BELOWGROUND CARBON SEQUESTRATION POTENTIAL OF APPLE TREES

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Abstract

Climate change is altering global weather patterns, threatening food security for the growing global population. Terrestrial soils have the potential to store twice as much carbon (C) as the atmosphere. It is, therefore, essential to explore factors that affect terrestrial C sequestration. Perennial crops such as apple trees sequester C belowground in their roots and surrounding soil and could play an important role in mitigating rising CO_2 levels and maintaining food production.

However, factors influencing belowground C sequestration in apple trees are not fully understood. The overall aim of my thesis was to determine what attributes influence this process. This project explored several factors that may enhance or impede apple tree's capacity to sequester C belowground throughout the orchard's lifecycle. Five factors were identified: 1) rootstock variety, 2) scion variety, 3) increasing atmospheric temperature, 4) orchard age, 5) stored soil C post-grubbing, each with specific experimental aims.

The results showed; 1) Rootstocks were not significantly different in amounts of soil C sequestered. Soil MBC showed significant declines across the three rootstocks between August and September's destructive harvest (P = <0.0001). 2) There were some significant differences between scions in soil total % C, but not in other C fractions. Soil total C significantly increased under COP (P = 0.03) and Dabinett (P = 0.01) over eighteen months for pot grown trees. 3) An increase in UK temperature by 2°C was linked to increased belowground C sequestration across different apple cultivars. The 2°C above ambient tunnel was consistently higher in TC than the ambient tunnel (P between 0.01 and <0.0001), and the 4°C above ambient (P between <0.01 and <0.00001). 4) A decline in most soil C fractions as orchards aged was observed. Most significant declines occurred in orchards 6 years of age and under. 5) After grubbing, an initial significant loss of soil C occurred in the former tree stands during the two months following soil disturbance

(Ruben P = 0.04 and Gala P =0.01), followed by potential recovery if grass re-colonises the former tree stands.

In conclusion, increasing atmospheric temperature by 2°C had a significant positive effect, whereas age of orchards and grubbing had significant negative effect on soil C. Grafted scions could have an impact but require longer-term studies. Rootstocks showed no significant effects on C sequestration across the five months. This study suggested the potential of apple trees to sequester C belowground may not be what is expected, compared with other studies. All these factors must be considered for accurately assessing apple orchards' belowground C sequestration potential, enabling growers to work towards C net zero.

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Declaration of Original Authorship

I declare that this research is my own original work and all citations from other sources have been acknowledged.

Date: Monday 18th March 2024

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Abbreviations

C	Carbon
CO_2	Carbon dioxide
AG	Aboveground
BG	Belowground
GHG's	Greenhouse gases
RZ	Root zone (soil within 1cm of the root)
RS	Rhizosphere (soil attached to the root)
B	Bulk (soil that is not close to the roots)
TC	Total Carbon
TN	Total Nitrogen
TIC	Total inorganic carbon
TOC	Total organic carbon
SOM	Soil organic matter
SOC	Soil organic carbon
POXC	Active carbon (permanganate oxidizable carbon)
MBN	Microbial biomass nitrogen
MBC	Microbial biomass carbon
ATP	Adenosine triphosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
COP	Cox's Orange Pippin
Pg C yr^{-1}	Petagram of carbon per year (1,000,000,000,000 Kg)
Gt C yr^{-1}	Gigatonnes of carbon per year (1,000,000,000,000 Kg)
Tg C yr^{-1}	Teragram (1,000,000,000 Kg)

Chapter 1. Introduction

1.1 Global carbon cycle

Carbon (C) is ubiquitous on Earth, occurring in many forms, which are converted as it is cycled through the atmosphere, oceans, soils, living organisms and dead organic matter, and a significant amount of C is cycled via geological activity (Farsang *et al.*, 2021) (**Fig 1.1**). Soil has been found to be the second largest active C cycling pool after the oceans (Fry, De Long and Bardgett, 2018), which can either be a source or sink of atmospheric carbon dioxide (CO_2). Either atmospheric CO_2 is absorbed by plants (photosynthesis) and exuded into the soil where it is stored (mitigation) or released back into the atmosphere as CO_2 through soil disturbances and soil enzymatic activity. Many studies have shown that terrestrial soils have the potential to store twice as much C than the atmosphere (Lal, 2004; Davidson and Janssens, 2006), with the upper 100 cm of soil being estimated to be able to store as much as 1,584 Petagrams (Pg) globally (Batjes, 1996; Singh *et al.*, 2018; Tiefenbacher *et al.*, 2021). It is believed that soils may be able to store more C than they currently hold, as they are not at full capacity (Stewart *et al.*, 2007; Dignac *et al.*, 2017; Chen *et al.*, 2019; Bossio *et al.*, 2020).

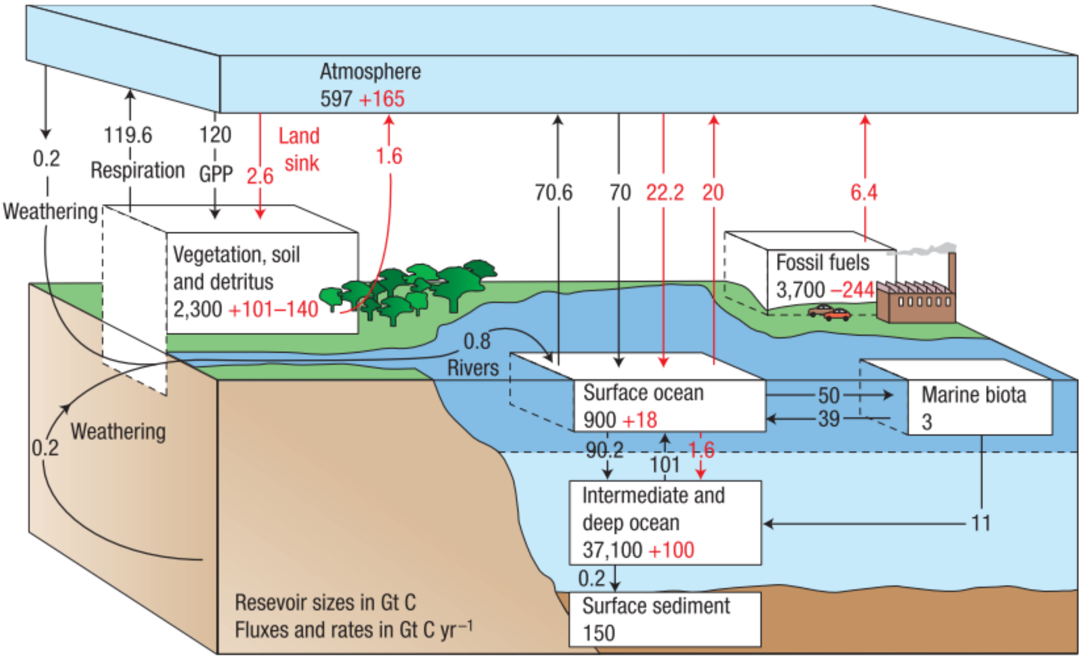


Fig 1.1. Global C cycle showing the estimated amounts of C passing through each stage of the cycle, with natural C flux in black and anthropogenic alterations in red using the units Gigatonnes (Gt) C yr^{-1}. (Taken from Reay *et al.*, 2008).

Since the industrial revolution in the 19th century, C emissions have been added to the atmosphere through anthropogenic activities including the burning of fossil fuels and deforestation. Carbon enters the atmosphere as different compounds, e.g. CO_2 and methane (CH_4) two of the greenhouse gases (GHGs), which also include nitrous oxide (N_2O) and sulphur hexafluoride (SF_6). Carbon can be fixed or stored for long periods in the oceans, rocks, and soil (including peat and the arctic permafrost), but soil disturbance and other human activity leads to CO_2 being released back into the atmosphere (Lenoir, Hattab and Pierre, 2017; Burrell, Evans and De Kauwe, 2020). Atmospheric CO_2 levels are still rising and becoming a major threat to life on this planet, through changing climatic conditions. In August 2023 global CO_2 levels peaked at 419.68 part per million (ppm), a rise of 93 ppm since 1969 (US Department of Commerce, 2023; Ades *et al.*, 2019) (**Fig 1.2)**. An effect of climate change is the increase in global temperatures which is resulting in the thawing of permafrost soils, which release CO_2, back into the atmosphere and has been estimated that in the worst case scenario 112.6 Pg C could be released (Koven *et al.*, 2015).

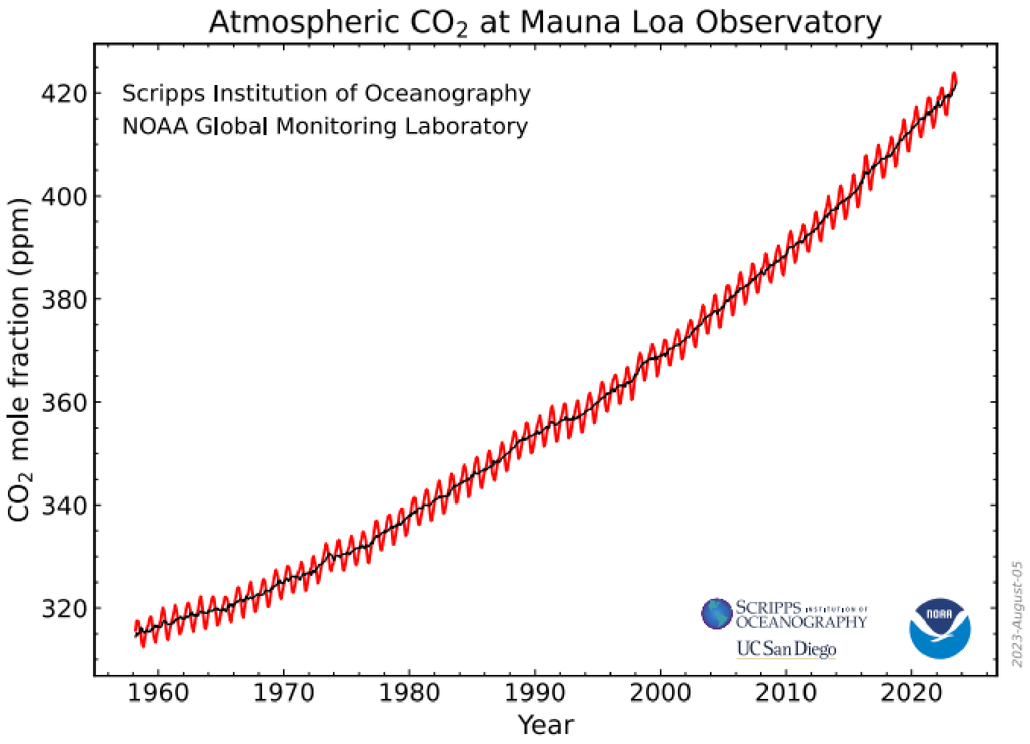


Fig 1.2. Atmospheric CO_2 levels from the Mauna Loa Observatory, Hawaii showing the global monthly mean increases since the 1960's to August 2023 (US Department of Commerce, 2023).

1.2. Terrestrial carbon stores

Different terrestrial environments and their ability of the soil to sequester C belowground is an ongoing area of research, from forests and orchards (George *et al.*, 2012; Wu *et al.*, 2012), grasslands (Moxley *et al.*, 2014; Scott, Baer and Blair, 2017) and cultivated land (Moxley *et al.*, 2014; Haddaway *et al.*, 2016). Previous research to determine amounts of soil C, has tended to focus on the first metre of soil or less, although some have sampled soil from greater depths (up to 500 cm), which are important for deeper rooted plants, including apple trees, which can grow to depths of 6 metres dependent on rootstock (Juan, 1933; Harper and Tibbett, 2013; Zhang *et al.*, 2021; M. Yang *et al.*, 2022). However, some research teams discard the top 10 cm of soil as it's not regarded as a 'good representation' of the C in the soil, because it contains leaf litter and shallow roots (Leinfelder, Merwin and Brown, 2012; Wu *et al.*, 2012). Different soil types, land use, soil pH, and soil moisture can affect C sequestration belowground into the soil and amounts of CO_2 which is released into the atmosphere (**Table 1.1**).

Table 1.1. Estimated carbon sequestration under different land uses.

Land use	**C sequestration potential per year (CO_2)**	**Country**	**Reference**
Peatland	390-455Pg	Wales	(Freeman, Fenner and Shirsat, 2012; Rydin and Jeglum, 2015; Dunn and Freeman, 2018; Fry, De Long and Bardgett, 2018)
Grassland	2.097Pg 0.01-0.30 Gt	UK Globally	(*New research on carbon sequestration and grassland | Farm Carbon Toolkit*, 2020; Ghosh and Mahanta, 2014)
Apple orchards	1.4 to 3.2Pg	China	(Wu *et al.*, 2012)
Data palms	1.748×10^{-11} - 4.001×10^{-11} Pg C ha $^{-1}$	Ethiopia	(Betemariyam and Kefalew, 2022)
Forests	1.25×10^{-13}Pg	India	(Phani Kumar *et al.*, 2010)

Apple, walnut, apricot	2.23x10^{-5}Pg C sequestered across all three.	India	(Phani Kumar *et al.*, 2010)
Hazelnuts orchards	1.3x 10^{-12}–3.3x10^{-12} Pg C ha–^{1} yr-^{1} 1.6x 10^{-11} ± 2.5x 10^{-12} Pg C ha^{-1}.	Italy Italy	(Granata, Bracco and Catoni, 2020; Pacchiarelli *et al.*, 2022)
Green roof systems	3.75x 10^{-13} Pg C·m^{-2}	USA	(Getter *et al.*, 2009)

Freeman et al (2012) stated that “one-third of soil C could be found in peatland,” which covers approximately 3% of the earth’s surface, and can store a higher density of C per unit area compared to other terrestrial systems such as forests or oceans. A report published by Rydin and Jeglum (2015) for the IUCN, estimated that the global amount of C sequestered by peatlands is 0.37 gigatonnes (Gt) of CO_2 each year, more than that of all other vegetation combined. The report discussed that damaging this vital C store releases 5% of global anthropogenic CO_2 per year. Angelo and Du Plessis (2017) discussed how maintaining, managing, and restoring peatland would have an immediate impact on sequestration and would reduce the CO_2 emission which occurs whenever peatland is damaged. This sequestration by peatland can last for centuries, rather than being limited to the life span of a tree, for example.

