman (2000) concluded that the C mineralisation rate was strongly depressed at a bulk density of 1.6 g/cm^3 on a loamy sand soil and the reduction in C mineralisation led to higher SOM accumulation. They also concluded that increasing soil compaction starting at a bulk density of 1.5 g/cm^3 affected some microbially driven processes. However, the soil BD in this study did not reach higher than 1.4 g/cm^3 at 0-30 cm. The average soil BD for CTF systems was 1.23 g/cm^3, LTP systems 1.37 g/cm^3 and STP systems 1.35 g/cm^3 at 0-30 cm. That is likely why soil compaction in our study had the opposite effect as described by Neve and Hofman (2000). The less compacted soil of CTF systems had higher SOM concentrations, likely favoured by a better soil structure with higher pores, pore connectivity (Millington, 2019) and biotic activity, which increased SOM physical protection and aggregate stability compared to LTP and STP systems. However, there are not many studies on this area (with more commonly achieved soil bulk densities in arable soils) and more research is needed. Another study by Carlesso *et al.* (2019), looking at how soil compaction affected the decomposition rate of litter bags on an arable field in the UK, also contradicted our results. They inserted litter bags (2mm and 0.02 mesh size) at 5 cm depth in three different areas with different soil BDs: the tramline (DB = 1.25 g/cm^3), crop area (DB = 1.02g/cm^3) and grass margin (DB = 0.89g/ cm^3). They reported that the greatest amount of littler remaining in the bags after 6 months was in the tramline and the least in the grass margin. These results suggest that the more compacted soil should have more crop residue remaining than the less compacted soil from CTF systems. However, the results of this study are limited because of the mesh size used, the duration of the experiment, it does not specify if the tramlines had crop growing on them, and the location of the bags at only 5 cm. All of these factors will affect the structure of the soil and the biotic characteristics affecting the litter in the bags, and therefore, it fails to look at the effects of the whole system on litter decomposition in arable soil.

While soil compaction might temporarily preserve SOM, the long-term effects of soil compaction in arable soils lead to a net decline in SOM. This could be due to many factors, such as the reduced plant-derived organic inputs due to reduced root growth and crop yield (Andersen, Munkholm and Nielsen, 2013; Kaczorowska-Dolowy, 2022). The reduced porosity limits nutrient movement via soil water, also affecting fertiliser absorption. It can also reduce aeration, which could lead to reaching anaerobic conditions, or waterlogging, which could shift microbial processes toward denitrification. The combination of soil compaction with frequent tillage further degrades soil structure and breaks soil aggregates, exposing protected SOM to decomposition. The soil in zero tillage systems can improve the soil structure, making it more resilient to compaction compared to conventional tillage, which creates a loose but unstable structure that recompacts easily under agricultural traffic (Kumar *et al*., 2022).

CTF systems also resulted in significantly higher POM-C (13.8% more) than STP and LTP systems and MAOM-C (3% more) than STP systems at 0-30 cm. Therefore, after 12 years of continuous traffic and tillage practices, the findings indicate that the higher SOM concentrations in CTF systems were driven by the POM fraction, despite POM-C accounting for only one-fifth of the total SOC. In CTF, the higher storage of POM-C likely resulted from the reduced soil compaction compared to STP and LTP systems.

9.1.3. THE OVERALL EFFECT OF ALL TILLAGE SYSTEMS

This study reported that Deep tillage systems stored significantly lower SOM concentrations than Zero and Shallow tillage systems at 0-30 cm. In temperate regions, Deep tillage systems often have a negative impact on soil carbon storage and soil physical characteristics (Topa, Cara and Jităreanu, 2021). However, this study showed that soil BD was not significantly affected by tillage depth, suggesting a re-compaction mechanism in Deep tillage systems over time. Therefore, the lower SOM concentrations of Deep tillage systems can be attributed mainly to soil disturbance, redistributing the OM across the soil profile and breaking soil aggregates, which increases SOM mineralisation. Deep tillage systems also stored significantly lower POM-C than Zero and Shallow tillage systems at 0-30 cm. However, MAOM-C was not significantly affected by the different tillage systems. These results are in line with Cotrufo *et al*. (2019), who reported that while tillage is known to accelerate POM-C decomposition, MAOM-C typically exhibits greater stability and persistence in the soil due to its strong chemical bonds with soil minerals and the protection of C within microaggregates.

Zero and Shallow tillage systems stored ~3% more SOM concentrations and ~7% more POM-C than Deep tillage systems. The fact that Zero and Shallow tillage had similar SOM concentrations could be due to the low tillage depth of only 10 cm and our Zero tillage system sometimes used discs (~5 cm) when drilling the new crop to break the surface crop residues from the previous crop and aid dropping the seeds for the next crop. The higher SOM can also enhance soil aggregate stability.

These findings also indicated that the overall effect of traffic or tillage alone did not significantly affect SOC stocks on ESM (0-30 cm), although it did affect SOM and SOC concentrations. This is why it is important to look at SOC stocks, especially when tracking C storage over time. Content alone cannot distinguish between carbon gains or artefacts of soil compaction (Smith *et al*., 2019). However, the quantification of SOC stocks is more expensive and time-consuming, therefore, many farmers are likely to do SOM analysis only, as it is part of different soil health frameworks. The work in this thesis reiterates the current literature, saying that SOC should not be estimated using pedotransfer functions from SOM due to errors. Therefore, quantifying SOC concentrations and soil DB with an appropriate sample strategy is recommended to track carbon gains over time.

Some studies (Haddaway *et al*., 2017; Cooper *et al.,* 2021) have shown that different tillage systems affected SOC stocks, with Zero tillage storing 4–6 Mg ha^{-1} more SOC stocks than conventional tillage practices after 6-10 years. However, other studies in temperate climate regions have shown the limited or variable role of tillage depth on SOC storage when considered in isolation from other related system variables such as residue management or cover cropping (Hermle *et al.,* 2008; Mary *et al.,* 2020; Martinez *et al*., 2016; Schjønning and Thomsen, 2013). A recent report by the British Ecological Society (2025) on regenerative agriculture in the UK also reported that the evidence for the benefits of no-till or minimum tillage associated with SOM increase was weak, while there was strong evidence for "minimising bare soil". This is because keeping plants and roots in the soil at all times by using cover crops or crop rotation will provide additional SOM inputs, while at the same time improving soil structure and nutrient cycling, supporting biodiversity (Giller *et al.,* 2021; Lal, 2020).

9.2. NATURAL ABUNDANCE ISOTOPE ^{13}C TECHNIQUE

The fate of millet's crop litter and root exudates in the different SOM fractions and soil depths was investigated through the natural abundance stable isotopic approach. The results of both the $\delta^{13}C$ values and the *f*C4 (proportion of millet derived C) aligned with previous results, indicating that carbon storage was driven by changes in POM and the treatments with significantly higher *f*C4 also exhibited significantly higher SOM concentrations, confirming the relationship between new carbon and its storage as influenced by the traffic and tillage management practices. However, the data after just one C4 crop year was small, and highly variable, this might be why other studies using this technique often have much longer time frames of C4 inputs (from 16 to 57 years) following a land use change of C3 plants (Deng *et al.,* 2016; Paul *et al.,* 2008; Poeplau *et al*., 2018).

The *f*C4 over time showed that the *f*C4 in POM remained constant after two years. However, the *f*C4 in MAOM was significantly lower after the second year. This result was unexpected because if there was any decomposition of POM-C4, it would go into MAOM-C4, increasing its content. However, this might be explained by preferential microbial processing, where microbes could prefer to decompose other POM material other than millet. This was also observed by de Almeida (2022), who reported that oats decomposed faster than millet.

9.3. CROP YIELD

9.3.1. EFFECTS OF THE INTERACTION BETWEEN TRAFFIC AND TILLAGE SYSTEMS

This study evaluated the 10th – 13th cereal crop seasons (2021-2024) for this long-term project. During this period, there were only significant traffic and tillage interactions on crop yield for spring oats (2023), where the highest yields were achieved in LTP Deep and CTF Shallow and Deep tillage systems. And the lowest yield was on STP Deep tillage systems. This result agrees with the long-term analysis of this project (Godwin *et al.,* 2022).

9.3.2. THE OVERALL EFFECT OF ALL TRAFFIC SYSTEMS

The CTF and LTP systems delivered a significantly higher yield (9.3% more for winter wheat and 6.8% more for spring oats) compared to STP systems. However, there were no significant differences in yield for the winter barley and millet crops. The data from the previous 8 years (from 2012 to 2020) at the same site reported that CTF resulted in higher yields (4% higher) in comparison to STP systems (Kaczorowska-Dolowy, 2022). This slightly higher response of CTF and the increase in yield of LTP systems could be due to the meteorological conditions affecting the crop response to traffic-induced compaction, as spring oats and winter wheat experienced unusually wet conditions in spring and autumn, likely due to climate change.

CTF systems in this study had a 30% trafficked area. If recalculated for a more commercially desirable 15% trafficked area for CTF systems, then an estimated mean yield increase of ~4% is suggested compared to the observed CTF trafficked yields. This result is consistent with the last eight-year study on the same site, where the effect of reducing the trafficked area to 15% produced an estimated increase in mean crop yield of 3% (Godwin *et al*., 2022).

These results are in line with the current literature, with CTF systems reporting 12-15% higher yields in combinable crops using CTF systems (DEFRA farming blog, 2025) or 30% higher yields for grain sorghum (Hussein *et al.,* 2021). These yield improvements are likely due to the reduced soil compaction (with this study showing 8% less soil bulk density) than STP and LTP systems, improving root growth and biological properties (Kaczorowska-Dolowy, 2022), allowing better nutrient uptake by crops as well as water infiltration and storage, improving rainfall and nitrogen fertiliser use efficiency (Hussein *et al.,* 2021). On top of that, CTF systems can also contribute to the reduction of labour and fuel costs (DEFRA, 2024).

9.3.3. THE OVERALL EFFECT OF ALL TILLAGE SYSTEMS

Tillage depth had no significant effect on yield. However, the first five years of the project the average Zero tillage yield was 10% lower than in Shallow and Deep tillage systems, but in year 7 it recovered, and the average Zero tillage yield was 4% greater than the average yield in Shallow and Deep tillage systems (Goodwin *et al*., 2022). Therefore, in the years 10 – 13, the average Zero tillage yield has levelled with Shallow and Deep tillage. However, Zero tillage while achieving comparable yields to Deep tillage systems, provides additional economic advantages (reducing labour hours and fuel consumption) (Godwin, 2014) and environmental benefits (reducing soil erosion and therefore environmental pollution, protecting water resources and increasing soil biodiversity) (Karayel and Šarauskis, 2019).

9.3.4. IMPLEMENTATION GUIDANCE FOR TRANSITIONING BETWEEN MANAGEMENT SYSTEMS

Transitioning to CTF and Zero tillage systems might require some initial investment, but this could be done over time as the equipment needs replacing. However, each farm situation is different. Therefore, individualised steps towards more sustainable agricultural practices could be chosen according to their specific needs to ensure success.

To ensure positive uptake by the farmers, CTF with Zero tillage management practices could be integrated into the current agri-environment policy as they promote soil health by increasing SOM concentrations and SOC stocks, which can be expected to increase resilience and, therefore, food security.