Soil C is important in maintaining soil health, and this could have significant implications for climate change mitigation, especially against rising atmospheric CO_2. Fry et al (2018) provided a conservative estimate for the global levels of C being stored terrestrially of 900-3000 Gt, with most of this occurring within 1 m depth from the surface. Davis (2018) showed that grasslands could sequester more C belowground than forests and trees as they are more resilient to droughts and wild fires but stated that trees still have a vital role in CO_2 mitigation and the fight against climate change. Pett-Ridge et al (2018) suggested that moving to the use of deeper-rooted plants could help to increase the levels of C being stored in the soil, as well as improving plants drought tolerance, as they would be able to acquire water from deeper in the soil, this contradicts Davis (2018). Perennial crops such as apple trees have been classed as effective at converting large amounts of atmospheric CO_2 into C stored within the plant biomass and soil, than annual crops including flowers (Robertson, Paul and Harwood, 2000), which could help mitigate the rise in atmospheric CO_2.

Forests are amongst the largest terrestrial C sinks (**Fig 1.3**) and have stored up to 30% of C from the atmosphere over the last couple of decades (Bellassen and Luyssaert, 2014). Estimates for orchards varies between studies and the data they are using for their calculations. Genard et al (2008) compared several types of C modelling developed for C allocation for fruit trees, none of which account for the C stored in the soil via root exudates. In an earlier article, Bouma et al (2001) commented that when modelling for C and nutrient cycling, root turnover needs to be included as part of the calculations.

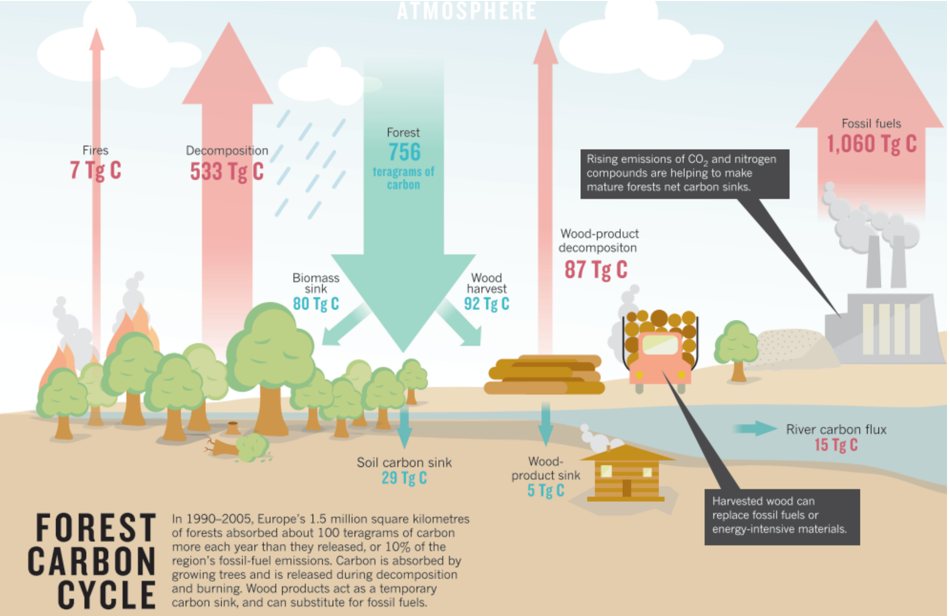


Fig 1.3. Carbon cycle of a forest with numbers (Tg (teragram)) showing the movement of C between different environments (Bellassen and Luyssaert, 2014).

1.2.1. Photosynthesis

Plants are a major pathway for C sequestration belowground through photosynthesis and root exudates into the soil, decomposition of leaf litter, and other plant biomass. Plants can be classified into three groups based on how they use solar radiation to photosynthesize and fix C, which developed from climatic and seasonal variations across the globe (Fry, De Long and Bardgett, 2018). The three types of photosynthesis pathways are; C3 (such as apple trees (*Malus domestica*)), C4 and crassulacean acid metabolism (CAM) (Ehleringer and Cerling, 2002; Meacham and Hensold, 2020). Only the C3 pathway will be discussed further, as this is the mechanism used by apple trees.

C3 plants use the method of photosynthesis known as the Calvin cycle (**Fig 1.4**), which is the dominant form of photosynthesis (85% of plants) across the globe (Burgess and Wang, 2023). It was suggested by Panzacchi et al. (2012) that apple trees use light efficiently in their production of photosynthetic products. Plants absorb atmospheric CO_2 through the stomata on the leaf surface which is then transferred into the mesophyll cells which contains chloroplasts (light independent reaction)(*10.5: The Light Independent Reactions (aka the Calvin Cycle) - Biology LibreTexts*, no date; Raines, 2003; Bartee, Shriner and Creech, 2017). At this point, the CO_2 molecule becomes attached to the compound ribulose-1,5-bisphosphate. This compound is then divided in half to form 2 3-phosphoglyceric acid (C fixation of the Calvin cycle). In the next step of the Calvin cycle, the reduction phase, this is where 3-phosphoglyceric acid become simple sugars, for this process plant cells use the reaction of ATP and NADPH to fix the C into the plants structure. Following this, the final phase of the cycle (regeneration), repeats the earlier steps to create glucose molecules and to create new ribulose -1,5 -bisphosphate (*Calvin Cycle: Definition, Function, Steps & Products | Biology Dictionary*, 2023). The Calvin cycle converts solar energy into carbohydrates, both starch and sugars, used by plants or exuded from the roots into the soil, and releases O_2 back into the atmosphere.

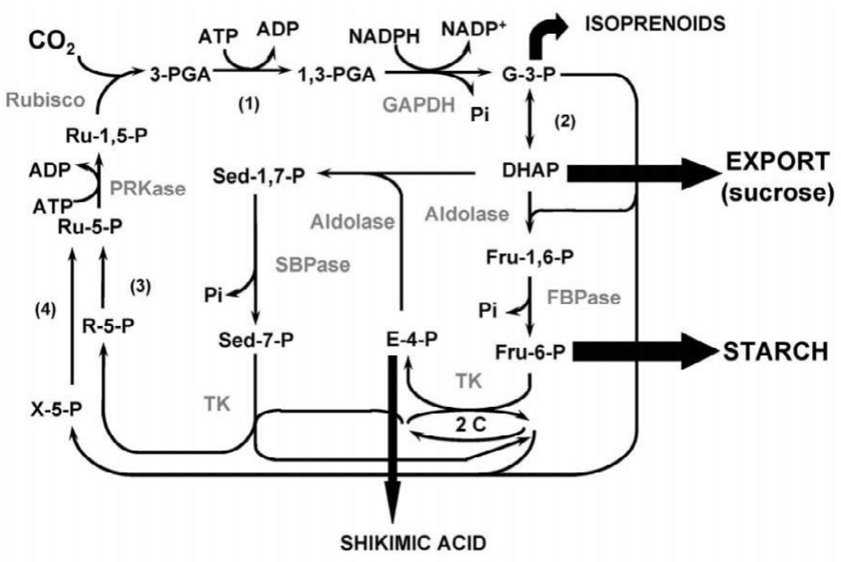


Fig 1.4. Diagram of the Calvin cycle for photosynthesis fixation of C, pathway used by C3 plants (Raines, 2003).

The carbohydrates derived from photosynthesis are used by plant’s aboveground (AB) or belowground (BG) biomass for growth. The roots can then exude C compounds that are transported belowground into the soil where microbial communities use the C for energy. In return, some microbes will provide trees with access to nutrients via a symbiotic relationship, as microbes are able to break down the soil releasing stored nutrients. Some absorbed C is returned to the atmosphere via soil respiration, and some will be transferred into long-term storage (**Fig 1.5**). The C sequestered by trees varies dependent upon tree types (such as fruiting trees, other non-fruiting deciduous and evergreen trees), and location of the storage of the C such as belowground in the roots and soil or above ground in the woody biomass or fruit, also climate, and the soil type.

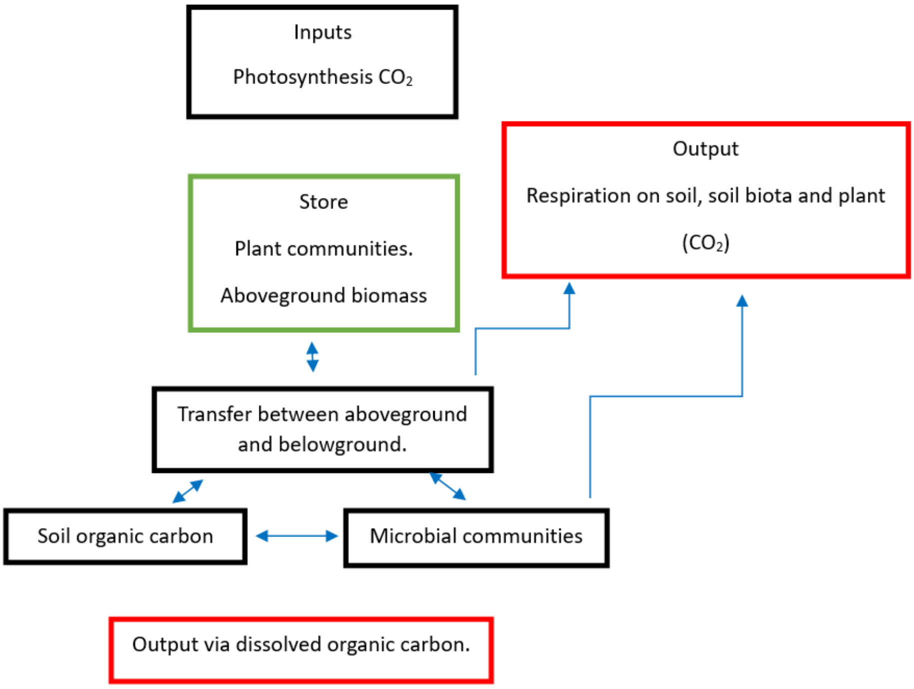


Fig 1.5. A diagram of how a plant community can impact C sequestration (addapted from Fry, De Long and Bardgett, 2018)

1.2.2. What influences soil C sequestration?

Soil is a complex but fragile environment that needs to be carefully managed to provide food security for the future in the current changing climate. The soil is a vital yet an under-used store for C that could help with mitigation in the fight against the rising levels of atmospheric CO_2 and other greenhouse gases. Soil C can be divided into pools which have different rates of turnover; fast, medium- and long-term storage (Dignac *et al.*, 2017). The terms given to the different C pools change between articles, such as liable, active and passive pools (stable)(Trumbore, 1997; Jandl *et al.*, 2007; Wieder *et al.*, 2018), or particulate and mineral associated organic matter (Sokol, Sanderman and Bradford, 2019; Witzgall *et al.*, 2021). These C pools are added to and can be accessed at different rates and under different conditions, such as climate or plant root depths. The transfers of C from short-term into longer-term C pools is very slow and generally occurs when the C is converted to mineral carbonates (Dignac *et al.*, 2017; Sokol *et al.*, 2022). The fast or active C pools are more rapidly turned over, new soil organic carbon (SOC) is constantly being added to the soil, where microbial communities consume it, environmental and management practices also impact CO_2 release from the soil back into the atmosphere (Sokol, Sanderman and Bradford, 2019). This active C pool will generally store C for a couple of years (Dynarski, Bossio and Scow, 2020), depending on the pressure being placed on the soil (e.g. compaction, climatic, or agricultural practises)(Deurer *et al.*, 2012). The medium-term passive pools can store C anywhere from tens of years up to hundreds of years. These medium-term pools can be converted back into active pools, through deeper-rooted plants and root exudates being released into the soil that can access nutrients and water locked up in the deep soil layers, when the upper layers have limited supply allowing soil microbes deeper access causing respirations and CO_2 release. The medium-term pools have the greatest potential to help to mitigate the rising CO_2 levels.

The C storage pools as described above have been hard for many research teams to fully quantify how the different types of C are transferred between pools, remain liable (active) or become stabilized in the passive pools for longer term storage (von Lützow *et al.*, 2007). Many different laboratory methods (chemical and physical) as described in the article by Von Lützow et al (2007) have been developed to try to understand separation of C pools and the types of C most likely to be found in each and the likely residency time, via C dating. These laboratory methods aim to help with C sequestration modelling within the soil, all of which have had varying success, but none have been definitive.

Carbon has been shown by research to be higher in soils that have limited to no tillage compared to farmland that is regularly tilled (Denef *et al.*, 2004), as soil disturbance damages the structure breaking the particulate organic matter and the mineral association bonds (in macro and microaggregates) which release C back into the atmosphere. The amount of C lost can be impacted by the amounts of clay found in the soil. Two models have been suggested for C cycling as means for SOM including leaf litter, microbial necromass, and fine roots to be transformed, transferred and stabilised into the various C storage pools. The first is the microbial C pump (Denef *et al.*, 2004; Liang, Schimel and Jastrow, 2017) which describes the role of the soil microbial communities (both active and necromass) and the two potential pathways that C can become stored longer term in stable pools via in vivo turnover and ex vivo modifications.

The second model is the mineral associated C pump described by Xiao et al (2023). The mineral C pump transforms organic C in several ways making it more stable in the soil and less accessible for consumption by soil microbial, fungal communities and enzymatic activity. This includes the formation of large organo-mineral complexes or forming bonds with clay and other soil mineral particles (such as iron) that forms recalcitrant organic C which can become entombed in the soil. Xiao et al (2023) concluded that the two C pumps were not mutually exclusive but working in conjunction with each other to stabilize and store C in longer term pools, through mineral - microbial interactions.

The amount of C that can be sequestered in the belowground environment can be impacted by internal factors within the soil, including nutrient availability, pore space, and numbers of soil microbes. Many different types of soil microbes are involved in the movement of C from the roots to the soil, including arbuscular mycorrhizal fungi (AMF), and other soil based fungi, bacteria, and other soil-borne organisms (Gougoulias, Clark and Shaw, 2014; Tomè *et al.*, 2016) this will be discussed in more detail in Section 1.2.3. Bacterial and fungal (microbial communities) in rhizosphere soils can promote and increase nutrients availability for uptake by plant roots from the soil, in return for root exudates which feed these soil communities (Kell, 2012).

Soil C when analysed can be classified into different subtypes each of which can be influenced by external land management and environmental conditions. The types of C studied provide an indication of the overall health of the soil and C sequestration which include; total carbon (TC), total organic C (TOC) or soil organic C (SOC), and inorganic C and active (liable) C (Amato and Ladd, 1988; Weil *et al.*, 2003; ‘Technical Information Advice Sheet 45: Soil Carbon Check Service’, 2021). Total C (%) is the combination of all the various types of C, whereas TOC is specific to just the organic C in the soil. Organic C is influenced by the environment (including soil moisture, pH, and temperature) and management of the land and are C based compounds that are derived from plant material, and soil microbes. Inorganic C soil content is made up of carbonates and bicarbonates, such as from weathered rocks. Active C is the fraction that can be used by soil microorganisms due to its ability to be broken down into smaller units and is easily oxidized and released into the atmosphere as CO_2.

Soil nitrogen (N) concentrations are directly linked to the soil’s ability to sequester C, through N ability to affect plant productivity, mineralisation of the N making more N available to the plants and soil microbes (Zaehle, 2013). Bala et al (2013) showed that as N is positively correlated with the ability of the soil to sequester C, although this is influenced by local ecosystem, plants, soil moisture and several other factors. They also suggested that there may be an upper limit to the amount of N and C that the soil can sequester. Several studies have investigated the influence of N on C sequestration and the influence of phosphorus on C:N interactions across the globe (Reay *et al.*, 2008; Lu *et al.*, 2021; Luo *et al.*, 2022). All these studies have shown that N enrichment increases the soils’ ability to sequester more C, but there may be a limit on how much extra C may be increased by. The increase of soil N can have negative impacts on soil phosphorus and therefore soil C, either negatively or positively, as plants release additional and different types of root exudates, in order to gain nutrients, which can either stabilise or increase decomposition of soil organic C (Luo *et al.*, 2022).

Other factors that can influence C sequestration include abiotic stresses such as droughts (increasing atmospheric and soil temperatures or the lack of rainfall) and flooding which are increasing in frequency with global climatic changes. These events can all impact the rate of photosynthesis, plant growth rates, C storage capacity, production of fruit, and CO_2 release from the soil. Climate change is not only influencing and altering flowering times and crop harvest, but it is also increasing the occurrence and spread of new invasive species of pests and pathogens in UK orchards (Brown and Maloney, 2015). This could have major economic impacts on fruit production in the future.

Soil pH has been shown to be an important factor that influences the diversity of bacteria that are found within the soil (Shen *et al.*, 2019), these can affect the levels of active C in the soil through either increasing or decreasing growth rates of soil bacteria and fungi (Malik *et al.*, 2018). Malik et al (2018) reported that near-neutral soil pH in lower-intensity land management has the greater potential to store C, than other soil types and land uses as these are the optimal conditions for microbial growth. Soil pH can also influence the availability of nutrients within the soil required for growth of trees and plants, as soil pH can be influenced and altered by root exudates to make the soil nutrients more available to the plant (Wang *et al.*, 2016; Leisso, Rudell and Mazzola, 2017; Vives-Peris *et al.*, 2020).

Soil moisture content can also influence soil C sequestration and cycling, due to its effect on photosynthesis, and can affect the levels of C sequestered year on year (Humphrey *et al.*, 2021). Humphrey et al. (2021) showed that soil moisture affects C sequestration in two ways. Firstly, by directly effecting net biome production, as photosynthesis is reduced when the soil moisture is lower than a certain level. Secondly, indirectly through temperature and the vapour pressure deficit, and land-atmosphere coupling, which feeds back to the net biome production, limiting C availability to the soil. Wieder et al. (2018) discussed different C modelling techniques and showed that C sequestration may be higher than previous models have shown, due to different estimations of climate changes, and microbial turnover rates. Some models have shown that soil moisture and temperature will affect C storage and release (effects of atmospheric temperature on soil C will be discussed in **Chapter 4**). As plants use the available soil moisture in the upper layers of the soil, deeper soil C pools become more important in the storage of C as these are not affected by the plants roots (Ceccon *et al.*, 2011; F. Yang *et al.*, 2022).

1.2.3. Root exudates

1.2.3.1. What are root exudates?

Root exudates are metabolites created by the plant which are secreted from the roots into the rhizosphere soil (the soil found closest to the roots). Root exudates are traditionally classed into two groups. The first class are low molecular weight compounds that include - amino acids, organic acids, sugars, phenolics, and secondary metabolites which are believed to make up most root exudates. The second group are high molecular weight compounds which includes proteins and polysaccharide sugars (Mucilage), (Walker *et al.*, 2003) (**Table 1.2**). All these compounds are built around C molecules absorbed during photosynthesis and are used either aboveground (AG) for energy and growth (including production of fruits and seeds) or transferred to the roots for growth or passed out into the soil as exudates. Root exudates are an important and significant pathway for C transfer from plants into the soil (Grayston, Vaughan and Jones, 1997; Walker *et al.*, 2003).

Table 1.2. Types and composition of root exudates, table taken from (Inderjit and Weston, 2003)

	Substances identified
Organic acid	Acetic, butyric, citric, glutaric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, succinic, tartaric, valeric
Amino acid and amide	α-Alanine, β-alanine, arginine, asparagine, aspartic acid, cystine ⁄ cysteine, glutamine, glycine, histidine, lysine, methionine, phenylalanine, proline, serine ⁄ homoserine
Enzyme	Amylase, invertase, phosphatase, protease, polygalacturonase
Growth factor	*p*-Amino benzoic acid, auxins, biotin, choline, inositol, *n*-methyl nicotinic acid, niacin, pantothenate, pyridoxine, thiamine
Phenolic acid and coumarin	Caffeic acid, cinnamic acid, coumarin, ferulic acid, salycilic acid, syringic acid, vanillic acid
Sugar	Arabinose, fructose, fucose, galactose, glucose, maltose, oligosaccharide, raffinose, rhamnose, ribose, sucrose, xylose
Others	Nucleotide, flavonone, fatty acids, proteins, sterols, lipids, aliphatics, aromatics, carbohydrates

1.2.3.2. Functions of root exudates

Our understanding as to the function of plant root exudates is still not fully understood but continues to be an active area of research. The grassland experiment by Herz and Colleges (2018) investigated the differences in root exudates produced by different plants. They concluded that the different plants produced different forms of root exudates, although the majority found were the same. Plants can modify the composition of exudates to help modify the soil around them to help ensure plant survival under adverse conditions (Vives-Peris *et al.*, 2020). They also play a vital role in influencing the availability of nutrients in the rhizosphere for plant uptake and growth (Grayston, Vaughan and Jones, 1997; Herz *et al.*, 2018). Exudates affect the soil environment in many ways including changing soil pH (Hu *et al.*, 2018; Vives-Peris *et al.*, 2020). Root exudates can attract microbes that are beneficial for plant growth, and can protect plants from pests and pathogens through releasing toxic substances, and chelating toxic compounds (Vives-Peris *et al.*, 2020). Root exudates influence plant growth in two ways, directly and indirectly. The direct impact is through the production of cations via chelation (which is where a molecule with a free binding site can attach itself to a metal ion such as iron) (McNear Jr., 2013), or indirectly through their influence on the soil microbial activity (Grayston, Vaughan and Jones, 1997). Root exudates also play a vital role in the transport of C from the plant into the soil (Grayston, Vaughan and Jones, 1997; Canarini *et al.*, 2019) and through the shedding of cells from the roots as they grow, and through the metabolites that are transferred in the plants phloem.

Research has shown that the mechanism of exudation differs for the two types of exudates. Primary exudates (low molecular weight, such as sugar, amino acid and organic acids) use diffusion via concentration gradients (Walker *et al.*, 2003; Canarini *et al.*, 2019), whereas the secondary exudates (high molecular weight) require energy through the use of a variety of transporters (**Fig 1.6**).

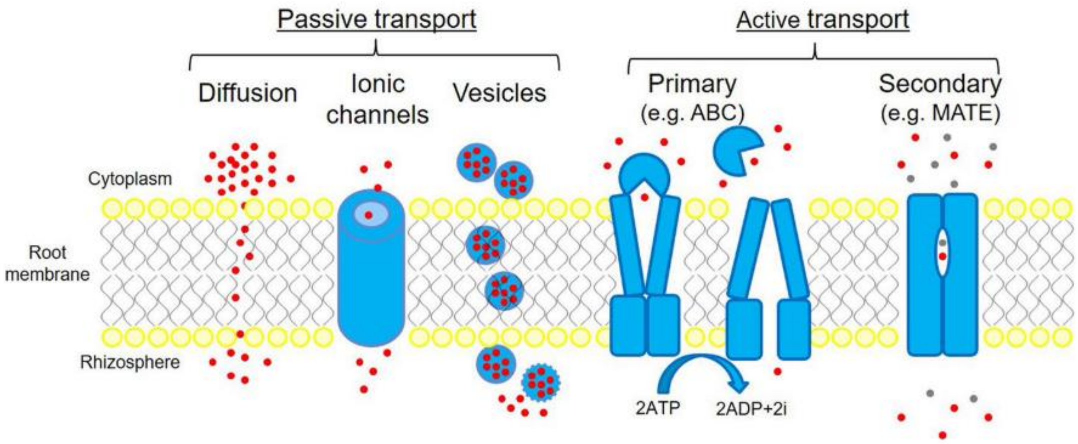


Fig 1.6. The two mechanisms by which root exudates are released into the rhizosphere (red circles are the released molecules). Taken from Vives-Peris et al (2020).

Exudates are thought to be secreted from the root tips, where cells remain undifferentiated, enabling exudate diffusion (Canarini *et al.*, 2019). Canarini et al. (2019) suggested that the root tips were able to sense the environmental conditions around them, such as the availability of nutrients, water, and microbial activities, and in response to differing conditions they were able to alter the quantity of exudates released. These adjustments can all lead to a change in the root system architecture through stimulating or inhibiting root growth and which roots are being developed (e.g. lateral root growth) and where C in the tree is allocated, such as movement to the reproductive organs. Root exudates can account for up to 40% of photosynthetic captured C (Canarini *et al.*, 2019), making this process vital to potential C sink and one that should not be underestimated (Scandellari *et al.*, 2007). Vives-Peris et al. (2020) are the only researchers who have stated that the 40% of photosynthetic captured C was exuded by the roots differs between plant species, age and nutritional status of plant and soil. Gargallo-Garriga et al. (2018) and other studies (Henry *et al.*, 2007; Chang, 2019) have mentioned oxalic acid as a root exudate, which is believed to directly release compounds from the soil (organo-mineral aggregates) to allow microbial communities better access to these compounds. However, this compound has a negative impact on stored C by enabling the breaking down of the soil by microbes, which release the stored C back into the atmosphere (microbe respiration and mineralisation).

Research is showing how important, and how close the relationship is between what plants release through their root exudates and the soil microbial communities (Williams and de Vries, 2019). Williams et al. (2019) stated that the quantity and quality of exudates can alter under different conditions, such as drought, which alters the rhizosphere environment to make extracting nutrients and water easier, reduce competition from other plants and pest attack, and the levels of C within the exudates. They also discussed how different plants produce different exudates in response to the same situation. Gargallo-Garriga et al. (2018) working on the Holm Oak (*Quercus ilex*) under drought conditions and recovery, found strong effects on the composition of root exudates. They found that 71% of metabolites produced under drought conditions were secondary metabolites such as phenolic, flavonoids and terpenoids. As the plant recovered, it shifted towards more primary metabolites such as amino acids, accounting for 81% of exudates. Plants that were under extreme drought conditions could not recover from this, as the exudates released from the roots had changed, and this change could not be reversed when the drought conditions had been stopped.