9.3.5. IMPLICATIONS FOR POLICY AND PRACTICE

There is a need for farmers to adapt their agronomic practices to address both economic gain and climate/ environmental objectives. Enhancing SOM storage is one of the follow-up outcomes mentioned in the UK Government’s 25-Year Environment Plan and, more recently, in the Soil Health Action Plan for England, where a key ambition is to manage soils sustainably by 2030 (UK Parliament Post, 2022). Some of the sustainable practices mentioned in this report are regenerative, organic and conservation agriculture (e.g. cover cropping, reduced and reduced reliance on fertilisers) could promote C storage and reduce greenhouse gas emissions. Reduced and zero tillage practices are proposed as potential strategies for enhancing soil carbon sequestration; however, evidence for zero tillage efficacy in the UK is mixed and may benefit only heavier clay soils. This study shows that the use of CTF with Zero tillage could also be a powerful strategy to minimise carbon losses, while at the same time maintaining crop yields and improving long-term economic returns (Godwin *et al*., 2022).

9.4. LIMITATIONS AND RECOMMENDATIONS FOR FURTHER STUDIES

- Due to logistical and budgetary constraints, carbon analysis was limited to the 0-30 cm stratum. However, significant SOC accumulation occurs in deeper horizons. Future investigations at this site should extend the sampling depth to 60-100 cm to provide a more comprehensive view of C storage.

- Long-term monitoring is essential for gathering robust data for decision-making and revealing insights into SOC accumulation dynamics over time. SOC stock gains are slow, and over time, the system tends to approach an equilibrium state. Therefore, future research on this site should quantify SOC storage over >5 years. Further knowledge of the time it might take the system to reach this equilibrium state after a management change in temperate climate regions would be useful for farmers and policymakers.
- SOC stock gains are not permanent. Knowledge gaps persist regarding the impact of isolated tillage events on SOC storage in systems following long-term conservation/ regenerative management practices. Further research is necessary to provide farmers with the data required for informed decision-making regarding occasional tillage events in systems with long-term conservation management practices. This will allow farmers and policymakers to know whether, for example, incorporating a root crop into a rotation sets those soils back marginally in terms of SOC stocks, or whether they are pushed back to “Square one”. This information could also affect the timeline needed for carbon credits to ensure the carbon stored in those 10 years is not released back into the atmosphere.
- Soil type, climate, vegetation cover and experimental duration and their interactions are all important factors affecting SOM dynamics and crop yields. This limits generalisation; therefore, more long-term experimental data is required to better understand the effects of different traffic and tillage systems on SOM dynamics and crop yield.
- The project and sample strategy were set up to study the different traffic effects of the three traffic systems imposed. However, due to the constraints of the 4 m plots and the wheel mark set-up, only the permanent crop bed of CTF systems was sampled for SOC quantification. Future research could incorporate the sampling of the permanent traffic lanes in CTF systems and the untrafficked areas of STP and LTP systems to have a better representation of the whole system.
- While increasing storage of SOC may mitigate CO_2 emissions, it is also important to take a full greenhouse gas (GHG) balance accounting for N_2O and CH_4 emissions. These two GHGs are 273 and 27 times more potent than CO_2, respectively (Arias *et al.,* 2021). Further research is needed to assess the full GHG impact of different traffic and tillage management practices that enhance SOC storage.

CHAPTER 10

CONCLUSIONS

10.1. CONCLUSIONS

This investigation focused on determining the combined effects of traffic and tillage management systems on soil organic matter (SOM) and its fractions, soil organic carbon (SOC) stocks and crop performance. The study was part of a long-term research programme conducted at Harper Adams University (Shropshire, United Kingdom) that combines, in a 3×3 factorial design, three traffic (controlled traffic farming and conventional traffic using machinery fitted with either standard tyre pressure or low-ground pressure tyres) and three tillage (zero-, shallow, and deep-tillage) systems. The experimental site was established in 2011 and sits on a sandy loam soil with a temperate climate and mean annual rainfall of 845 mm (range: 760-1000 mm year). The work reported in this thesis builds upon earlier work, which focused on understanding the interacting effects of traffic and tillage on crop productivity and farm economics, and the technical feasibility of implementing such systems in the context of UK agriculture, but which has not addressed the associated effects on SOM and SOC. This is an important scientific consideration that will help the UK agricultural sector meet its net-zero ambitions by 2050 by (1) identifying farming practices that maintain or improve both food security and soil carbon stocks, and (2) devising a pathway for their adoption. The main conclusions derived from this research are summarised below each of the objectives previously formulated:

Objective 1: To determine the individual and interacting effects of three traffic and three tillage management systems on SOM, SOM fractions and SOC stocks through the quantification of:

1. SOM concentration and microbial biomass carbon (MBC) in soil,
2. SOC content, SOC stocks, and soil bulk density (BD),
3. SOM fractions: Particulate (POM-C) and Mineral associated (MAOM-C) organic matter carbon, and soil carbon-to-nitrogen ratio (C/N ratio), and
4. ^{13}C and ^{12}C isotope analyses to determine the pathways of photosynthetic C moving into the different SOM fractions.

To answer objective 1, the following conclusions were observed:

1. The overall effect of all traffic systems significantly affected SOM concentrations, with the non-trafficked crop area of Controlled Traffic Farming (CTF) systems exhibiting higher SOM concentrations compared to Standard tyre pressure (STP) systems at 0-30 cm depth. Low tyre pressure (LTP) systems were not statistically significant. After 12 years from establishment, the non-trafficked crop area of CTF systems stored 2.7% higher SOM concentrations (increasing from 3.9% to 4.05%) than STP systems and had 9.6% lower soil BD than STP and LTP systems at 0-30 cm depth, while also storing 13.9% more POM-C and 2.9% more MAOM-C within the same soil profile. This suggests that the non-trafficked area of CTF soil was likely healthier, as both increased SOM concentrations and reduced levels of compaction are associated with increased soil health.
2. The overall effect of all tillage systems: Zero and Shallow tillage systems stored 3% higher SOM concentrations (increasing from 3.92% to 4.04%) and 7.4% higher POM-C than Deep tillage systems at 0-30 cm depth. Tillage did not significantly affect MAOM-C concentrations and soil BD within the same soil profile. Therefore, the lower SOM concentrations in Deep tillage systems can be attributed to soil disturbance and subsequent SOM mineralisation, mainly from the POM fraction. This suggests that repeated Deep tillage will reduce the concentration of POM down the soil profile, leading to depletion of SOM over time.
   Neither traffic nor tillage systems showed significant main effects on SOC stocks, suggesting that these individual management practices on their own were insufficient to influence soil C storage. However, they did affect SOM concentrations as mentioned above, which measurement is required for farmers to participate in some UK agricultural government schemes, and it is often recommended for enhancing soil health.
3. The interaction between traffic and tillage systems: after 12 years, the non-trafficked crop area of CTF with Zero tillage exhibited 6.4% higher SOM concentrations at 0-30 cm compared with other treatment combinations. This resulted in the storage of 5 Mg/ha more SOC stocks for an equivalent soil mass (ESM) at 0-30 cm depth than all of the alternative traffic and tillage treatment combinations, except for LTP with Shallow and Deep tillage systems. This is explained by the fact that the non-trafficked crop area of CTF with Zero tillage systems stored both the highest POM-C (25.6% more) and MAOM-C (5.8% more) at the same profile depth than the remaining treatment combinations. Therefore, the POM fraction was the primary driver of SOC storage. Higher POM-C concentrations were attributed to reduced soil

disturbance in the absence of both traffic and tillage. However, if tillage practices were to resume, POM-C levels may decline due to it being a labile SOM fraction. This suggests that caution is required if applying different tillage and traffic management systems for the purpose of soil C trading; the 10-year duration of most carbon credit schemes in the UK limits their effectiveness as a long-term climate change mitigation strategy, as these C gains are vulnerable to changes in management practices. However, increasing the C content of the soil through building SOM can provide significant benefits to farmers, improving productivity and farming system resilience. Therefore, management practices that protect and promote POM should be implemented where possible.

The Microbial Biomass Carbon (MBC) analysis found that only the effect of soil depth was significant, with MBC decreasing with increasing soil depth: the 0-10 cm layer showed the highest MBC, followed by the 10-20 cm layer, and the lowest values were observed in the 20-30 cm layer. No other treatment effects were evident on the MBC.

The C/N ratio of the POM fraction was ~19, and the MAOM fraction was ~8. These ratios reflect their formation and the relative contributions of crop residues (i.e. mainly straw with a higher C/N ratio) for POM and mainly microbial-derived metabolites and necromas (which have a lower C/N ratio) for MAOM. There were no significant traffic and tillage effects on either POM and MAOM C/N ratios; only the overall effect of Depth was significant. While MAOM C/N ratio remained constant over time, POM C/N ratio was not, demonstrating that POM is more variable in the system.

The natural abundance ^{13}C isotope technique confirmed that crop residues are the primary source of C entering the system, predominantly accumulating in the POM fraction. This finding also aligned with the POM-C and MAOM-C results, indicating that carbon storage was driven by changes in POM. The results for the *f*C4 (proportion of millet derived C) in the POM and MAOM fraction also aligned closely with previous findings, demonstrating that the treatments with significantly higher *f*C4 values also exhibited significantly higher SOM concentrations.

Objective 2. To determine the individual and interacting effects of three traffic and three tillage systems on crop performance for the following crops:

1. Winter barley cv. Belfry (*Hordeum vulgare* L.)
2. Millet cv. White (*Panicum miliaceum L.)*

3. Spring oats cv. Isabel (*Avena sativa L.*)
4. Winter wheat cv. Extase (*Triticum aestivum* L.)

In recent years, there has been an increase in extreme weather events driven by climate change affecting both crop establishment and yield. To answer objective 2, the following conclusions were observed:

1. The overall effect of all traffic systems on plant establishment was only significant for winter wheat (2023-24), where CTF systems had higher plants per m^2 than STP systems. The overall effect of traffic systems on combine harvested yield was only significant for winter wheat and spring oats, where CTF and LTP systems had significantly higher yields (9.3% more for winter wheat and 6.83% more for spring oats) compared to STP systems. However, there were no significant differences for the winter barley and millet crops. These differential responses suggested that the timing of establishment and meteorological conditions affected the crop response to traffic-induced compaction. The data of the previous 8 years (from 2012 to 2020) at the same site reported ~4% higher yields for CTF compared with STP systems. This again suggests that the interaction between soil structure and precipitation can combine to influence plant available water with consequences for plant growth and crop yields.
   CTF systems in this study had a 30% trafficked area. If recalculated for a more commercially desirable 15% trafficked area for CTF systems, an estimated mean yield increase of 3.9% would be expected compared with the yield observed for CTF with 30% of the field cropped area under traffic.
2. The overall effect of all tillage systems on plant establishment significantly affected winter barley (2020-21), millet (2022) and spring oats (2023), with different results. For winter barley, Zero tillage systems had higher plants per m^2 than Deep tillage systems despite having the same seed rate. For millet, Shallow tillage had higher plants per m^2 than Deep tillage systems, and for spring oats, both Shallow and Deep tillage had higher plants per m^2 than Zero tillage. This difference can be due to meteorological conditions after drilling; spring oats had the wettest month on record (exceeding the 2000-2020 historical average of precipitation of 44.4 mm by 116%), while Millet had the driest month on record after drilling. This suggests that soil structure and the amount of precipitation can interact to influence plant germination and establishment, as well as plant growth, as mentioned previously. Tillage depth had no significant effect on combine harvested yield. While Zero tillage systems achieved comparable yields to Deep tillage systems, they provide additional

economic advantages (reducing labour hours and fuel consumption) and environmental benefits (reducing soil erosion and enhancing water infiltration).

3. The interaction between traffic and tillage systems on plant establishment and combined harvested yield was significant only for spring oats (2023), coinciding with below-average precipitation in May 2023. For the plant establishment, LTP Shallow had the highest plants per m^2, and STP Zero had the lowest. For the combine harvest yield, the highest yields were achieved in LTP Deep, followed by CTF (Shallow and Deep), while the lowest yield was on STP Deep systems. This agrees with earlier work and suggests that soil structure interacts with soil moisture to affect plant available water with consequences for plant growth and crop yields, in agreement with a large body other literature on this topic.