Fry et al. (2018) discussed how low microbial activity such as those found in waterlogged conditions, can preserve root exudates in the form of Dissolved Organic C (DOC) and so increase the storage ability for C. They showed that lignin can remain in the soil as decomposition is slow, allowing C sequestration in woodlands to be at a reduced rate. They discuss the length of time C in the soil can remain if the soil is protected and how regularly tilling the soil can lose large amounts of organic matter so affecting the C sequestration ability.

Holz et al. (2018) investigated how hairs on barley roots effected root exudation, focusing on the amount of C released into the soil. They discovered that the root hairs increased the plants' ability to release exudates and increased the area of the rhizosphere by up to three times, compared to plants with no root hairs. They concluded that this increase in the size of the rhizosphere could enhance the nutrient cycling in the soil and could be of benefit to soil that had a limited supply of nutrients.

The production of root exudates has been studied for their possible role in signalling, as attractants for beneficial microbes or defence mechanisms for plants that are under attack from pest or pathogens (Hofmann *et al.*, 2009; Hu *et al.*, 2018; Zwetsloot, Kessler and Bauerle, 2018). Walker et al. (2003) suggested that root exudates are not just a target for soil organisms but they could control the growth/competition from other plants (such as Knapweed), to ensure access to nutrients. They discussed how the types and amounts of exudates that a tree produces may change over time, as roots and plants age. Hu et al. (2018) discovered that Benzoxazinoids (a secondary metabolite that is linked to plant defence) released from the roots of cereal crops could alter the microbial communities and defend the plant from herbivores and pests. Yuan et al (2018) research showed that root exudates can help protect the aboveground biomass through recruiting beneficial rhizosphere communities in response to pathogens. Their results showed that plants which had been grown in conditioned soil showed a significant increase in Jasmonic acid. In contrast, infected plants increased production of amino acids, nucleotides, and long-chain organic acids, and reduced the number of sugars, alcohol, and short-chained organic acids produced.

Valentinuzzi et al. (2019) investigated how root exudates of apple trees responded to iron and phosphorous deficiencies in the soil. They found that the production and release of oxalates and flavonoids from the roots increased when there was a lack of available phosphorus in the soil. In the case of iron, flavonoid levels were observed to be lower in the deficient plants compared to the control trees, but the oxalate levels were slightly elevated. Their work showed there are multiple genes involved in up-regulation and down-regulation of root exudation levels, to respond to the changing needs of the plant's. The plants release of root exudates do not just respond to soil nutrient content, but to other chemical, biological, and physical factors interacting with the plant, **(Fig 1.7).**

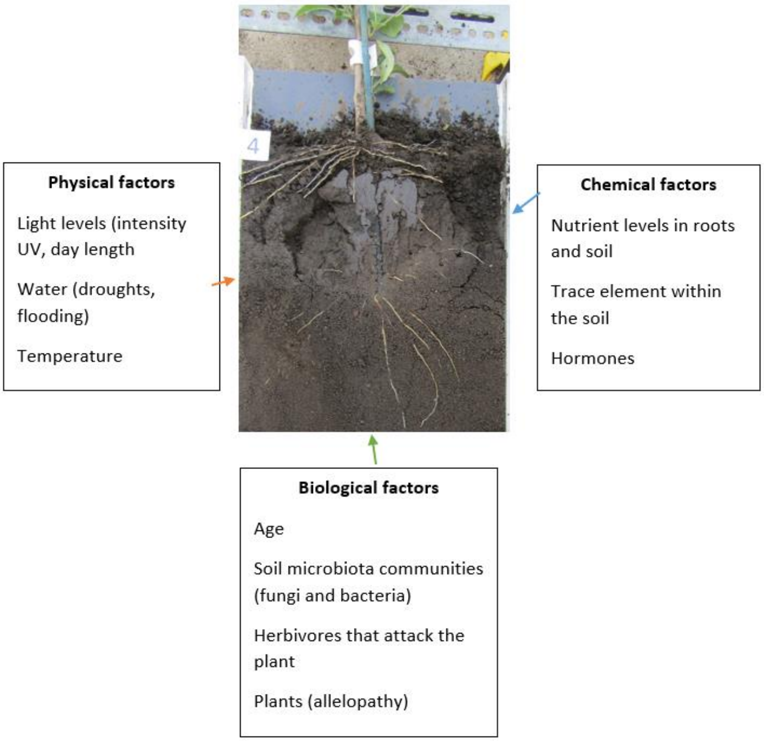


Fig 1.7. Factors that can affect the process of root exudation adapted from Vives-Peris et al (2020).

1.2.4. Soil microbes

Soil micro and meso communities are invaluable to the world plants and C sequestration in the soil. Without them, plant diversity, new soil development and soil health could not happen. These soil communities are very much inter linked with the plant root exudates that they feed upon and help create suitable and nutrient-rich environments for the plants to grow.

Several research teams have shown how C within the soil limits microbial growth, and C enters the soil from plants through leaf litter, dead roots, cells from roots and rhizodeposits (root exudates), (Grayston, Vaughan and Jones, 1997; Gargallo-Garriga *et al.*, 2018; Canarini *et al.*, 2019). Within the soil there are different species of fungi and bacteria (microbes) as well as insects, worms, nematodes, and many others (mesofauna such as hexapods) that help to break down leaf litter, and root exudates into simpler chemical components (Fierer, 2017; Dubey *et al.*, 2019). These can be used by plants, transferred into longer-term storage, or used as foods for soil communities. Some of these microbial communities can directly benefit plants by forming symbiotic relationships, where the plant exudes a variety of chemical compounds in exchange for help to access nutrients (McNear Jr., 2013; Canarini *et al.*, 2019).

It has been suggested that bacteria and fungi account for up to 90% of the total soil microbial biomass in the natural ecosystem and no-till agriculture and are linked to the amount and quality of soil organic matter (SOM) (Six *et al.*, 2006). This may change between different managed systems such as tilled agricultural systems, orchards, and even managed forests, through the application of nitrates and other fertilizers, pesticides, and other controls. The ratio of bacteria and fungi are sensitive to environmental changes such as droughts, heat, flooding, soil nutrient availability, and this can affect rates of C sequestration/respiration balance, and therefore increase the turnover of soil C and the health of the soil can deteriorate over time.

Microbes are essential to global and local C and N cycles but can be affected by climate changes either directly or indirectly (Bardgett, Freeman and Ostle, 2008). Microbes are important in the regulation of soil organic matter and nutrient availability, also the storage of C and N in the soil and recycling of CO_2 back to the atmosphere. The colonisation of root surfaces by microbes generally only covers 15-40% of the root surface (McNear Jr., 2013). The number and composition of microbes on the roots are linked to the levels of nutrients available and the physicochemical variations along the root surface. Root exudates can serve as chemoattractants for microbes, encouraging them to the roots and help form the microbial communities (McNear Jr., 2013).

1.2.4.1. Soil Fungi

Soil fungi can be divided into three functional classes: biological controllers, ecosystem regulators and decomposition/compound transformers (Frac *et al.*, 2018), however, Davis et al. (1992) classified fungi into 4 major groups: phycomycetes, ascomycetes, basidiomycetes and fungi imperfecti. Fungi are a very diverse group of soil microbiota, and found in different types of soil environments, so classification is difficult especially due to their size, as scientists use different methods to study them including DNA (Gardi *et al.*, 2009). Fungi can form symbiotic relationships with the root systems of plants and trees, some are plant-specific, and others form attachments with multiple types of plants (**Table 1.3**) (Bridge and Spooner, 2001; Tedersoo *et al.*, 2014; Barman *et al.*, 2016; Eldridge and Delgado-Baquerizo, 2018). The fungi that attach themselves to the roots vary, some fungi can enter the roots of the plant, some fungi/plant relationships are beneficial, and others are detrimental to the plant as shown in **Figure 1.8,** and these relationships between roots and plants can be species specific.

Table 1.3. Types of Soil mycorrhizal fungi (Founoune *et al.*, 2002; Nath and Kumar Gandhi Rajendra Prasad, 2020)

Type	**Location where fungi is most likely to be found**	**Form**
Ectomycorrhiza	Found mostly on woodland trees, such as birch and willow	A compact external sheathes of mycelium filaments on root surface
Endomycorrhiza	Found mostly in herbaceous plants and some trees	Able to enter plant roots using hyphae, arbuscules and vesicles
Arbuscular mycorrhizal fungi	Most widespread and can be found on apple trees	Form arbuscules that penetrate cells and need a plant to survive, site of C, P and H_2O exchange
Ericaceous	Found in acidic soils which support blueberries and azalea.	Enter roots but do not create arbuscules, form hyphal coils on outside of roots. Help in Fe, Mg and Al acquisition
Arbutoid	Generally found with blueberries and rhododendrons, also found in acidic soils.	Looks like ectomycorrhizal, forms fungal sheath but can also penetrate cortical cells of plants
Orchidaceous	Found on orchids	Required for seed germination
Yeasts	Everywhere	Single cell

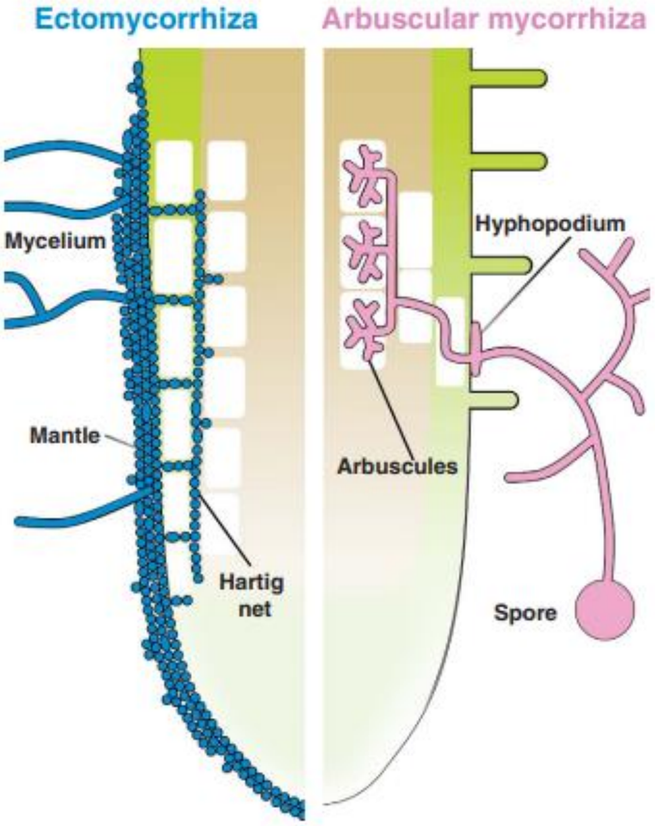


Figure 1 | Illustration of root colonization structures in ectomycorrhizal (blue) and arbuscular mycorrhizal (pink) interactions. The ectomycorrhizal fungus surrounds the root tip with a thick mantle of closely appressed hyphae, whereas the Hartig net develops around epidermal cells (green). In the case of arbuscular mycorrhizas, the root tip is usually not colonized. Hyphae develop from a spore and produce a hyphopodium on the root epidermis. Intraradical colonization proceeds both intra and intercellularly and culminates with the formation of arbuscules, little fungal trees, inside inner cortical cells (brown).

Fig 1.8. Difference between Ectomycorrhizal and Arbuscular interaction with plant roots taken from Bonfante Genre (2010)

1.2.4.2. Soil Bacteria

Soil bacteria can be divided into four functional groups as seen in **Table 1.4** and **Table 1.5** (Hoorman, 2011, 2016; Ingham, 2019; Australian Government, 2020). Most soil microbes grow optimally when pH is neutral (Rousk *et al.*, 2010; Hoorman, 2016). Two research teams stated that a teaspoon of soil from cultivated land would have more living organisms than there are people in the world (Hoorman, 2011; Wendal, 2018). Estimates of bacteria range between 100 million – 1 billion, but could be 1000-2000 times more in rhizosphere soil than general bulk soil (Hoorman, 2011; McNear Jr., 2013). Within the SOM, bacteria is able to convert energy into other forms that is then freely available for other organisms within the soil to use (Hoorman, 2011). In tilled soil, bacteria has been found to be the most dominant microorganism and that they have a higher N content than C (3 to 10 C:N ratio), and that they can only recycle 20-30% of C they encounter (Hoorman, 2011). With bacteria being microscopic it allows them to adapt and grow more rapidly in response to changes in the soil environment than larger and more complex organisms such as fungi (Hoorman, 2016; Fang *et al.*, 2021).

Table 1.4. Functional groupings of soil bacteria (Hoorman, 2016).

Type	**Description**
Decomposers	Consume simple C eg from root exudates and leaf litter. Very important in retaining nutrients.
Mutualists eg Nitrogen fixers	Forms symbiotoic relationships with plant roots creating visable nodes on root hairs. Some covert N_2 from the air into available nitrogen for the plant. 4 types of bacteria convert atomspheric N2 into N for plants, not all need plant host to carry out their function
Pathogens eg Xymomonas and Erwinia	Form galls on root hairs, can produce antibiotics that protect the plant from disease
Lithotrophs or Chemoautotrophs	Obtain energy from nitrogen, sulfur, iron or hydrogen some are important to nitrogen cycling or degredation of pollutants

Table 1.5. Soil bacterial classifications addapted from Hoorman (2016).

Bacteria	Types	Role in the soil
Classified on shape	Rods (called bacilli), spheres (called cocci), spherical (called spirilla) and a slim branching filament called actinomycetes. There are others with more complex shapes	
Aerobic and anaerobic	Oxygen required (aerobic) - *Aerobacter* genus are widely distributed in the soil and actinomycetes bacteria genus *Streptomyces*. Non oxygen dependent (anaerobic). Majority of Bacteria require oxygen for survival	Aerobic bacteria use oxygen to decompose most C compounds. Pathogenic bacteria are anaerobic and can out compete or even kill aerobic bacteria.
Gram +/-	Gram negative are the smallest bacteria. Gram positives have thicker cell walls, larger in size and have an external negative charge.	Gram Negative are sensitive to water stress. Gram positives are more resistant to water stress.
Autotrophic and heterotrophic bacteria	Very important class. Autotrophic include algae and cyanobacteria. Heterotrophic include *Arthrobacter*	Autotrophic absorb C from CO_2, can directly create sugars from CO_2 using sunlight. Heterotrophs gain carbohydrates from the environment and cells around them and can include nitrogen nitrification
Classification based on phyla	Divided into 12 types of phyla and live in a wide range of environments from sea floors and around sulphur, to the ice cold of the arctic.	

1.3. What is the role that apple orchards could play in C mitigation.

Apples have been cultivated for thousands of years, originating in the Middle East (Turkistan) and over the centuries they have spread globally becoming a major fruit crop. The timeline in Appendix 1 shows the history of apple cultivation in the UK (*History of Apples and Cider in the UK ‹ Real Cider and Perry website | Old Scrump's Cider House*, accessed 2019). The first apples were wild varieties, which would include the European Crab apple (*Malus sylvestris*)(Cornille *et al.*, 2012). Since then, crosses have been made, selecting for desirable traits of flavour, size, and texture, and developing varieties suitable for different soils. These cultivated crosses have created the large, diverse range of apples we see today, including sweet dessert apples such as Sunburst, cooking apples like the Bramley and bitter apples used in cider making such as Kingston Black (a bitter sharp) or Dabinett (a bittersweet). The number of cultivated apple varieties is still increasing as new crosses are made to keep up with what consumers are looking for, but only a limited selection is available in supermarkets. It is not just the apple varieties that have been a focus of breeding programmes (Brown and Maloney, 2015), but the rootstocks on which they grow. Recent apple rootstock breeding programmes have focused on enhancing C uptake by the fruit and promoting yield while keeping the aboveground woody biomass limited, through the development of dwarfing rootstocks (Webster, Tobutt and Evans, 2000; Wang *et al.*, 2019). Dwarfing rootstocks produce smaller, more compact trees than the standard ones, allowing for denser planting, higher yields, and ease of harvesting. This reduction in aboveground biomass also limits the amounts of C available to the roots, as apple trees will partition and favour the transport of carbohydrates to the production of fruit rather than the roots, limiting exudation and soil C sequestration. Rootstocks have great impact on the overall tree in many ways including size and resistance to pest and diseases which will be discussed further in **Chapter 2**. Most fruit tree production globally rely on the combination of rootstocks for height and disease resistance and a grafted-on fruiting section (scion) of the desired variety, as this reduces genetic variation of fruit, compared to apples that have been grown from seed (discussed further in **Chapter 3**).

The life cycle of apple orchards varies between dessert, culinary, cider, and traditional orchards. Traditional orchards can reach over 100 years old and typically feature trees grafted on to more vigorous rootstocks. These trees are less densely planted as they are not being intensively grown for commercial profits and could potentially have animals grazing under the tree canopy (*BE5: Creation of traditional orchards - GOV.UK*, no date; England, Information and Tin, 2010). In commercial dessert orchards, scions (the fruiting part of the trees which bears the desired variety of apple, grafted on to rootstocks before planting) are grown on dwarfing or semi-dwarfing rootstocks, such as M9 (Webster, Tobutt and Evans, 2000; Ma *et al.*, 2013). This allows trees to be more densely planted, than trees on more vigorous rootstocks. Dessert orchards have a shorter life span than cider orchards (age of orchards influence on C sequestration is discussed in more detail in **Chapter 5**). Dessert orchards are typically grubbed after 15 to 20 years (which is discussed further in **Chapter 6**), whereas a commercial cider orchard's lifespan can be much longer (up to 80 years). This is due to the reduction in yield after this time, resulting in loss of income and increasing attacks from pests and pathogens ('High Density Apple Orchard Management | NC State Extension Publications',1998). More modern cider orchards are also being grown on semi-vigorous or semi-dwarfing rootstocks to enable denser planting and increased yield.

In 2022, the total area of orchards in the UK was 22,281 hectares (ha)(*Orchard Fruit Survey - data.gov.uk*, 2022) the majority of this (13,719 ha) producing apples, followed by pears and the remainder of cherries, plums and other top fruit, including nuts. This represents a decrease of 3000 ha of land under apple orchard production in the UK since 2012, with cider orchards accounting for almost half of the total apple orchards. The survey also revealed that in 2012 the hectarage of dessert apples such as Cox's and Discovery declined by -5.6% and -11.4%

respectively, whereas Gala was on the rise (+49.5%). The land given over to culinary apples had also dropped by approximately 32.8%. Since the 2012 report, the number of orchards planted with Cox's and Discovery have continued to decline, whereas Gala is still increasing and has become the dominant apple variety grown commercially in the UK with 2,771 ha under cultivation.

Apples are a globally important crop with the cost of exported apples internationally in 2019 totalling 7 billion US dollars (*Apples Exports by Country 2019*). According to worldstopexports.com (*Apples Imports by Country 2019*) Germany imported the highest value of apples in 2018 approximately worth 482.2million US dollars, with the United Kingdom in second place **(Table 1.6)**. The UK imported a considerable proportion of apples, in 2018 from over 15 countries worldwide, with the greatest numbers coming in from France, South Africa and New Zealand. Globally China produces the most apples, with around 31 million tonnes in the year 2018/2019, with the European Union in second place producing just over 14 million metric tons (*Global top apple producing countries 2019 | Statista*, 2019). In 2017 the amount of land under orchard cultivation in the EU (*Agricultural production - orchards - Statistics Explained*, 2014) totalled 1,295,407 hectares (ha), with the largest number under apple cultivation at 473,550 ha. Poland had the greatest area under apple production with 160,844 ha, the UK had 5,743 ha, and Cyprus had the smallest area (listed) of apples at 337 ha. The UK came in about 14th in the land used for apple production, as seen in the **Table 1.7**.

Table 1.6. Top 10 importers of apples and their monetary value (*Apples Imports by Country 2019*) work done by Workman. D, *2019* under worldstopexports.com

Country	Import values (US $)	% Of total imported apples
Germany	482.2 million	6.7%
United Kingdom	423.8 million	5.9%
Russia	394 million	5.5%
Vietnam	352.9 million	4.9%
Netherlands	267.9 million	3.7%
Taiwan	255.1 million	3.6%
India	253.1 million	3.5%
Hong Kong	252.2 million	3.5%
United states	239.3 million	3.3%
China	219 million	3%

Table 1.7. Land under fruit tree cultivation in Europe in 2017 (information taken from Eurostat, (Eurostat, 2014)

Country	Total land given to fruit orchards (ha)	Apple orchards (ha)	Pear orchards (ha)
United Kingdom	7,242	5,743	1,499
Spain	422,809	27,532	18,108
Italy	279,281	55,810	28,623
Poland	167,315	160,844	5,032
France	698,32	38,298	5,250
Romania	62,489	55,050	3,231
Portugal	38,332	11,306	11,306
Hungary	36,291	25,044	2,335
Germany	36,118	33,981	2,137
Netherlands	16,691	6,950	9,742
Total for 28 countries in the EU	1,295,407	473,500	100,383

The UK left the European Union at the end 2020 and in so doing came out of the Common Agricultural Policy (CAP), which allowed for free trade and subsidies to farmers for crops, loss of livestock and environmental schemes. The UK government laid out the Agricultural bill (DEFRA, 2020) that sets out how the government plans to support farmers and what subsidies will be provided to replace those supplied under CAP. These will include plans to reduce C emission and environmental CO_2, such as through offsetting schemes such as the planting of orchard/or plantations encouraging belowground C sequestration (**Fig 1.9**) and will be discussed in sections 1.4.2.

Land management has a crucial role in determining the amounts of C that is sequestered, the addition of fertilizers and the farming ethos (organic or non-organic) could have an impact on the soil C content (Moxley *et al.*, 2014; Aguilera, Guzmán and Alonso, 2015). Liu et al. (2013) discussed how understorey planting can enhance nutrients availability for apple trees resulting in higher C in to the soil. Canarini et al. (2019) discussed how external stressors can impact the plants' ability to store C and to modify it into suitable forms for use and storage.

With fruiting plants, the fruit is also a C sink but are removed every year, so the C does not remain fixed in the plant. Although a proportion of fruit that is either not appropriate for the market, or wind falls may be left to rot in the orchards and so enters the soil as it decomposes (**Fig 1.10**). Ledo et al. (2020) observed that around 30% of land is planted with perennial crops like apple orchards globally. They suggest that over the lifespan of perennial crops they become C neutral or C negative as they continually absorb and store C, as the soil is undisturbed and not releasing CO_2 back into the atmosphere (**Table 1.8**).

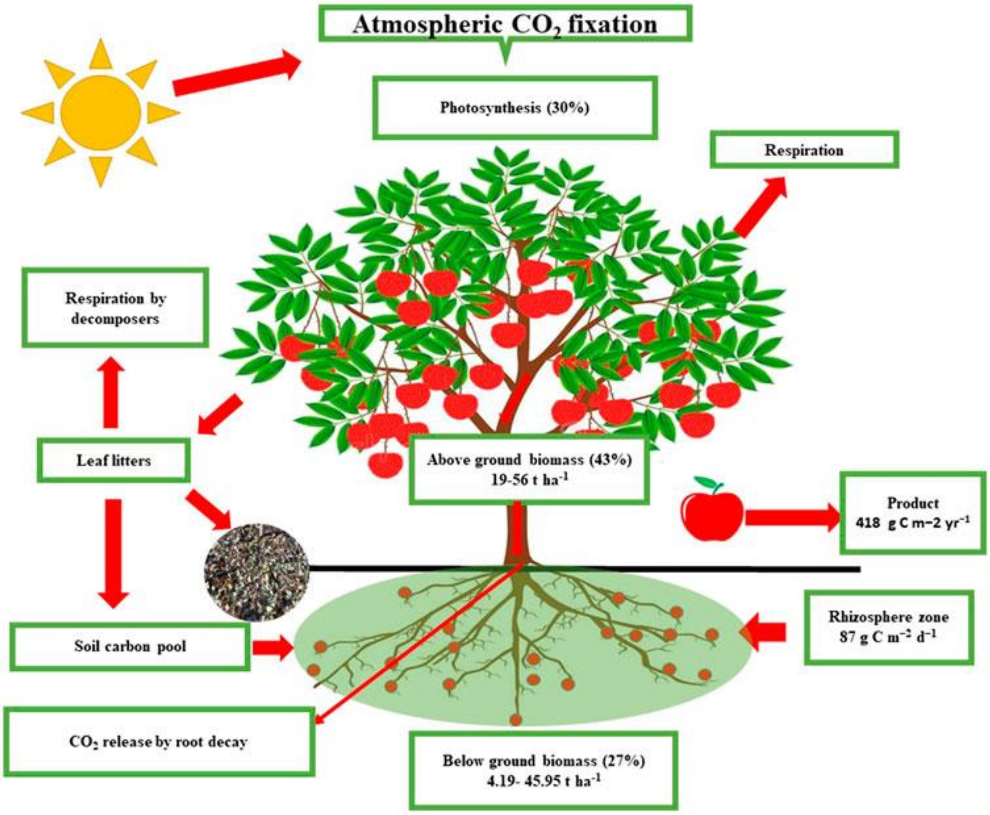


Fig 1.9. The C pools within and around an apple tree, taken from Sharma. S et al (2021).

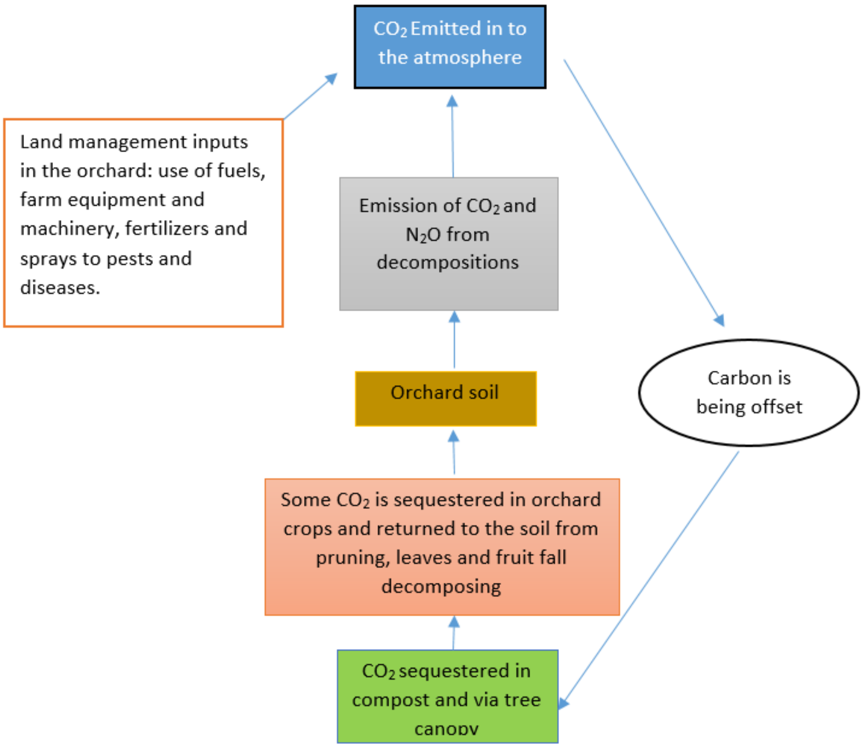


Fig 1.10. Diagram of the C cycle of an orchard adapted from Page and Kelly (2011).