10.2. PRACTICAL RECOMMENDATIONS

Traffic and tillage management system recommendations for maximising SOC storage (0-30 cm) for cereal crops on sandy loam soil under UK climatic conditions, ranked by effectiveness:

1. Integration of CTF with Zero tillage for higher carbon stock storage.
2. Adoption of CTF systems for higher SOM.
3. Adoption of reduced tillage practices (Zero and Shallow tillage systems) for higher SOM.

This long-term study incorporated crop residues and only two winter cover crops. However, to maximise the effects of the recommended traffic and tillage management practices, it would be best to use them in combination with other practices that promote C inputs into the soil. The literature strongly suggests that the use of practices that promote C inputs into the soil such as conservation or regenerative agricultural management practices are also very important for improving SOC storage and soil health, such as keeping the soil covered at all times by using cover crops, companion crops and crop rotation or increase crop residues, manure or compost.

Traffic and tillage management recommendations for maximising crop yield, ranked by effectiveness:

1. Adoption of CTF and/ or LTP systems, STP systems always had the lowest yield and were significantly lower in 2 out of 3 years.

2. Adoption of Zero tillage systems, as they deliver similar yield to Deep and Shallow systems but can offer further advantages such as less labour hours, fuel consumption and wearing of metal, plus higher SOM concentrations.
3. Avoid STP Deep, it consistently had the lowest yield, although significantly in 1 out of 4 crops of this study. The interaction effect of traffic and tillage systems on crop yield was less clear; there were only significant effects on crop yield for spring oats, where LTP Deep and CTF with Shallow and Deep had higher yields compared to STP Deep.

10.2. SCIENTIFIC RECOMMENDATIONS

Long-term monitoring is essential for gathering robust data, therefore, future research on this site should quantify SOC storage over >5 years. This will also help clarify if the system might be reaching an equilibrium state. If possible, future investigations should extend the sampling depth to 60-10 cm to provide a more comprehensive view of C storage. Future research on this site could also incorporate sampling of the permanent traffic lanes in CTF systems to have a better representation of the whole system. Knowledge gaps persist regarding the impact of isolated tillage events on SOC storage in systems following long-term conservation/ regenerative management practices. This site could investigate this in a small portion of the plots to provide farmers with the data required for informed decision-making regarding occasional tillage events in systems with long-term conservation management practices. While increasing storage of SOC may mitigate CO_2 emissions, it is also important to take a full greenhouse gas (GHG) balance accounting for N_2O and CH_4 emissions. Further research is needed to assess the full GHG impact of different traffic and tillage management practices that enhance SOC storage.

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CHAPTER 12 APPENDICES

12.1 FOR CHAPTER 3. APPENDIX 3.

Table A3.1. - Traffic or compaction protocol established by Smith (2017) and amended by Millington (2019). Applied at the end of each cropping season.

	Front weight 540 kg, rear weight 1400kg							
	Sequence 1	✓		**Sequence 2**	✓		**Sequence 3**	✓
1	Set High Pressures		34	Set LH to low pressure		53	Set pressures to low	
2	**Go to plot 1**		35	**Go to plot 1**		54	**Go to plot 2**	
3	Drive AB line		36	Drive Offset (1200)		55	Drive AB line	
4	Return		37	**Go to plot 6**		56	Return	
5	Drive Offset (600)		38	Drive Offset (1200)		57	Drive Offset (600)	
6	Return		39	**Go to spare 1**		58	Return	
7	Drive Offset (600)		40	Drive Offset (1200)		59	Drive Offset (600)	
8	**Go to plot 18**		41	**Go to plot 15**		60	**Go to plot 10**	
9	Drive AB line		42	Drive Offset (1200)		61	Drive AB line	
10	Return		43	**Go to plot 18**		62	Return	
11	Drive Offset (600)		44	Drive Offset (1200)		63	Drive Offset (600)	
12	Return		45	**Go to plot 20**		64	Return	
13	Drive Offset (600)		46	Drive Offset (1200)		65	Drive Offset (600)	
14	**Go to plot 25**		47	**Go to plot 25**		66	**Go to plot 21**	
15	Drive AB line		48	Drive Offset (1200)		67	Drive AB line	
16	Return		49	**Go to plot 29**		68	Return	
17	Drive Offset (600)		50	Drive Offset (1200)		69	Drive Offset (600)	
18	Return		51	**Go to plot 31**		70	Return	
19	Drive Offset (600)		52	Drive Offset (1200)		71	Drive Offset (600)	
20	**Go to plot 31**					72	**Go to plot 32**	
21	Drive AB line					73	Drive AB line	
22	Return					74	Return	
23	Drive Offset (600)					75	Drive Offset (600)	
24	Return					76	Return	
25	Drive Offset (600)					77	Drive Offset (600)	
26	**Go to plot 9**					78	**Go to plot 5**	
27	Drive Offset (600)					79	Drive Offset (600)	
28	**Go to plot 13**					80	**Go to plot 12**	
29	Drive Offset (600)					81	Drive Offset (600)	
30	**Go to plot 23**					82	**Go to plot 27**	
31	Drive Offset (600)					83	Drive Offset (600)	
32	**Go to plot 33**					84	**Go to plot 35**	
33	Drive Offset (600)					85	Drive Offset (600)	

Table A3.2. - Cultivation protocol after Millington (2019).

1	**Set Pressures High**			24	**Set Pressures Low**	
2	**Set Topdown for Deep Tillage**			25	**Keep Topdown for Shallow Tillage**	
3	**Go to Spare 1**	SPARE 1		26	**Go to Plot 5**	LGP SHALLOW
4	Drive			27	Drive	
5	**Go to Plot 1**	RTF DEEP		28	**Go to Plot 8**	CTF SHALLOW
6	Drive			29	Drive	
7	**Go to Plot 18**	RTF DEEP		30	**Go to Plot 11**	CTF SHALLOW
8	Drive			31	Drive	
9	**Go to Plot 25**	RTF DEEP		32	**Go to Plot 12**	LGP SHALLOW
10	Drive			33	Drive	
11	**Go to Plot 31**	RTF DEEP		34	**Go to Plot 19**	CTF SHALLOW
12	Drive			35	Drive	
13	**Set Topdown for Shallow Tillage**			36	**Go to Plot 27**	LGP SHALLOW
14	**Go to Spare 2**	SPARE 2		37	Drive	
15	Drive			38	**Go to Plot 28**	CTF SHALLOW
16	**Go to Plot 9**	RTF SHALLOW		39	Drive	
17	Drive			40	**Go to Plot 35**	LGP SHALLOW
18	**Go to Plot 13**	RTF SHALLOW		41	Drive	
19	Drive			42	**Set Topdown for Deep Tillage**	
20	**Go to Plot 23**	RTF SHALLOW		43	**Go to Plot 2**	LGP DEEP
21	Drive			44	Drive	
22	**Go to Plot 33**	RTF SHALLOW		45	**Go to Plot 4**	CTF DEEP
23	Drive			46	Drive	
				47	**Go to Plot 10**	LGP DEEP
				48	Drive	
				49	**Go to Plot 17**	CTF DEEP
				50	Drive	
				51	**Go to Plot 21**	LGP DEEP
				52	Drive	
				53	**Go to Plot 22**	CTF DEEP
				54	Drive	
				55	**Go to Plot 32**	LGP DEEP
				56	Drive	
				57	**Go to Plot 36**	CTF DEEP
				58	Drive	

Table A3.3. - Drilling protocol after Millington (2019).

CLOSE COULTERS 1 and 24	
Front weight 1400 kg	
TGW 49.5	
Seed rate	
CLOSE COULTERS 1 and 24	
Set Pressures High	
WHEEL MARK ERADICATORS DOWN	
Set Spirit for Shallow.	
Go to Spare 2	SPARE 2 - Shallow
Drill	
Go to Plot 9	RTF SHALLOW
Drill	
Go to Plot 13	RTF SHALLOW
Drill	
Go to Plot 23	RTF SHALLOW
Drill	
Go to Plot 33	RTF SHALLOW
Drill	
Set Spirit for Deep.	
Go to Spare 1	SPARE 1 - Deep
Drill	
Go to Plot 1	RTF DEEP
Drill	
Go to Plot 18	RTF DEEP
Drill	
Go to Plot 25	RTF DEEP
Drill	
Go to Plot 31	RTF DEEP
Drill	
WHEEL MARK ERADICATORS UP	
Set Spirit for ZERO. Drill 124 kg/ ha	
Go to Spare 3	SPARE 3 - Zero
Drill on the whole length up to the end of block 3	
Go to Plot 6	RTF ZERO
Drill	
Go to Plot 15	RTF ZERO
Drill	
Go to Plot 20	RTF ZERO
Drill	
Go to Plot 29	RTF ZERO
Drill	
Draft force gauge settings:	
Full bridge	
Custom unit 0.00221mv/v	
GF=2.130	
Auto Axis off	

Pressures Low	
KEEP WHEEL MARK ERADICATORS UP	
Continue ZERO tillage. Keep seed rate 124kg/ha	
Go to Plot 3	CTF ZERO
Drill	
Go to Plot 7	LGP ZERO
Drill	
Go to Plot 14	CTF ZERO
Drill	
Go to Plot 16	LGP ZERO
Drill	
Go to Plot 24	CTF ZERO
Drill	
Go to Plot 26	LGP ZERO
Drill	
Go to Plot 30	LGP ZERO
Drill	
Go to Plot 34	CTF ZERO
Drill	
WHEEL MARK ERADICATORS DOWN	
Set Spirit for Shallow.	
Go to Plot 5	LGP SHALLOW
Drill	
Go to Plot 8	CTF SHALLOW
Drill	
Go to Plot 11	CTF SHALLOW
Drill	
Go to Plot 12	LGP SHALLOW
Drill	
Go to Plot 19	CTF SHALLOW
Drill	
Go to Plot 27	LGP SHALLOW
Drill	
Go to Plot 28	CTF SHALLOW
Drill	
Go to Plot 35	LGP SHALLOW
Drill	
Set Spirit for Deep. Drill 124kg/ha	
Go to Plot 2	LGP DEEP
Drill	
Go to Plot 4	CTF DEEP
Drill	
Go to Plot 10	LGP DEEP
Drill	
Go to Plot 17	CTF DEEP
Drill	
Go to Plot 21	LGP DEEP
Drill	
Go to Plot 22	CTF DEEP
Drill	
Go to Plot 32	LGP DEEP
Drill	
Go to Plot 36	CTF DEEP
Drill	

Table A3.4. – All the crops since the beginning of the experiment (after Smith, 2017; Millington,, 2019 and Kaczorowska–Dolowy, 2022).

Crop	Variety	Date of Drilling	Target seed rate m^{-2}	Target seed rate m^{-2} on Zero tillage plots	Combine harvest date
Winter wheat	Duxford	09/11/2012	325	325	01/09/2013
Winter barley	Cassia	26/09/2013	No data	No data	22/09/2014
Winter barley	Cassia	20/10/2014	No data	No data	27/08/2015
Cover crop *TerraLife-N-Fixx*	n/a	03/09/2015	n/a	n/a	n/a
Spring oats	Aspen	25/04/2016	350	450	08/09/2016
Spring wheat	Mulika	04/04/2017	400	520	29/08/2017
Winter bean	Tundra	10/22/2017	22	28	10/08/2018
Winter wheat	Graham	05/10/2018	325	417	27/08/2019
Winter barley	Orwell	23/10/2029	400	500	29/07/2020

12.2 FOR CHAPTER 4. APPENDIX 4.

Figure A4.13 – Main effects of the Traffic and Tillage treatments on SOM over time at 0-10 cm depth. Lines show means (n=12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors. The interaction between Traffic and collection date was not statistically significant ($p = 0.27$).

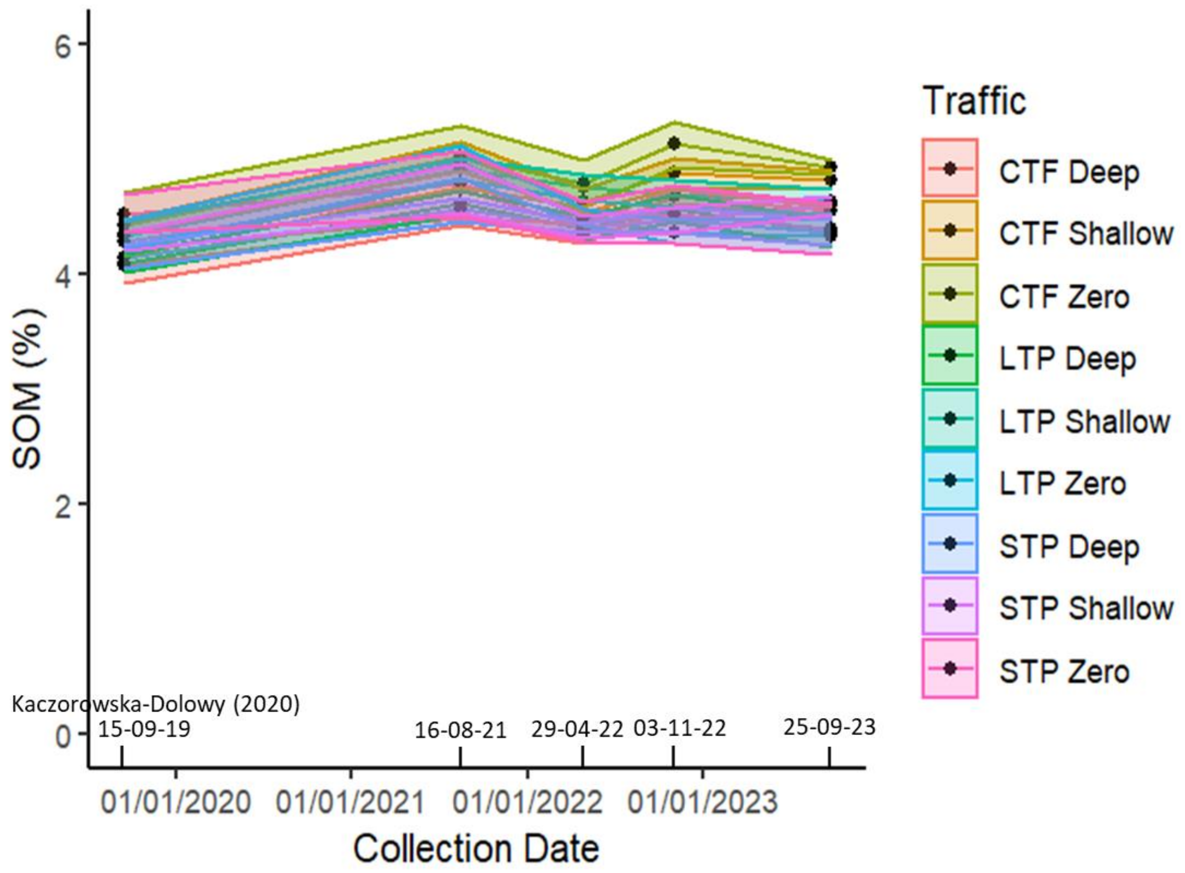
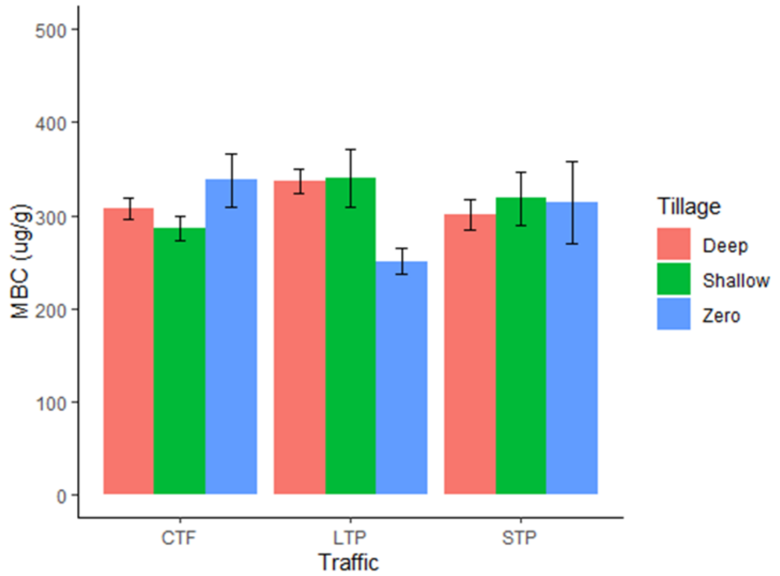


Table A4. 1. - Represents the results of SOM at 0-10 cm for ANOVA (mixed effects model with Traffic-Tillage and Collection date as fixed effects and block as a random effect). This analysis includes data from Kaczorowska-Dolowy (2020). The interaction between the Traffic and Tillage systems and the Collection dates was not statistically significant (p=0.27).

```
lme1 <- lmer(SOM ~ Traffic-Tillage * Collection + (1|Block), data = data)
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                    Sum Sq Mean Sq  NumDF DenDF  F value   Pr(>F)
Traffic-Tillage     4.3541 0.54427    8   132 10.1016   6.308e-11 ***
Collection          4.7577 1.18944    4   132 22.0759   5.715e-14 ***
Traffic:Collection  2.0056 0.06267   32   132  1.1632    0.2724
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure A. 4.2. MBC and its statistical analysis ANOVA, 0-10 cm




```
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                 Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
Tillage          1599.1   799.5   2    24  0.5335    0.59335
Traffic            20.3    10.1   2    24  0.0068    0.99327
Tillage:Traffic 25087.0  62714        24  4.1848    0.01036 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

<u>*Pos hoc* Traffic*Tillage</u>

```
Pos-hoc
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

$contrasts
 contrast                   estimate   SE df t.ratio p.value
 Zero CTF - Shallow CTF        51.65 27.4 24   1.887  0.6283
 Zero CTF - Deep CTF           30.77 27.4 24   1.124  0.9645
 Zero CTF - Zero LTP           87.47 27.4 24   3.196  0.0769
 Zero CTF - Shallow LTP        -2.07 27.4 24  -0.076  1.0000
 Zero CTF - Deep LTP            1.19 27.4 24   0.044  1.0000
 Zero CTF - Zero STP           24.27 27.4 24   0.887  0.9917
 Zero CTF - Shallow STP        19.86 27.4 24   0.726  0.9978
 Zero CTF - Deep STP           37.26 27.4 24   1.361  0.9014
 Shallow CTF - Deep CTF       -20.88 27.4 24  -0.763  0.9970
 Shallow CTF - Zero LTP        35.82 27.4 24   1.309  0.9190
 Shallow CTF - Shallow LTP    -53.72 27.4 24  -1.962  0.5811
```

```
 Shallow CTF - Deep LTP      -50.46 27.4 24  -1.843  0.6553
 Shallow CTF - Zero STP      -27.38 27.4 24  -1.000  0.9822
 Shallow CTF - Shallow STP   -31.79 27.4 24  -1.161  0.9572
 Shallow CTF - Deep STP      -14.39 27.4 24  -0.526  0.9998
 Deep CTF - Zero LTP          56.70 27.4 24   2.071  0.5134
 Deep CTF - Shallow LTP      -32.84 27.4 24  -1.200  0.9488
 Deep CTF - Deep LTP         -29.58 27.4 24  -1.080  0.9717
 Deep CTF - Zero STP          -6.50 27.4 24  -0.237  1.0000
 Deep CTF - Shallow STP      -10.91 27.4 24  -0.398  1.0000
 Deep CTF - Deep STP           6.49 27.4 24   0.237  1.0000
 Zero LTP - Shallow LTP      -89.54 27.4 24  -3.271  0.0657
 Zero LTP - Deep LTP         -86.28 27.4 24  -3.152  0.0841
 Zero LTP - Zero STP         -63.20 27.4 24  -2.309  0.3753
 Zero LTP - Shallow STP      -67.61 27.4 24  -2.470  0.2941
 Zero LTP - Deep STP         -50.21 27.4 24  -1.834  0.6608
 Shallow LTP - Deep LTP        3.26 27.4 24   0.119  1.0000
 Shallow LTP - Zero STP       26.34 27.4 24   0.962  0.9860
 Shallow LTP - Shallow STP    21.93 27.4 24   0.801  0.9957
 Shallow LTP - Deep STP       39.33 27.4 24   1.437  0.8724
 Deep LTP - Zero STP          23.08 27.4 24   0.843  0.9940
 Deep LTP - Shallow STP       18.67 27.4 24   0.682  0.9986
 Deep LTP - Deep STP          36.07 27.4 24   1.318  0.9162
 Zero STP - Shallow STP       -4.41 27.4 24  -0.161  1.0000
 Zero STP - Deep STP          12.99 27.4 24   0.474  0.9999
 Shallow STP - Deep STP       17.40 27.4 24   0.636  0.9992

Degrees-of-freedom method: kenward-roger
P value adjustment: tukey method for comparing a family of 9 estimates
```

Appendix 4.3. MBC (0-10 cm) 4th soil sample collection. Statistical analysis:

```
lme1 <- lmer(MBC ~ Tillage * Traffic + (1|Block), data = data)
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
Tillage         2960.1  1480.1     2    24  0.9648  0.3953
Traffic         5315.8  2657.9     2    24  1.7327  0.1982
Tillage:Traffic 9290.2  2322.5     4    24  1.5140  0.2297
```

12.3. FOR CHAPTER 5. APPENDIX 5.

A.5. EFFECTS OF TRAFFIC AND TILLAGE ON SOIL SOC CONCENTRATION

A.5.1 SOC CONCENTRATION AT 0-10 CM DEPTH

The main effect of traffic ($p < 0.001$), tillage ($p < 0.001$) and the interaction between traffic and tillage ($p < 0.001$) on SOC concentration were all statistically significant.

Within the traffic systems, CTF (21.75 g/kg, CV = 7.46%) had significantly higher SOC concentration compared to LTP (20.61 g/kg, CV = 6.55%) and STP (20.24 g/kg, CV = 8.50%). CTF contained a 5.5% higher content of SOC compared to LTP and 7.5% higher than STP (Fig. 5.1).

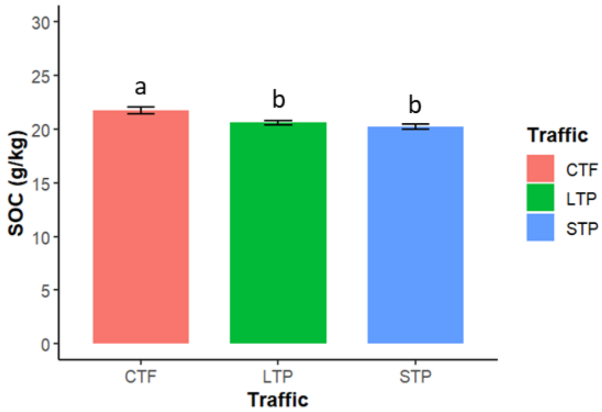


Figure 5. 1 – Main effects of the different traffic systems on SOC concentration at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

Within tillage treatments, Zero (21.13 g/kg, CV = 8.75%) and Shallow (21.28 g/kg, CV = 7.30%) had significantly higher SOC concentration compared to Deep (20.18 g/kg, CV = 6.46%) tillage (Fig. 5. 2). Zero and Shallow tillage systems contained a 5.1% higher SOC concentration than Deep tillage systems.