Table 1.8. Changing C fluxes under organic apple orchards of differing sizes. Data taken from Page and Kelly (2011).

C process (tons/ha/year)	**Apple orchard of 800 trees /ha**	**Apple orchard of 1250 trees /ha**
Total CO_2 sequestration	23.6	26.3
CO_2 sequestration in the biomass	23.6	26.3
Total CO_2 emission	18.9	21.3
CO_2 emissions from the soil	15.1	16.5
CO_2 emissions from energy used	3.8	4.8
Net CO_2 sequestration	4.7	5.0

Other researchers have been investigating how orchard trees can be useful after their growing life ends (grubbing discussed in more depth in **chapter 6**) or pruning's, by using the stored C in the aboveground biomass as a soil amendment, commonly known as biochar. Rosemary Anthony investigated this at Bangor University (Anthony, 2013) and others more recently tested it as soil amendments as a form of soil management practises and long term C storage (Eyles *et al.*, 2015; Zhao, Ta and Wang, 2017; Tan *et al.*, 2020; Yang *et al.*, 2020) and this is still a vital and ongoing area of research.

The flow of soil C and what influences the amounts of belowground C sequestration and methods of modelling is still an ongoing area of research within orchards (single trees), agriculture, and forestry, as the different plant species under investigating differ in amount of C sequestration in the soil (Subedi, Ma and Liang, 2006; Génard *et al.*, 2008; Smeglin *et al.*, 2020). Apple trees are often classed as single trees as they do not form a continues canopy unlike those of forests. This research needs to consider the multiple factors that can influence C sequestration of plants not only above but belowground. They need to incorporate all the information available to produce accurate assessments of C stored, estimate the C storage capacity of soil type available and CO_2 released from the soil from microbial and soil disturbances.

1.4. International and national agreements on reducing the impact of climate change.

1.4.1. International agreements

Since the signing of the Kyoto agreement in 1998 (French, 1998) and the Paris agreement in 2015 (United Nations, 2018), many countries have agreed that action needs to happen to mitigate the effects of climate change. This has included a consensus to reduce the amount of GHG's being pumped into the atmosphere and limit the increase in global temperatures, raising salinity of the world's oceans and changing weather patterns which have seen an increase in extreme events like droughts, wildfires, and floods across the globe.

The meeting of world leaders in Kyoto in 1998 laid out in a document showing what the signatory countries agreed to do to reduce GHG's and the targets for each country to achieve (French, 1998). The commitment of each country included the protection and the enhancement of C sinks and reservoirs. They also agreed to work more towards sustainable agriculture to maintain food security under climate change, and to fund research for new and renewable sources of energy (moving away from the reliance on fossil fuels) and innovative technologies that promote CO_2 sequestration. Article 12 of the Kyoto protocol discussed the Clean Development Mechanism that will help to arrange funding projects or activities that enable emission reduction.

The Paris agreement (United Nations, 2018) was the next major international agreement of over 125 countries to unite in the global fight against climate change which came into force in November 2016 (UNFCCC, 2014, 2020; United Nations, 2018). The main aim of this agreement was to keep rising temperatures this century under 2°C of that of pre-industrial levels. Also, within this agreement they hope to help countries to find ways to deal with and become more resilient to climate change and continue to lower emissions of GHG's. Through this agreement a clear framework of assessments was set out to help measure each country's effort. The aims to help mitigate and store C were discussed, and they acknowledged that it would take longer for developing countries to reduce their reliance on fossil fuels and move to cleaner technologies, than those in developing countries and this is down to economics. The United Nations have an annual conference known as the Conference of the Parties (COP) to discuss the previously agreed commitments, discuss and set new targets to help the world to effectively target climate change (United Nations, 2022).

1.4.2. What is the UK doing to reduce and mitigate CO_2 emissions?

In the Climate Change Act 2008 (UK Parliament, 2008) the UK government, pledged over £3 billion to help support low C initiatives over 6 years under the Clean Growth Strategy and the Industrial Strategy Challenge Fund. It continued to discuss how the UK, alongside other European countries, would try to help developing countries to reduce C emission and develop cleaner technologies in the fight against climate change through the ICF (International Climate Fund), including the reduction of deforestation.

Two climate change schemes were designed by the UK government to encourage industries including agriculture, to reduce both their energy usage, and CO_2 production which led to tax savings. These schemes were the Climate Change Levy (CCL) (OFGEM, 2016; Gazprom, 2021) which were set up in 2001 and is a tax on the amount of energy a business consumes, and the Climate Change Agreement (CCA) (Environment Agency, 2014), which came into effect on the 1st April 2013. These encouraged industries, in partnership with the Environment Agency, to plan and agree on ways to decrease energy usage and CO_2 emissions through targets which would be assessed at bi-annual intervals. If the targets were achieved this allowed the company to have a reduction in the CCL, so reducing energy bills.

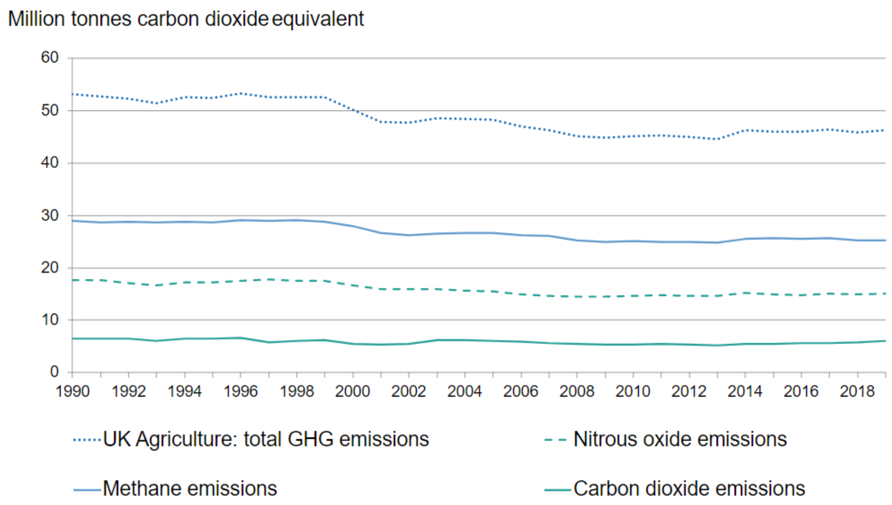
Three funds were available for those who wish to plant fruit trees as part of hedging (TE3 Planting fruit trees) which were released in 2015 (*TE3: Planting fruit trees - GOV.UK*, 2021). There are two linked to planting and maintaining orchards planted in the traditional style (not high-density commercial orchards) (*BE4: Management of traditional orchards - GOV.UK*, 2022), both of which provide over £200 per hectare of land under this style of orchard. All three of these funds stipulate what a traditional orchard is, the types of trees and rootstocks that can be used, and the height branches are kept at.

The UK Government introduced a Woodland C Fund in 2018, with the hope to help establish new productive woodlands with the specific aim of sequestering C into the soil with a one-off payment of £1000 per hectare of trees planted and guaranteed to be kept for a minimum of 5 years (*Woodland Carbon Guarantee - GOV.UK*, 2019). This has been complimented with the £50 million woodland C Guarantee scheme, which aims to encourage farmers and landowners to plant trees and allowed the sale of Woodland C units to the government for C sequestration as part of the 25-year environment plan (*Woodland Carbon Guarantee - GOV.UK*, 2019). It talked about selling C credits for the C captured and sequestered by the trees. Neither of these two forestry funds consider what the top-fruit industry could do to help mitigate climate change and reduce food miles by increasing the land used for orchards. All these are part of the Countryside Stewardship scheme (*The Countryside Stewardship (England) Regulations 2020*,2020; DEFRA, 2023) that provide grants to a variety of schemes designed to protect the countryside and farmland.

In a step to reduce the UK C emissions the UK government in November 2015 announced the end of coal fire power station by 2025 (Department for Business, 2019), this has since been brought forward a year to October 2024 in a press release from the government in February 2020 before the COP26 and 2021 UN Climate Change Conference (Department for Business, 2019). In the same press release it showed that the UK has achieved a 43% reduction in GHG's emissions since the 1990's, and it showed that between 2017-18 UK GHG emissions had fallen by 2.1%.

With the law for the UK to become C net zero by 2050, it falls on all UK industries, including farming, to find ways of not only reducing their C outputs, but also to help mitigate against rising CO_2 levels. For farmers this will require looking at their practices, including the use of machinery, no plough technique where you do not plough but plant straight into the soil to minimise soil disturbance (this is because soil disturbance releases C back into the atmosphere) and replanting mixed hedgerows. This could help maintain and even improve soil health and protect future food production with an ever-increasing population and changing weather patterns. Currently the apple industry removes the pruning's and whole trees (especially at the end of the commercial life of the orchard) from the orchards to help prevent the transfer of any pests and pathogens. These are often then burnt as this is currently one of the most economical ways to dispose of this material. This burning therefore releases C that is stored in the aboveground biomass and will be discussed further in C**hapter 6**.

The UK government in October 2021 (DEFRA, 2021) stated that agriculture currently produces 10% of the UK's GHG emissions (**Fig 1.11**), similar to that of the global scale of between 10-12%, although this figure is different to that published by the FAO (FAO, 2014), who put the CO_2 emissions from agriculture, forestry and other land use globally of all GHG's 24% (FAO, 2014) if forestry and other land usage is included.



Source: Department for Business, Energy & Industrial Strategy

Fig 1.11. UK agriculture greenhouse gas emission 2021 (DEFRA, 2021).

The report by the UK government 2019 (*Climate Change and Agriculture - POST Note - UK Parliament*,2019) discusses how climate change worldwide will affect agriculture through food security in the UK as we import approximately 40% of what we consume. The report continues to discuss how to mitigate emissions from food production by changing how farming is done and the overall land management through best practices. One of the ways suggested was by taking land out of food production and moving it into land which could help mitigate climate change. It does not state how the land will be used to mitigate climate change, how it will change the demand for food production, or how it will continue to provide food for an ever-increasing global population.

At the beginning of 2020 the UK started the processes of passing a new Agriculture Bill ready for leaving the EU (DEFRA, 2020). This bill set out the government’s plans for this sector and new grants which would be offered to farmers and their focus. As part of the bill, they proposed changing the focus of grants to environmental and animal welfare impacts instead of basing them on the amount of land owned/being farmed, which favoured bigger farms. This will encourage them to cut emissions and ensure or even boost productivity. This would include putting measures in place such as planting trees to help reduce flood events and improve water and air quality. In the agriculture bill the government has legislated for direct payments to farmers as the UK comes out of the EU’s CAP to ensure they still receive the grants and subsidies for the next few years until the Agriculture Bill payments become fully active (Coe, 2020).

One major issue with the tree planting or C mitigation schemes is that they do not specifically mention planting fruit trees in commercial orchards, especially apples, which is the UK’s principal orchard crop. Another issue with this scheme is the lack of specificity on tree varieties to plant as non-native trees could have a negative impact on the wider ecosystem. Not only could the tree fruit industry contribute to the UK becoming C zero through sequestering C into the soil, but it could also give the apple-producing industry a boost in production, thus reducing the reliance on imports.

1.5 Project aim

Currently there is still insufficient research on apple trees/ orchards C sequestration ability, and the research that has been conducted vary in their estimations on C stored within the orchards, in both above and belowground biomass and soils. Different research teams have focused on different areas, but when developing models or estimations, various factors are left out that may be important. This therefore leaves areas for further research to help better understand and determine levels of C sequestration for apple trees, both above and below ground. This information can help to build more accurate models that can help estimate soil C concentrations in different growing regions and how this is likely to alter with climate change to help safeguard the future of farming and long-term soil health.

This projects overall aim was to investigate factors that may impede or enhance the ability of apple trees to sequester C belowground into the soil over their lifespan and encompassing two classifications of apples (dessert and cider). Five factors were selected each with their own research questions on how they influence soil C sequestration, and these will be further presented in the individual chapters. The factors selected for investigation into their possible impact on belowground C sequestration were – (i) Apple rootstocks that are commercially used (**Chapter 2**), (ii) scions (fruiting variety) and their influence on M9 rootstock (**Chapter 3**), (iii) increasing atmospheric temperatures (**Chapter 4**), (iv) soil C changes under increasing ages of orchards (**Chapter 5**), and finally, (v) the grubbing of an orchard (**Chapter 6**). These factors were selected because they cover the planning, growing and end of the productive life stages of orchards, which many studies seem to ignore.

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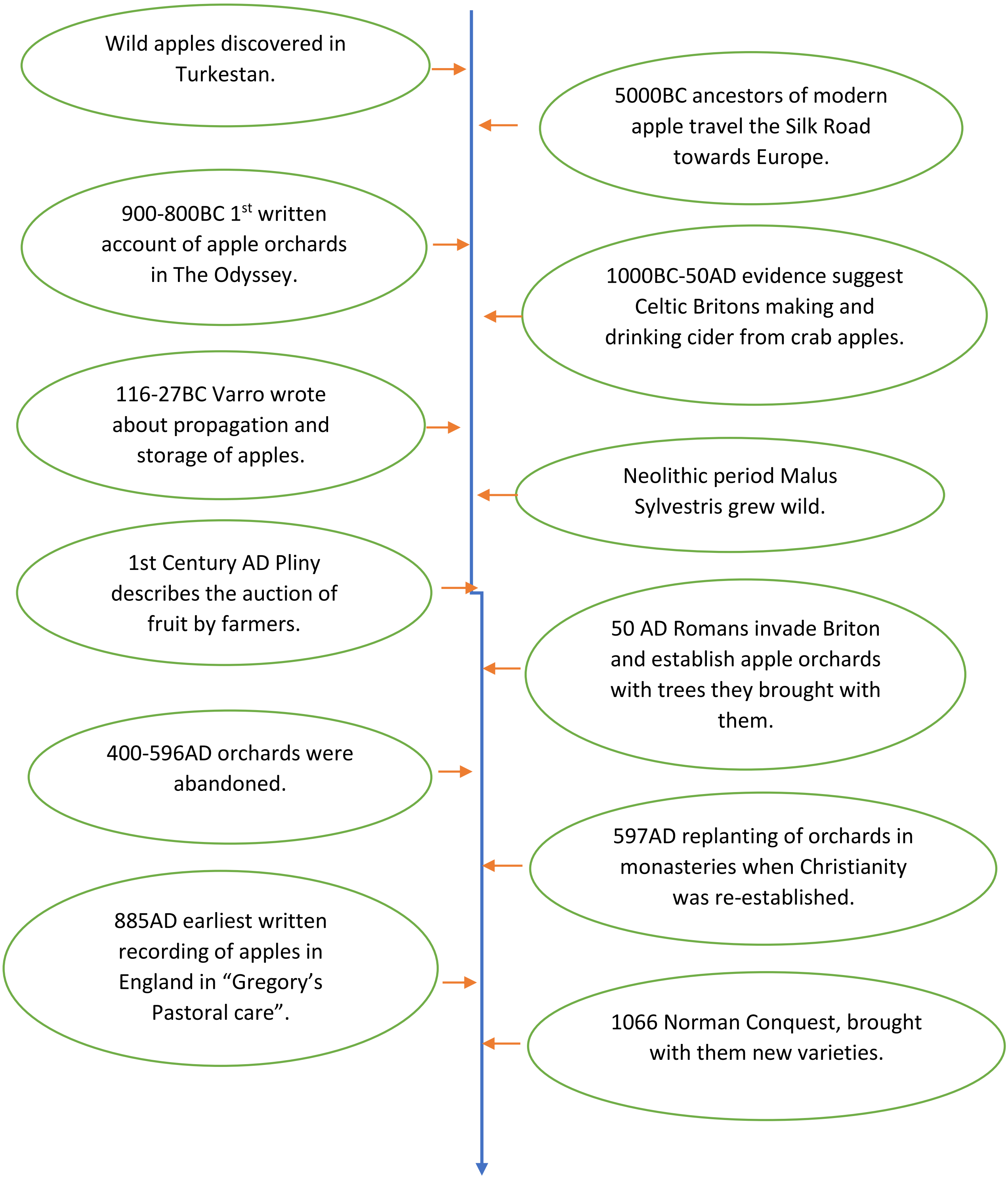
1.6. Appendix

Timeline of apple growing in the UK (*History of Apples and Cider in the UK ‹ Real Cider and Perry website | Old Scrump's Cider House*, no date; *Simply... A History Of The Apple | New Internationalist*, no date).



Wild apples discovered in Turkestan.

5000BC ancestors of modern apple travel the Silk Road towards Europe.

900-800BC 1st written account of apple orchards in The Odyssey.

1000BC-50AD evidence suggest Celtic Britons making and drinking cider from crab apples.

116-27BC Varro wrote about propagation and storage of apples.

Neolithic period Malus Sylvestris grew wild.

1st Century AD Pliny describes the auction of fruit by farmers.

50 AD Romans invade Briton and establish apple orchards with trees they brought with them.

400-596AD orchards were abandoned.

597AD replanting of orchards in monasteries when Christianity was re-established.

885AD earliest written recording of apples in England in "Gregory's Pastoral care".

1066 Norman Conquest, brought with them new varieties.

1347-1485 War of the Roses and the Black Death saw the decline in production of apples.

1491-1547 Henry VIII encouraged orchard planting and even got new varieties from France.

16th and 17th Century extensive planting of orchards in Kent and West Country (mostly for cider) and expanding of market for cider due to the canals.

1763 Lord Bute introduced the first tax on cider causing riots.

18th Century Thomas A. Knight undertook experiments in pollination leading to improved varieties, influencing 19th century Thomas Laxton.

Napoleonic wars saw high import taxes on fruit and lead to orchard expansion.

1837 reduction to import taxes causing collapse in apple market.

1876 Bramley seedling was first established as a culinary apple from a pip grown in1809.

1894 first centre devoted to fruit experiments set up at Woburn by Duke of Bedford and Spencer Pickering but did not last long.

Late 19th and early 20th century large number of new varieties were developed.

1903 Long Ashton research station in Bristol was set up as the National Fruit and Cider Institute before linking with Bristol University.

1913 East Malling Research Station was established.

After WW2 investment began in R&D for smaller trees.

1973 UK became member of EEC more competition from high yielding varieties, traditional orchards were removed from production.

1990s New Zealand varieties were introduced to UK market, trail orchards planted with great success.

2000's- present day, new varieties have been introduced and planted, cider consumption has increased and a rise land under orchards.

Chapter 2. The impact of commercial apple rootstocks on belowground C sequestration

2.1. Abstract

Apple rootstocks are a fundamental part of the apple trees, as they have many important functions that can impact tree growth (nutrient and water acquisition) and imparting genetic effects on tree vigour (dwarfing to very vigorous), pest and disease resistance. This five-month project investigated what effects different commercial rootstocks (M.9, M.116, and M.M.106) had on belowground C sequestration, where the soil C was being stored, and if rootstocks affected the diversity of soil microbial communities. The results showed that soil organic matter under M.M.106 had significantly increased between 13- and 19-weeks following planting, but microbial biomass C significantly declined under all rootstocks over the same period. Soil total C did not show any significant change over time or between rootstocks. Significant differences were found in soil bacteria and fungi across the three rootstocks. In conclusion, this investigation did not find that any of the three rootstocks were able to significantly affect amounts of belowground C sequestration. Soil regions do exhibit significant differences in the concentration of C found, with the soil closest to the roots having the highest concentrations.

2.2. Introduction

Apple rootstocks are the basis of apple production, and provide several functions including, anchoring the tree in the soil, to allow the root system to explore, and exploit the soil for nutrients, and water. The nutrients and water are supplied to the leaves and fruit via the vascular network in the trunk (Pregitzer, 2008; Ogura *et al.*, 2019). In reverse, photosynthesis supplies the roots with energy in the form of carbohydrates, and enables the tree to grow (De Neergaard, Porter and Gorissen, 2002). Some of the compounds produced through photosynthesis are transferred to the roots and released into the soil as exudates, which can in turn be used by symbiotic and non-symbiotic soil bacteria, fungi, or pathogenic species (**Chapter One**). After death some of the C contained in these microbial cells can become fixed in the soil, and some will be lost as CO_2 back to the atmosphere (Rillig *et al.*, 2001; Six *et al.*, 2006; Liang, Schimel and Jastrow, 2017; Buckeridge *et al.*, 2020).

Rootstocks not only provide these necessary functions to keep the tree alive, and productive, but they can influence the tree's height, from dwarfing trees that can make hand harvesting easier, to other trees that are several meters tall and harvested mechanically (Wang *et al.*, 2019). Rootstocks can also help the tree to adapt to environmental conditions, such as droughts or flooding. Rootstocks also have an effect on the amount of winter chill needed for the tree to come out of dormancy which is likely to become increasingly important in the coming decades with the effects of climate change (Olesen and Bindi, 2002; *Met Office*, accessed 2020; Lal, 2016; Atkinson *et al.*, 1999; *University of Reading News*, 2018). Rootstocks can also impart resistance to certain pests and diseases that are prevalent around the world, including woolly apple aphid, collar rot, and fire blight, (**Fig 2.1 A, B, and C**) all of which are detrimental to productivity and fruit quality (Fazio, Robinson and Aldwinckle, 2015; Webster, Tobutt and Evans, 2000; Fazio *et al.*, 2012).

Fig 2.1 A, B, and C. Pest and diseases that can impact apple trees growth and fruit quality, which some rootstocks are more resistant to than others. **A:** Damage caused by woolly apple aphids, at the start of colonisation with the wax and the galls they create. Image taken by Cindayniah Godfrey. **B:** Collar rot on an apple tree (*Crown rot and collar rot – additional information | Apple Best Practice Guide*, 2022). **C:** Fire blight damage (*Fire Blight | Stevens County | Washington State University*, 2016).

The use of RNA sequencing and reverse transcriptase PCR to identify factors that restricted growth via vascular enriched gene expression was conducted by Foster et al. (2017). Their work investigated polar auxin inhibitor 1-*N*-naphthylphthalamic acid, cytokinin, the levels of Abscisic acid and Gibberellic acid in the xylem, and their effect on both roots and scions for dwarfing and vigorous trees. They identified several genetic pathways were down regulated, and others were up regulated in dwarfing rootstocks. Lipid and cell wall biosynthesis were down regulated whereas degradation pathways (root death) were up regulated. They summarised that the molecular markers they observed in dwarfing rootstocks MdAUX1 and MdLAX2 helped to reduce the auxin transport, which controls plant development and growth (Zazimalová, Petrasek and Benková, 2014). This limiting of plant growth and the up regulation of the degradation pathways in dwarfing rootstock could impact the ability of these dwarfing trees to sequester C belowground, and so would need further investigation.

Deakin et al. (2019) investigated the impact of replanting orchards with the same or different rootstocks on the soil microbiome in relation to apple replant disease (ARD). They investigated whether the soil microbiome communities differed between rootstock genotypes and their possible effects on ARD. Their results showed that arbuscular mycorrhizal fungi (AMF) varied between planting positions, and they were significantly different between rootstock genotypes. The differences between microbial communities in soil regions reduced by 6 to 7 months post tree planting. Deakin et al's work did not investigate what effect the AMF could be having on soil C. Little research has been done to investigate how rootstocks of different sizes and vigour may impact soil C sequestration and how AMF may be influencing the rootstocks' ability to sequester C. Therefore, there is a gap in our knowledge regarding soil microbial communities and how they may impact soil C sequestration.

The aim of this investigation was to determine whether apple rootstock varieties influence belowground C sequestration and rhizosphere microbial community composition. The key objectives were to assess (1) the differences in sequestration ability of different commercial rootstocks used in the UK apple industry, (2) C sequestration in different soil zones (bulk and rootzone (1 cm around the roots)) (3) whether the diversity and composition of microbial communities (both fungal and bacterial) differed significantly between rootstock varieties and soil regions (rootzone and rhizosphere (brushed of the roots)).

2.3. Materials and Methods

The experimental work was conducted at NIAB East Malling, Kent, UK (51.286359, 0.452572) over a five-month period from May 2020. Three rootstocks from the Malling/ Malling Merton series were used: M.9, M.116 and M.M.106. M.9 is mostly used for the dessert apple industry and is a dwarfing variety which allows for a higher planting density within orchards and to make hand harvesting of fruit easier. M.116 produces a medium-sized tree, and although it is not commonly used in the dessert apple industry, some cider growers are choosing to use this rootstock. The largest rootstock included in this experiment was the semi-vigorous M.M.106, which is most widely used in the cider industry as the trees can withstand mechanical shaking during fruit harvest. The rootstocks used in this experiment were two years old, with a root system between 9 and 11 cm in length and supplied by F P Matthews, Tenbury Wells, Worcestershire UK. The rootstocks were all grafted with Cox's Orange Pippin to eliminate any potential effect of the scion on root growth and C capturing ability.

2.3.1. Experimental set up

Fifty-four rootstocks (eighteen of each rootstocks used) were grafted in early 2020 with Cox's Orange Pippin. The fifty-four trees were randomly assigned a harvesting point, four of each rootstock were kept in a cold store (below 5°C) and not planted, to provide baseline biomass C. The remaining forty-two trees were planted into rhizotrons and grown in a glasshouse compartment without supplementary lighting, but with daily fertigation (**Fig 2.2 a, b,** and **Fig 2.3**). Rhizotrons were chosen for growing the trees in, as the original plan was to assess the root system architecture but there was a lack of roots visible at each assessment, so data was not adequate for assessment.