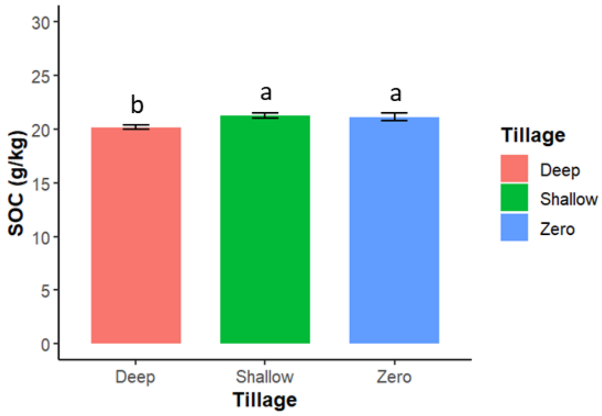


Figure 5. 2 – Main effects of the different tillage systems on SOC concentration at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 48). Bars show standard errors.

There was a significant interaction between the traffic and tillage treatments. CTF Zero (23.19 g/kg, CV = 9.50%) had the highest SOC concentration, which was significantly higher compared to the other treatment combinations. CTF Shallow (21.62 g/kg, CV = 6.59%) had significantly higher SOC concentration compared to LTP Deep (19.93 g/kg, CV = 7.04%) and STP Zero (19.73 g/kg, CV = 9.89%). And LTP Shallow (21.42 g/kg, CV = 5.74%) had significantly higher SOC concentration than STP Zero (19.73 g/kg, CV = 9.89%) (Fig. 5.3).

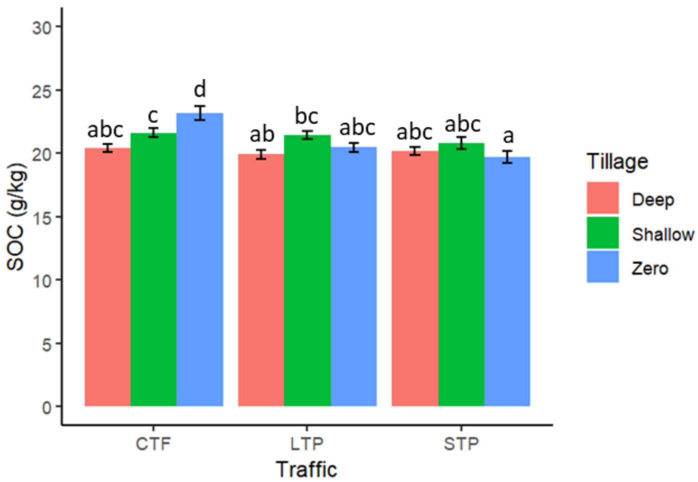


Figure 5. 3 – Main effects of the different traffic and tillage systems on SOC concentration at 0-10 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 16). Bars show standard errors.

A.5.1.1. SOC CONCENTRATION AT 0-10 CM OVER TIME

The effects of traffic over time: all, traffic (p <0.001), collection date (p = 0.02) and the interaction between traffic and collection date (p = 0.02) led to significant differences in SOC concentrations within the top depth of soil (0-10 cm). Within the Traffic systems, the observed results were the same as the main traffic effects above (Fig. 5. 1).

When comparing traffic effects across within collection dates, the collection on 03/11/2022 (21.25 g/kg, CV = 7.82%) and 25/09/2023 (21.23 g/kg, CV = 7.47%) had significantly higher SOC concentration when compared to the collection on 29/04/2022 (20.30 g/kg, CV = 6.81%).

When comparing the effects of the interaction between traffic and collection date, CTF on 25/09/2023 (22.85 g/kg, CV = 6.64%) and CTF on 03/11/2022 (22.64 g/kg, CV = 8.04%) had significantly more SOC concentration compared to all the other treatment combinations. And CTF on 03/11/2022 (22.64 g/kg, CV = 8.04%) had significantly higher SOC concentration compared to LTP on 29/04/2022 (22.64 g/kg, CV = 6.92%), STP on 25/09/2023 (20.49 g/kg, CV = 8.06%), STP on 16/08/2021 (20.46 g/kg, CV = 11.43%), STP on 03/11/2022 (20.31 g/kg, CV=9.47%), LTP on 25/09/2023 (20.33 g/kg, CV = 7.73%) and STP on 29/04/2022 (19.69 g/kg, CV = 5.44%) (Fig. 5. 4).

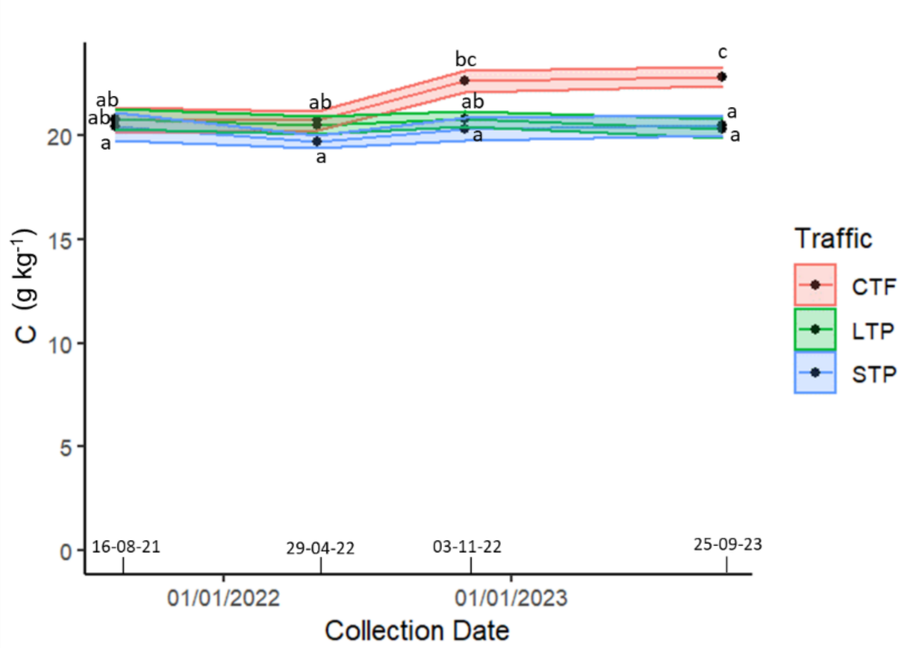


Figure 5. 4 – Main effects of the traffic treatments on SOC concentration over time at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors.

The effects of tillage over time: at 0-10 cm, both tillage (p = 0.002) and collection date (p = 0.03) had significant effects on SOC concentration, but the interaction between tillage and collection date (p = 0.90) was not statistically significant (Fig. 5. 5).

Within the tillage systems, the results were the same as the main tillage effects above.

The *Pos-hoc* analysis between collection dates showed borderline not significant differences between dates (p = 0.06 and p = 0.08).

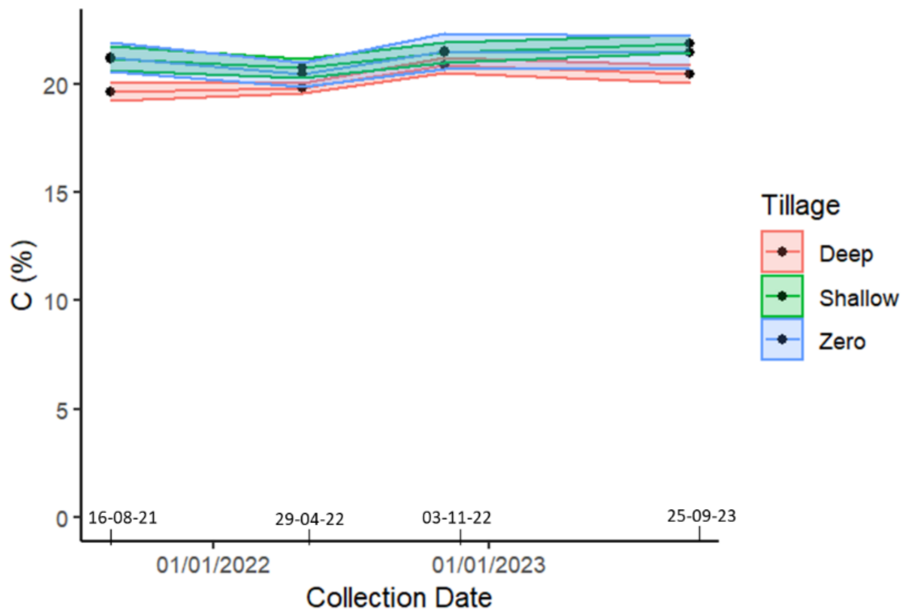


Figure 5. 5 – Main effects of the tillage treatments on SOC content over time at 0-10 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors.

The interaction between traffic and tillage over time: at 0-10 cm there was a significant traffic-tillage (p < 0.01) and significantly changed over time (i.e. collection date; p = 0.004). However, the interaction between traffic-tillage and collection date (p = 0.12) was not statistically significant (Fig. 4. 27).

Withing the traffic-tillage interaction the observed results were the same as the main effects of the traffic-tillage interaction above.

Across the Collection dates, the sampled collection on 03/11/2022 (21.25 g/kg, CV = 7.82%) and 25/09/2023 (21.23 g/kg, CV = 7.47%) had significant average higher SOC concentration than 29/04/2022 (20.30 g/kg, CV = 6.81%) and 16/08/2021 (20.67 g/kg, CV = 8.03%).

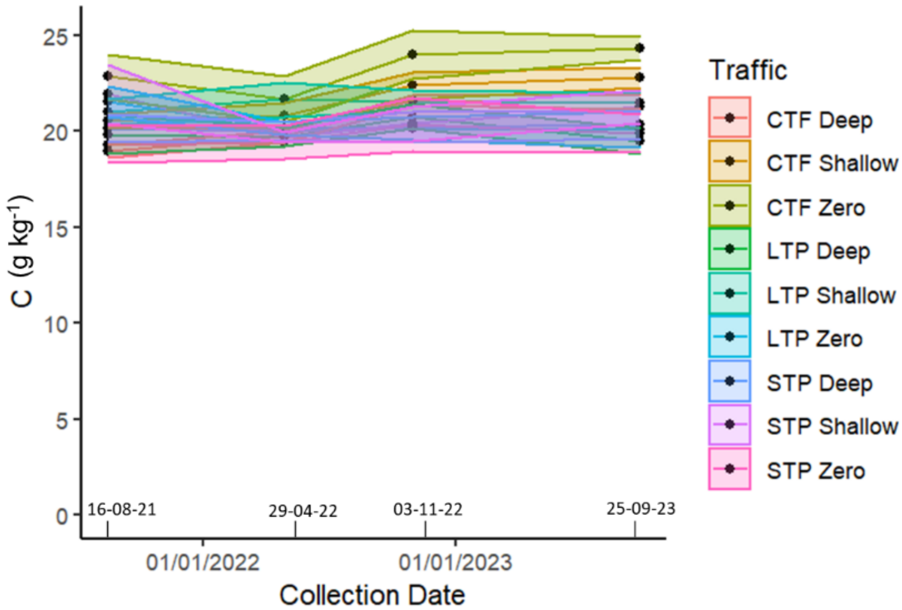


Figure 5. 6 – Main effects of the traffic-tillage interaction on SOC over time at 0-10 cm depth. Lines show means (n = 4). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors.

A. 5. 2. SOC CONCENTRATION AT 10-20 CM DEPTH

There were no statistically significant changes in SOC concentration at 10-20 cm depth (traffic p=0.36; tillage p = 0.89; the interaction between traffic and tillage p = 0.09) (Fig. 5. 7). The average SOC concentration was 17.17 g/kg.

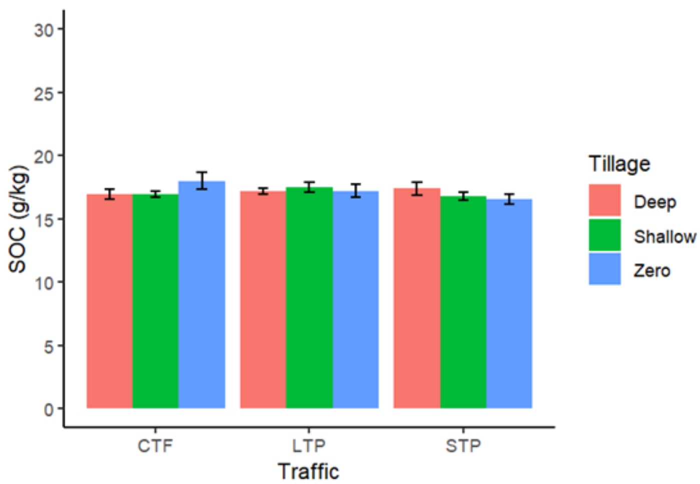


Figure 5. 7 – Main effects of the different traffic and tillage systems on SOC concentration at 10-20 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 16). Bars show standard errors.

A.5.2.1 SOC CONCENTRATION AT 10-20 CM OVER TIME

The effects of traffic over time: there were not statistically significant changes in SOC concentration at 10-20 cm depth across Collection dates (p = 0.18), Traffic (p = 0.37) and the interaction between traffic and collection (p = 0.60) (Fig. 5. 8).

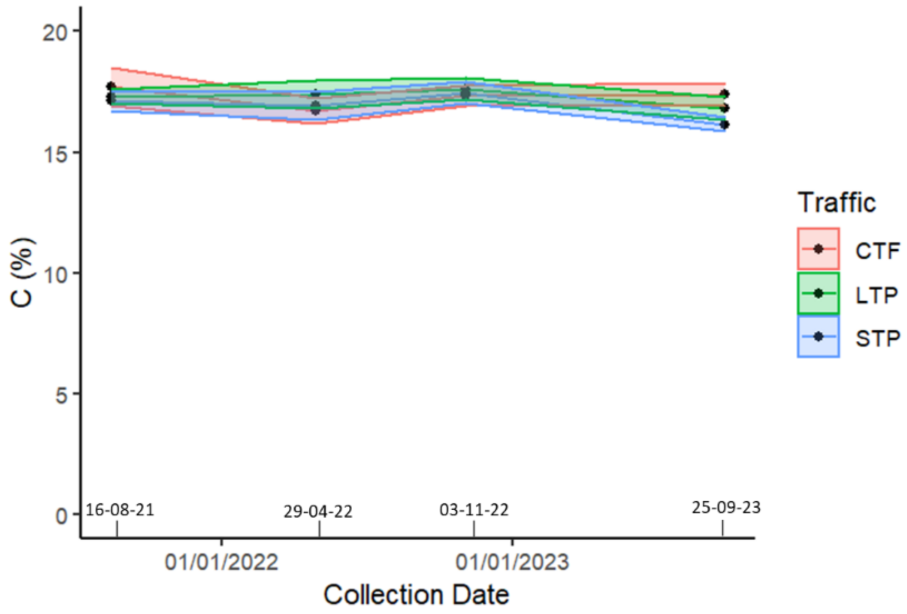


Figure 5. 8 – Main effects of the traffic treatments on SOC concentration over time at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors.

The effects of tillage over time: there were no statistically significant changes in SOC concentration at 10-20 cm depth across collection dates (p = 0.18), tillage (p = 0.89) and the interaction between tillage and collection date (p = 0.16) (Fig. 5. 9).

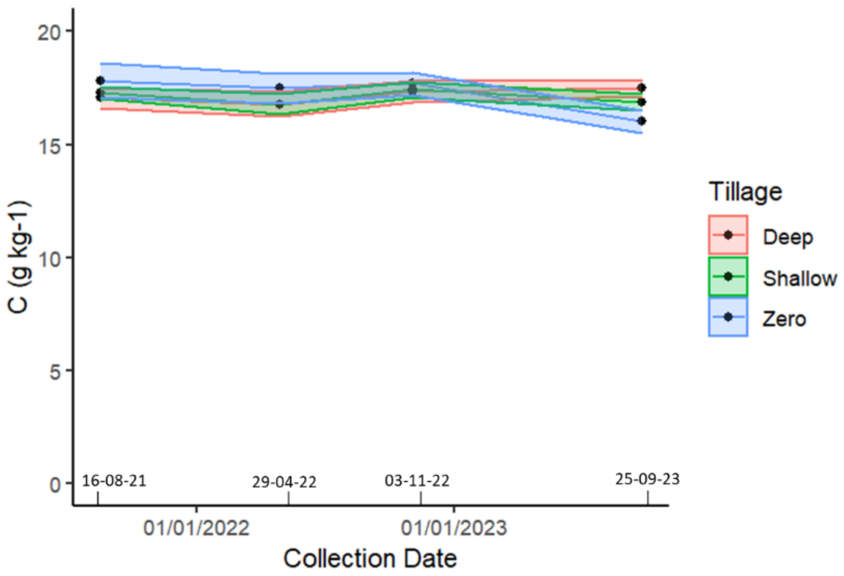


Figure 5. 9 – Main effects of the tillage treatments on SOC concentration over time at 10-20 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors.

The effects of the traffic-tillage interaction over time: there were no statistically significant changes in SOC concentration at 10-20 cm across collection dates ($p = 0.17$), traffic-tillage ($p = 0.25$), and the interaction between traffic-tillage and Collection date ($p = 0.40$) (Fig. 5.10).

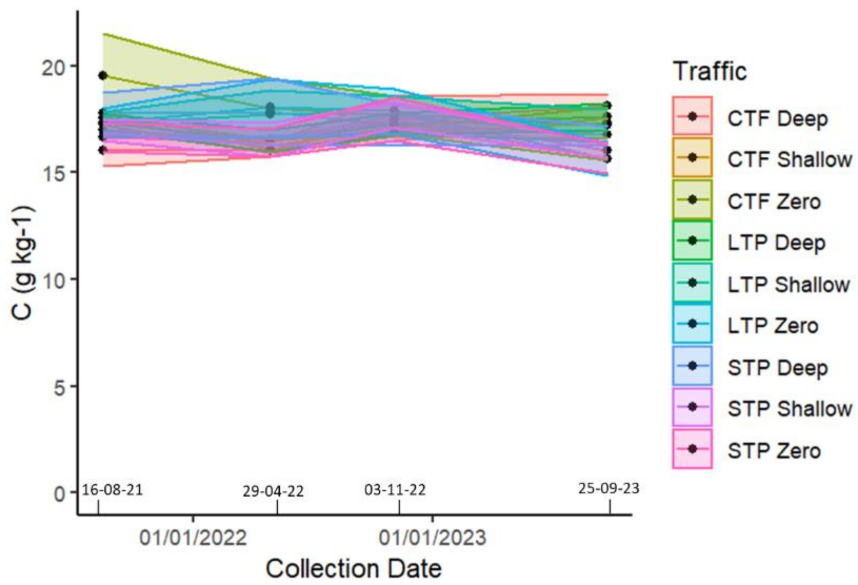


Figure 5. 10 – Main effects of the traffic-tillage interaction on SOC concentration over time at 10-20 cm over depth. Lines show means (n = 4). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors.

A. 5.3. SOC CONCENTRATION AT 20-30 CM DEPTH

Significant differences in SOC concentration were observed at 20-30 cm only for the interaction between traffic and tillage (p = 0.005) (Fig. 5.11). Traffic (p = 0.52) and tillage (p = 0.09) did not have a statistically significant effect on SOC content at this depth. The average SOC was 13.69 g/kg.

Within the interactions between traffic and tillage treatments; CTF Zero (14.92 g/kg, CV = 9.12%) tillage had significantly higher SOC concentration when compared to CTF Shallow (13.08 g/kg, CV = 15.17%) and STP Deep (12.95 g/kg, CV = 9.62%) (Fig. 5. 11).

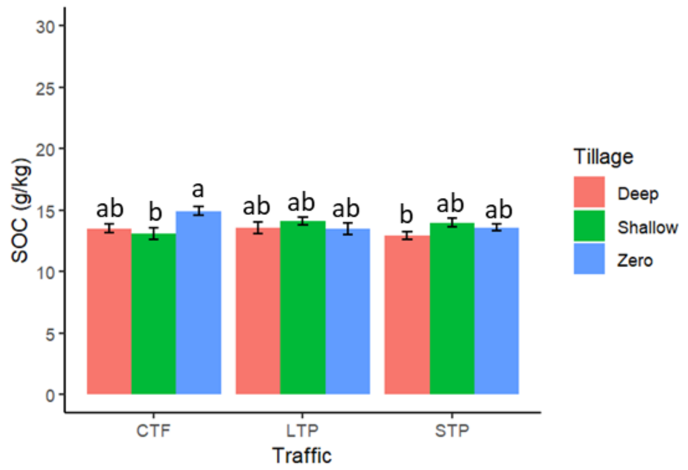


Figure 5. 11 – Soil organic carbon concentration at 20-30 cm depth. Data from four soil sample collection events. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 16). Bars show standard errors.

A.5.3.1 SOC CONCENTRATION 20-30 CM OVER TIME

The effects of traffic over time: significant differences in SOC concentration were observed at 20-30 cm across the collection date (p < 0.001) (Fig. 5.12). Traffic (p =0.52) and the interaction between traffic and collection date (p = 0.31) were not statistically significant.

Across the collection dates, the collection on 25/09/2023 had significantly higher SOC concentration (14.37 g/kg, CV = 8.66%) compared to the collection on 29/04/2022 (12.92 g/kg, CV = 12.20%).

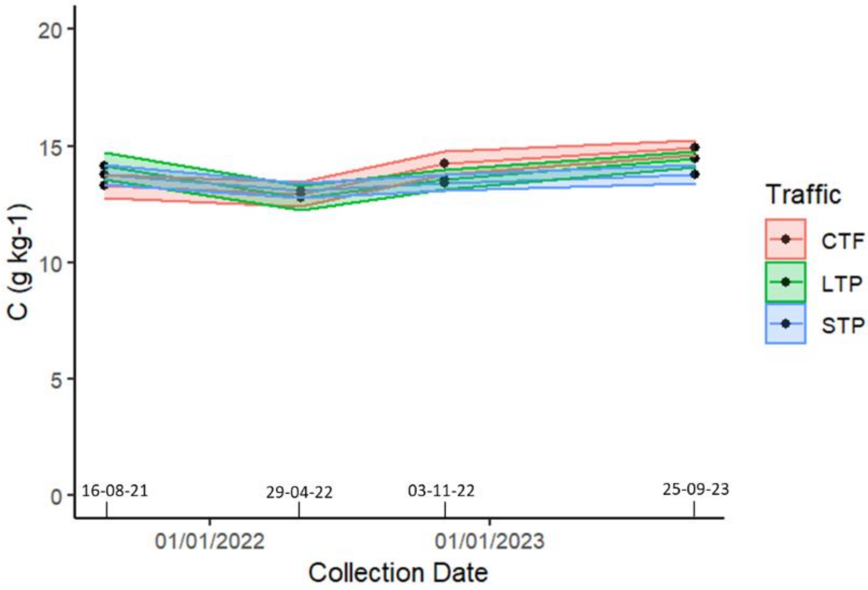


Figure 5. 12 – Main effects of the traffic treatments on SOC concentration over time at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on (p < 0.05). Ribbons show standard errors.

The effects of tillage over time: significant differences in SOC concentration were observed at 20-30 cm across the collection date (p < 0.001) (Fig. 5. 13). Tillage (p = 0.08) was borderline not statistically significant and the interaction between tillage and collection date (p = 0.16) was not statistically significant.

Across the Collection dates, the observed results were the same as above for Traffic over time.

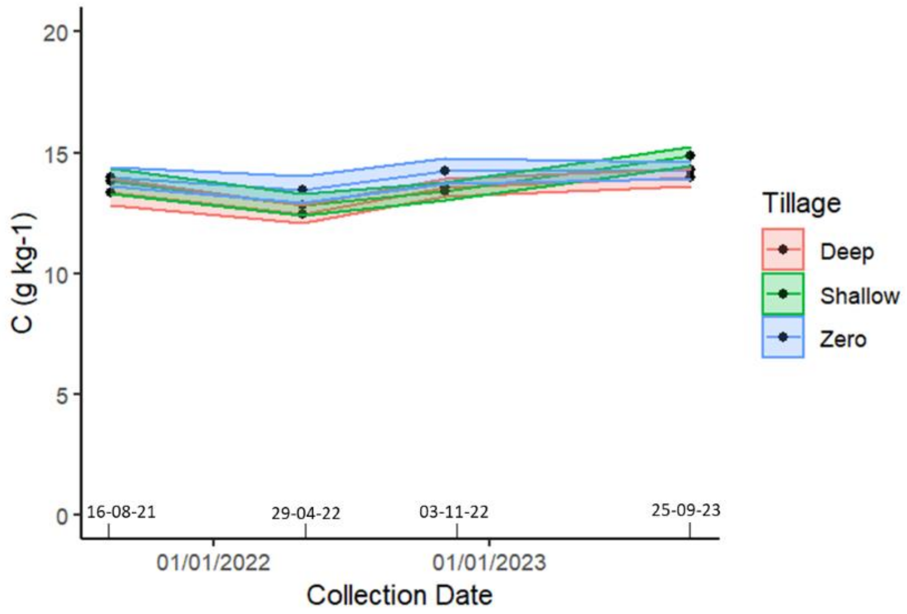


Figure 5. 13 – Main effects of the tillage treatments on SOC concentration over time at 20-30 cm depth. Lines show means (n = 12). Letters indicate significant differences based on ($p < 0.05$). Ribbons show standard errors.

The effects of the traffic-tillage interaction over time: The interaction between traffic and tillage was statistically significant at 20-30 cm depth ($p = 0.003$). There were significant changes in SOC concentration across Collection date ($p < 0.001$). However, the interaction between traffic and tillage and collection date was not statistically significant ($p = 0.26$) (Fig. 5. 14).

Within the traffic-tillage interaction, the results were the same as above for the main interaction effects between traffic and tillage (Fig. 5.11).

Across the collection dates, the collection on 25/09/2023 (14.37 g/kg, CV = 8.66%) and the collection on 03/11/22 (13.74 g/kg, CV = 9.45%) had significantly more SOC concentration when compared to the collection on 29/04/2022 (12.92 g/kg, CV = 12.20%).

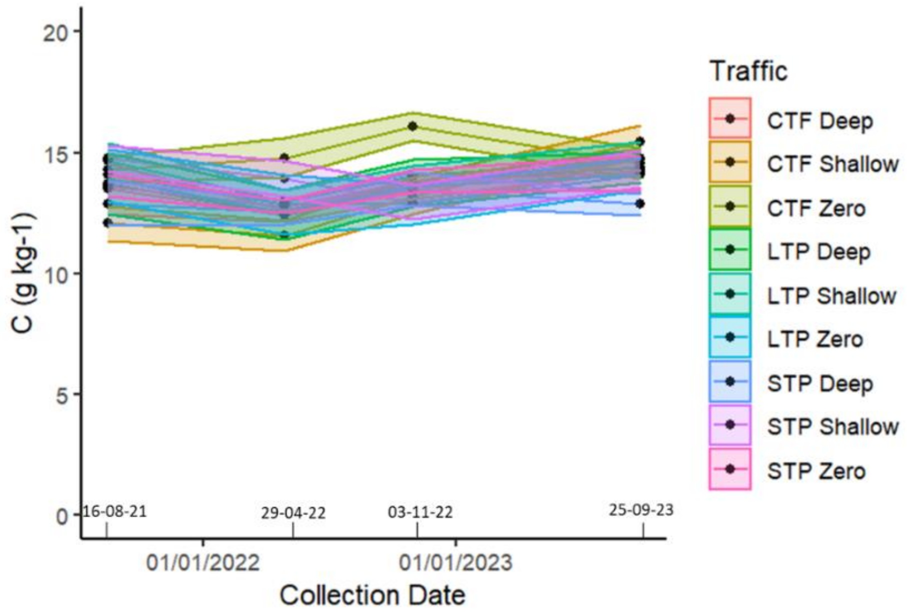


Figure 5. 14 – Main effects of SOC concentration for three traffic systems combined with three tillage systems over four soil sample collections at 0-10 cm depth. Lines show means (n = 4). Ribbons show standard errors.

A. 5. 4 SOC CONCENTRATION AT 0-30 CM DEPTH

The main effects of traffic ($p < 0.001$), tillage ($p = 0.01$) and depth ($p < 0.001$) were all significant. The interaction effects between traffic and tillage ($p < 0.001$), traffic and depth ($p = 0.01$) and tillage and depth ($p = 0.05$) were also significant. However, the interaction effect between traffic, tillage and depth ($p = 0.24$) was not significant.

Within the traffic systems, CTF (17.63 g kg^{-1}, CV = 20.86%) had significantly higher SOC concentration than LTP (17.20 g/kg, CV = 18.75%) and STP (16.89 g/kg, CV = 18.78%) (Fig. 5. 15).

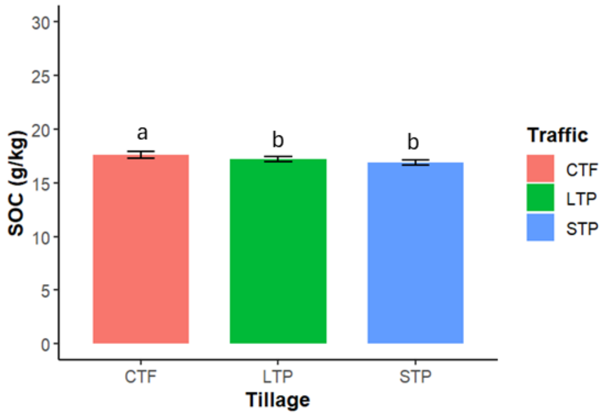


Figure 5. 15 – Main effects of the different traffic systems on SOC concentration at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 144). Bars show standard errors.

Within the Tillage systems, Zero (17.46 g/kg, CV = 19.72%) and Shallow (17.36 g/kg, CV = 19.88%) tillage had significantly higher SOC concentration than Deep (16.90 g/kg, CV = 18.79%) tillage systems (Fig. 5. 16).

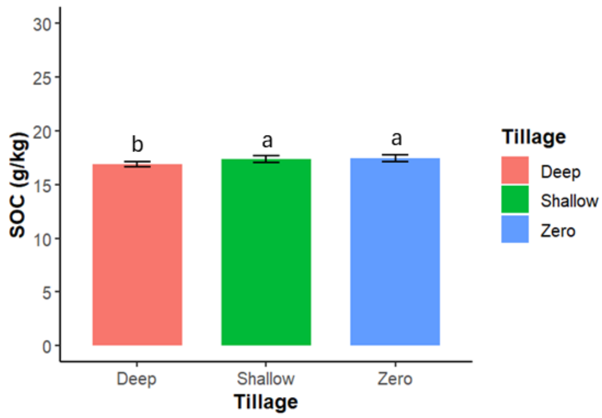


Figure 5. 16 – Main effects of the different tillage systems on SOC concentration at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on (p < 0.05). Columns show means (n = 144). Bars show standard errors.

Within the different soil depth layers, SOC was significantly greater at 0–10 cm depth (20.86 g/kg, CV = 8.36%) than 10–20 cm depth (17.16 g/kg, CV = 9.86%), which was, in turn, significantly greater than at 20–30 cm depth (13.69 g/kg, CV = 11.53%) (Fig. 5. 17).

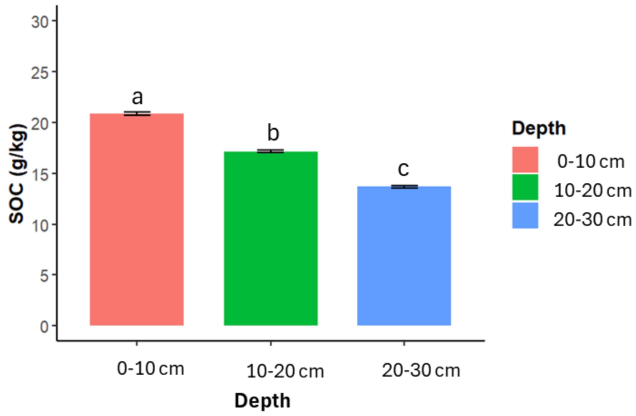


Figure 5. 17 – Main effects of the different soil depth layers on SOC concentration at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 144). Bars show standard errors.

Within the interaction between traffic and tillage systems, CTF Zero (18.70 g/kg, CV = 21.65%) had significantly higher SOC concentration than the rest of the treatment combinations (17.06 g/kg, CV = 19.19%). And LTP Shallow tillage had significantly more SOC concentration compared to STP Zero (16.63 g/kg, CV= 20.32%). CTF Zero tillage had 8.7% more SOC concentration than the rest of the treatment combinations. And LTP Shallow tillage had 3.9% more SOC concentration than the rest of the treatment combinations (apart from CTF Zero) (Fig. 5. 19).

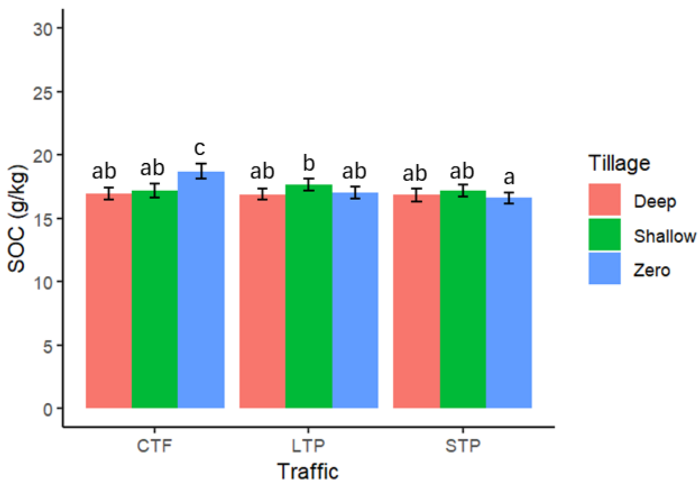


Figure 5. 19 – Main effects of the different traffic and tillage systems on SOC concentration at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p < 0.05$). Columns show means (n = 48). Bars show standard errors.

The effects of the interaction between traffic with depth over 0-30 cm: traffic ($p <$ 0.001), depth ($p < 0.001$) and the interaction between traffic and depth ($p = 0.02$) were significant.

Within traffic systems, CTF (17.63 g/kg, CV = 11.02%) had significantly higher SOC concentration compared to STP (16.89 g/kg, CV = 9.24%).

Within traffic systems at the different soil depth layers, CTF (21.75 g/kg, CV = 9.22%) at 0-10 cm was significantly higher than all the other traffic systems and soil depths. LTP (20.61 g/kg, CV = 7.07%) and STP (20.24 g/kg, CV = 8.78%) at 0-10 cm were significantly higher than all the traffic systems at 10-20 cm (avg. 17.16 g/kg, CV = 9.86%) and they were in turn, significantly higher than all the traffic systems at 20-30 cm (avg. 13.69 g/kg, CV = 11.53%) (Fig. 5. 20).

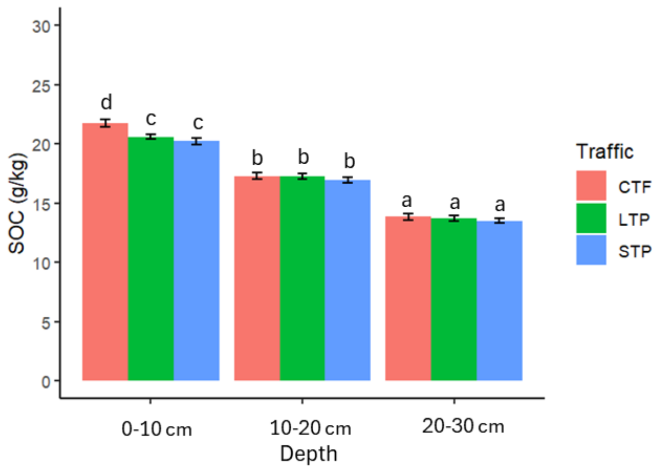


Figure 5. 20 – Main effects of the different traffic systems over different depth layers on SOC concentration at 0-30 cm. Data from 2021-2023. Letters indicate significant differences based on ($p <$ 0.05). Columns show means (n = 48). Bars show standard errors.

A. 5.5. DIFFERENCES IN SOC CONCENTRATIONS USING A 4MM AND A 2 MM SIEVE

To assess potential differences in SOC concentrations between the 4 mm (first soil sample collection) and 2 mm (rest of the soil collections) sieved soils, both sieved soil fractions were analysed in the final sampling collection.

Table 5.1. - Represents the results of SOC at 0-10 cm for ANOVA (mixed effects model with traffic, tillage and collection (4mm and 2 mm sieved sizes) as fixed effects and block as a random effect). At 0-10 cm depth, a marginally non-significant interaction ($p = 0.08$) was observed between the traffic-tillage systems and sieve sizes (collections).

```
lme1 <- lmer(TC ~ Tillage * Traffic * Collection + (1|Block), data = data)
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                             Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
Tillage                       8.437  4.218     2 51.043  2.7057    0.07641 .
Traffic                      71.558 35.779     2 51.043 22.9484    7.773e-08 ***
Collection                    2.485  2.485     1 51.043  1.5941    0.21247
Tillage:Traffic              16.279  4.070     4 51.043  2.6103    0.04615 *
Tillage:Collection            7.399  3.700     2 51.043  2.3729    0.10342
Traffic:Collection            2.518  1.259     2 51.043  0.8075    0.45159
Tillage:Traffic:Collection   13.775  3.444     4 51.043  2.2087    0.08104 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 5.2. - Represents the results of SOC at 10-20 cm for ANOVA (mixed effects model with traffic, tillage and collection (4mm and 2 mm sieved sizes) as fixed effects and block as a random effect). At 10-20 cm depth, the interaction between the Traffic and Tillage systems and the sieved sizes (collections) was not statistically significant (*p* = 0.83).

```
lme1 <- lmer(TC ~ Tillage * Traffic * Collection + (1|Block), data = data)
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                             Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
Tillage                      30.164 15.0821    2 51.039  9.7356    0.000262 ***
Traffic                      46.211 23.1055    2 51.039 14.9148    7.929e-06 ***
Collection                    0.501  0.5015    1 51.039  0.3237    0.571876
Tillage:Traffic               5.140  1.2851    4 51.039  0.8296    0.512606
Tillage:Collection            1.009  0.5044    2 51.039  0.3256    0.723604
Traffic:Collection            9.166  4.5829    2 51.039  2.9583    0.060868 .
Tillage:Traffic:Collection    2.218  0.5544    4 51.039  0.3579    0.837352
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 5. 3. - Represents the results of SOC at 20-30 cm for ANOVA (mixed effects model with traffic, tillage and collection (4mm and 2 mm sieved sizes) as fixed effects and block as a random effect). At 20-30 cm depth, the interaction between the traffic and tillage systems and the sieved sizes (collections) was not statistically significant (p = 0.99).

```
lme1 <- lmer(TC ~ Tillage * Traffic * Collection + (1|Block), data = data)
> anova(lme1)
Type III Analysis of Variance Table with Satterthwaite's method
                          Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)
Tillage                   5.1645  2.5822     2 51.004  3.2044   0.04887 *
Traffic                  19.0886  9.5443     2 51.004 11.8439 5.958e-05 ***
Collection                4.1873  4.1873     1 51.004  5.1962   0.02685 *
Tillage:Traffic           6.3850  1.5963     4 51.004  1.9809   0.11143
Tillage:Collection        1.2000  0.6000     2 51.004  0.7445   0.48004
Traffic:Collection        1.1306  0.5653     2 51.004  0.7015   0.50056
Tillage:Traffic:Collection 0.1876 0.0469     4 51.004  0.0582   0.99352
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

A.5.6. SOIL PH

Table 5. 4. - Results for the soil pH at 0-30 cm for the first soil sample collection (17/08/2021). pH (mean 5.4) at 0-30 cm.

				1st soil sample collection		
Block	Block	Traffic	Tillage	Plot	Depth	pH
1	1	STP	Deep	P 1	1	5.49
	1	STP	Deep	P 1	2	5.24
	1	STP	Deep	P 1	3	5.61
1	1	LTP	Deep	P 2	1	5.25
	1	LTP	Deep	P 2	2	5.14
	1	LTP	Deep	P 2	3	5.71
1	1	CTF	Zero	P 3	1	4.98
	1	CTF	Zero	P 3	2	5.15
	1	CTF	Zero	P 3	3	5.55
1	1	CTF	Deep	P 4	1	4.69
	1	CTF	Deep	P 4	2	5.08
	1	CTF	Deep	P 4	3	5.73
1	1	LTP	Shallow	P 5	1	4.92
	1	LTP	Shallow	P 5	2	5.2
	1	LTP	Shallow	P 5	3	5.71
1	1	STP	Zero	P 6	1	5.04
	1	STP	Zero	P 6	2	5.41
	1	STP	Zero	P 6	3	5.42
1	1	LTP	Zero	P 7	1	5.12

	1	LTP	Zero	P 7	2	5.16
	1	LTP	Zero	P 7	3	5.64
1	1	CTF	Shallow	P 8	1	4.64
	1	CTF	Shallow	P 8	2	5.26
	1	CTF	Shallow	P 8	3	5.69
1	1	STP	Shallow	P 9	1	4.86
	1	STP	Shallow	P 9	2	5.41
	1	STP	Shallow	P 9	3	5.73
2	2	LTP	Deep	P 10	1	5.04
	2	LTP	Deep	P 10	2	5.75
	2	LTP	Deep	P 10	3	5.79
2	2	CTF	Shallow	P 11	1	4.65
	2	CTF	Shallow	P 11	2	5.24
	2	CTF	Shallow	P 11	3	5.59
2	2	LTP	Shallow	P 12	1	4.74
	2	LTP	Shallow	P 12	2	5.46
	2	LTP	Shallow	P 12	3	5.73
2	2	STP	Shallow	P 13	1	4.73
	2	STP	Shallow	P 13	2	5.44
	2	STP	Shallow	P 13	3	5.5
2	2	CTF	Zero	P 14	1	4.48
	2	CTF	Zero	P 14	2	4.84
	2	CTF	Zero	P 14	3	5.56
2	2	STP	Zero	P 15	1	4.73
	2	STP	Zero	P 15	2	5.01
	2	STP	Zero	P 15	3	5.39
2	2	LTP	Zero	P 16	1	4.95
	2	LTP	Zero	P 16	2	5.25
	2	LTP	Zero	P 16	3	5.39
2	2	CTF	Deep	P 17	1	4.57
	2	CTF	Deep	P 17	2	4.82
	2	CTF	Deep	P 17	3	5.44
2	2	STP	Deep	P 18	1	4.88
	2	STP	Deep	P 18	2	5.01
	2	STP	Deep	P 18	3	5.72
3	3	CTF	Shallow	P 19	1	5.15
	3	CTF	Shallow	P 19	2	5.01
	3	CTF	Shallow	P 19	3	5.51
3	3	STP	Zero	P 20	1	4.95
	3	STP	Zero	P 20	2	5.3
	3	STP	Zero	P 20	3	5.59
3	3	LTP	Deep	P 21	1	4.8
	3	LTP	Deep	P 21	2	5.31
	3	LTP	Deep	P 21	3	5.94
3	3	CTF	Deep	P 22	1	5.13

	3	CTF	Deep	P 22	2	5.73
	3	CTF	Deep	P 22	3	5.72
3	3	STP	Shallow	P 23	1	4.89
	3	STP	Shallow	P 23	2	5.34
	3	STP	Shallow	P 23	3	5.89
3	3	CTF	Zero	P 24	1	4.84
	3	CTF	Zero	P 24	2	5.42
	3	CTF	Zero	P 24	3	5.44
3	3	STP	Deep	P 25	1	5.34
	3	STP	Deep	P 25	2	5.37
	3	STP	Deep	P 25	3	5.88
3	3	LTP	Zero	P 26	1	4.98
	3	LTP	Zero	P 26	2	5.38
	3	LTP	Zero	P 26	3	5.86
3	3	LTP	Shallow	P 27	1	5.02
	3	LTP	Shallow	P 27	2	5.26
	3	LTP	Shallow	P 27	3	5.61
4	4	CTF	Shallow	P 28	1	5.08
	4	CTF	Shallow	P 28	2	5.49
	4	CTF	Shallow	P 28	3	6.23
4	4	STP	Zero	P 29	1	5.1
	4	STP	Zero	P 29	2	5.85
	4	STP	Zero	P 29	3	6.1
4	4	LTP	Zero	P 30	1	5.22
	4	LTP	Zero	P 30	2	5.59
	4	LTP	Zero	P 30	3	6.01
4	4	STP	Deep	P 31	1	5.17
	4	STP	Deep	P 31	2	5.86
	4	STP	Deep	P 31	3	5.84
4	4	LTP	Deep	P 32	1	5.47
	4	LTP	Deep	P 32	2	5.83
	4	LTP	Deep	P 32	3	6.55
4	4	STP	Shallow	P 33	1	4.93
	4	STP	Shallow	P 33	2	5.95
	4	STP	Shallow	P 33	3	6.04
4	4	CTF	Zero	P 34	1	4.68
	4	CTF	Zero	P 34	2	5.82
	4	CTF	Zero	P 34	3	6.1
4	4	LTP	Shallow	P 35	1	5.65
	4	LTP	Shallow	P 35	2	5.78
	4	LTP	Shallow	P 35	3	6.29
4	4	CTF	Deep	P 36	1	5.21
	4	CTF	Deep	P 36	2	5.16
	4	CTF	Deep	P 36	3	5.83
						5.370556