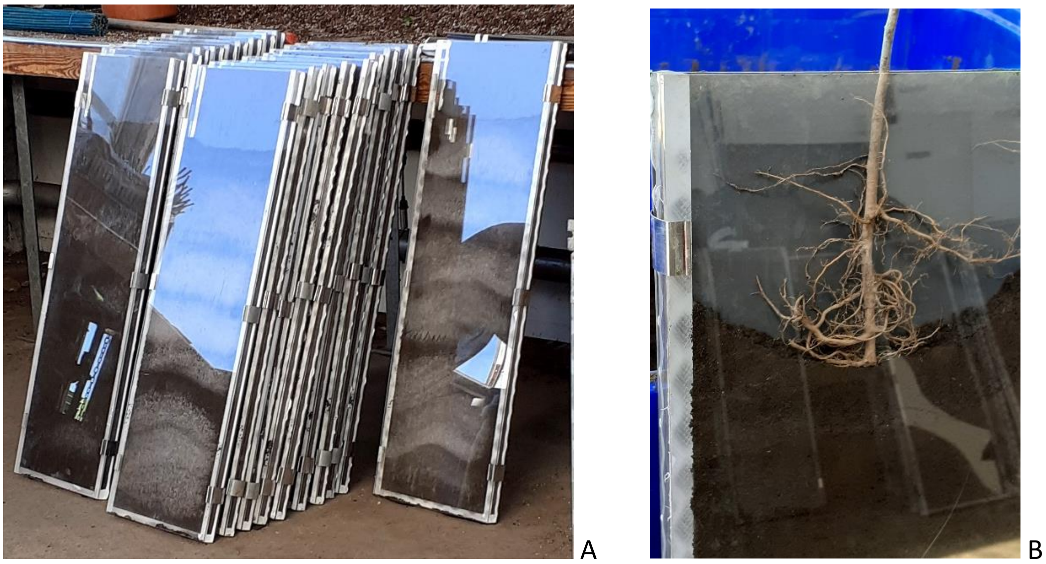


Fig 2.2 A and B. A) Soil filled rhiztrons (1-meter tall) ready for planting showing the clear Perspex panels being held on by metal clips, B) Indication of the planting depth of the trees which were planted by the glass house team at NIAB East Malling. (Photos by Catherine Chapman 19th May 2020)

Fig 2.3. The planted rhizotrons a month after planting, which were grown in a glasshouse compartment and were place angled at 30°, on metal frames with irrigation lines laid across the top, in a random block design at NIAB East Malling. (Photo by Catherine Chapman 19th June 2020)

The trees were planted on 19th May 2020, into rhizotrons. The rhizotrons (1 m tall, 30 cm wide and 5 cm deep), were constructed from Perspex panels. The front panel was a clear Perspex which was removable and held on the rest of the frame by clips to allow observation of the roots and the front and sides were wrapped in plastic sheeting to prevent light getting to the roots and soil (**Figs 2.2 and 2.3)**.

The soil was collected from a field at NIAB East Malling, which had been fumigated the previous year, and allowed to air dry before it was homogenised and sieved (5 mm) by the glass house team. The soil was a clay loam (analysis by NRM UK by laser diffraction) and an average pH_{CaCl2} of 6.78. Soil was added in layers to the rhizotrons and frequently tamped down to ensure even distribution and soil compaction. The trees were then planted, and the remainder of the rhizotron filled, leaving a 5 cm gap at the top. These were placed in the glasshouse compartment on metal frames at a 30° angle to encourage the roots to grow closer to the front of the box for easy observation of growth (**Fig 2.3**). The rhizotrons were placed in the glasshouse in a randomised block design to account for variations in the environmental conditions across the compartment. The rhizotrons were drip irrigated (using three separate lines - one per rootstock and at a rate of 1.2 L per hour for a maximum of 5 minutes three time a day), with drippers placed either side of the trunk, approximately 5 cm away. Fertigation with Universol Green (a nitrogen-based feed with a nitrogen to potassium ratio of 2:1, with phosphorus, magnesium and trace elements) at a dilution of 1 g per litre was supplied on a timer and the volume of daily irrigation was altered as the trees grew and their water requirements increased. There was no artificial lighting used and the glasshouse vents were used to help regulate the air temperature. Four soil-filled rhizotrons were left unplanted to function as blank controls and provide a baseline comparison for tree C sequestration.

Soil samples and tree biomass data were collected at four time points; pre-planting, then following planting at six and 13 weeks when four replicates of each rootstock were destructively harvested, and the final collection 19 weeks (September 2020) after planting when the remaining six of each rootstock were destructively harvested. Prior to planting, four trees of each rootstock and three soil samples were collected from the homogenised, sieved soil, to provide a base-line assessments for C and N concentrations. At each destructive harvest following planting, three soil samples (bulk, rootzone (1cm from roots), and rhizosphere (brushed of the roots)) were collected and analysed for C and N concentrations. The aboveground growth was defined as anything above the top of the rhizotrons (**Fig 2.4)** rather than using the graft union as the point of reference since these varied from tree to tree.

Fig 2.4. Rhizotron following the removal of aboveground tree biomass at the top of the rhizotron, in preparation of soil sampling (photo taken by Catherine Chapman on 2nd September 2020).

2.3.2. Soil and plants analysis:

The soil texture analysis was conducted by NRM (Reading UK) using laser diffraction method. The total % C and N analysis of both soil and roots was performed using the method described in the AOAC official methods of analysis (*Official Methods of Analysis, 21st Edition 2019*) and carried out by Forest Research UK.

At NIAB East Malling soil moisture content was determined using the gravimetric method described by Forster (1995) and soil pH using the calcium chloride method of Schofield and Taylor (1955). The potassium permanganate method was used to determine soil active carbon (POXC) following the methods described by Weil et al. (2003) and Culman et al. (2014). The ninhydrin assay described by Amato and Ladd. (1988) was used to determine both the microbial biomass nitrogen and carbon (MBN/MBC) within the soil, following soil fumigations as described by Vance et al. (1987). The soil nitrate concentration was analysed using the method described by Cataldo et al. (1975). Some alterations were made to the ninhydrin and nitrate assay methods, these alterations are noted below. Aboveground and belowground biomass C was measured following the method and calculations described by De Oliveira et al. (2019) , Petersson et al. (2012), and Manickam et al. (2014).

DNA was extracted using the Qiagen DNeasy PowerSoil Kit and protocol, and sent to LGC Genomics GmbH, Berlin, Germany for analysis for fungi and bacteria (ITs and 16s) using next generation sequencing.

Soil Nitrate – The standard solutions were increased from 0-10 mL (µg NO_3^- -N mL^{-1}) to 25 mL in increments of 4 or 5 mL. Some samples needed diluting up to 70 % with deionized water to allow for absorbance reading to be obtained.

Soil microbial biomass C –The standard solutions were decreased in concentrations from 10 to 6 mL (or 1000 - 600 µm NH_2-N L^{-1}) and made up in increments of 1 mL. The amount of extracted soil sample was decreased from 1 mL to 0.6 mL, and the citric acid buffer was increased from 1 ml to 1.4 mL.

2.3.3. statistical analysis

The data was statistically analysed with Microsoft Excel 365 using pairwise one-way ANOVA's, following normalisation of the data to ensure assumptions were met. This method was used to determine significant differences between the three commercial rootstocks used in this investigation, differences between the three soil regions (bulk, rootzone (1 cm around the roots), and rhizosphere (brushed of the roots for DNA)), and changes over time for the soil and tree biomass harvests. P values of <0.05 indicated significant differences. Pearson's correlation coefficient for the soil C and N, plant biomass C and the root total C and N contents.

R studio 4.1.1. was used for the DNA analysis, OTU counts diagrams, and alpha diversity plots using vegan 2.3-1 package in R as described by Oksanen et.al (2018)(conducted by Greg Deakin - Statistician at NIAB East Malling).

2.4. Results

The three regions of the soil were sampled at each harvest point. Bulk and rootzone soils were used for pH, moisture, carbon, and nitrogen analysis. Due to the lack of soil from the rhizosphere this was only used for the DNA analysis alongside the rootzone, as these two soil regions were most likely to have the higher concentrations of soil microbes and fungi.

2.4.1 soil pH

The pH_{CaCl2} in the bulk soil significantly increased over the course of the experiment becoming more neutral from the initial 6.78 to 7.11 or 7.15 (P = <0.00001). At 19 weeks post planting, the soil pH was significantly different between the two bulk and rootzone (RZ) under rootstocks M.116 and M.M.106 (P = 0.01 and P = 0.02 respectively) (**Fig 2.5**).

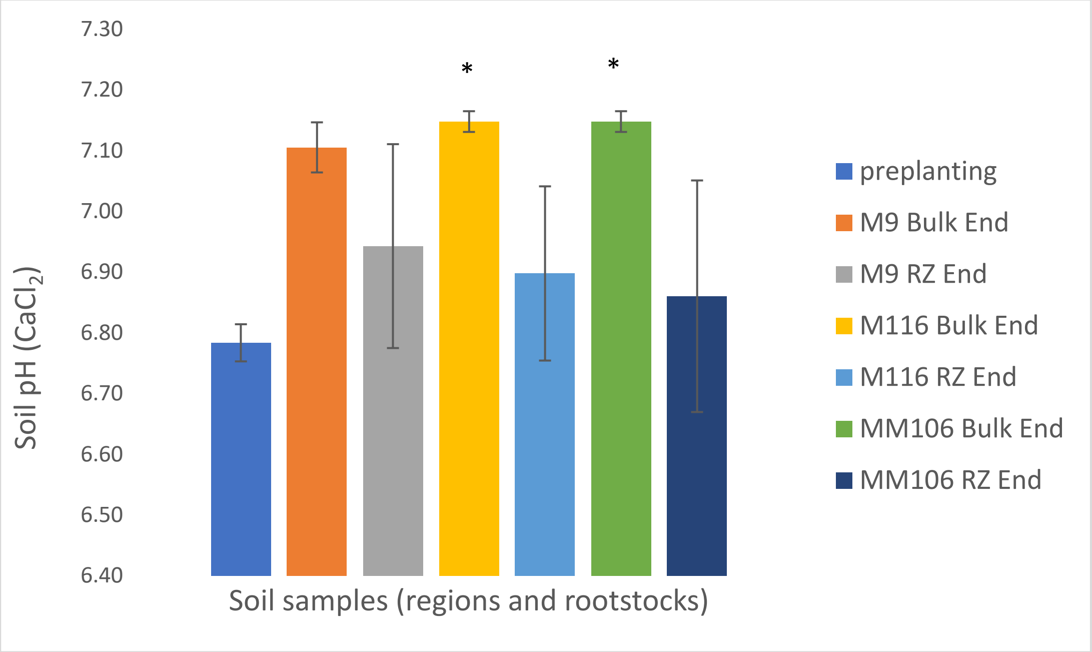


Fig 2.5. The mean soil pH before planting and at the final destructive harvest (End of the experiment September 2020) under the three rootstocks and the two sampled soil regions, with standard deviation bars. Two rootstocks with significant differences (P <0.05) between soil regions are marked with an asterisk (*).

2.4.2. Soil Carbon

The mean amounts of total percentage of C (TC), total organic C (TOC) total inorganic C (TIC) and organic matter for the three destructive harvest which are summarised in **Table 2.1**. The soils organic matter under the M.M.106 rootstock showed significant increases between 13- and 19-weeks and pre to 19 weeks (P = 0.04 and P = 0.01 respectively) and were significantly higher in concentrations than those of the M.9 rootstock at nineteen weeks (P = 0.04). The TIC significantly decreased (P = 0.03) under the M.M.106 from soil collected prior to planting to the second destructive harvest at thirteen weeks. The soils TOC significantly increased (P = 0.01) under M.M.106 from before planting to the soil analysed after nineteen weeks of growth. The TC in the soil under the three rootstocks did not significantly change over nineteen weeks of tree growth.

Table 2.1. Mean total percentage of soil C (TC) and its fractions (total organic carbon (TOC) and total inorganic carbon (TIC) and standard errors (±) for three collection point for all rootstocks. Asterisk (*) indicate significant changes over time and blue triangles indicate significant differences between rootstock at individual harvests.

Harvest, rootstock, and soil region	**TC %**	**TOC %**	**TIC %**	**Organic matter %**
Pre - *bulk soil*	5.378 (± 0.05)	4.386 (± 0.09) *	0.992 (± 0.04) *	7.56 (± 0.15) *
13 weeks (rootzone soil)				
M.9	5.410 (± 0.09)	4.547 (± 0.12)	0.863 (± 0.03)	7.84 (± 0.20)
M.116	5.507 (± 0.20)	4.617 (± 0.16)	0.890 (± 0.06)	7.96 (± 0.28)
M.M.106	5.361 (± 0.10)	4.513 (± 0.10)	0.848 (± 0.02) *	7.78 (± 0.18) *
19 weeks (rootzone soil)				
M.9	5.606 (± 0.18)	4.676 (± 0.18)	0.930 (± 0.04)	7.78 (± 0.15) ▲
M.116	5.761 (± 0.19)	4.878 (± 0.18)	0.882 (± 0.05)	7.86 (± 0.22)
M.M.106	5.684 (± 0.12)	4.774 (± 0.06) *	0.910 (± 0.09)	8.26 (± 0.08)*▲

At six weeks after planting (harvest point 1) the microbial biomass carbon (MBC) of the bulk soil was significantly higher than the rootzone (P = 0.03) under the M.116 trees. The bulk of M.M.106 was significantly lower in concentrations of MBC than M.116 (P = 0.04). Comparing soil regions for each rootstock between the first and second harvest, M.116 and M.M.106 rootzone soils had significant increases (P = 0.01 and P = 0.02) respectively. By 19 weeks (final harvest), the levels of MBC in the soil had significantly declined across all three rootstocks and bulk and rootzone soil regions from the 13-week destructive harvest. (**Fig 2.6 A and B**).

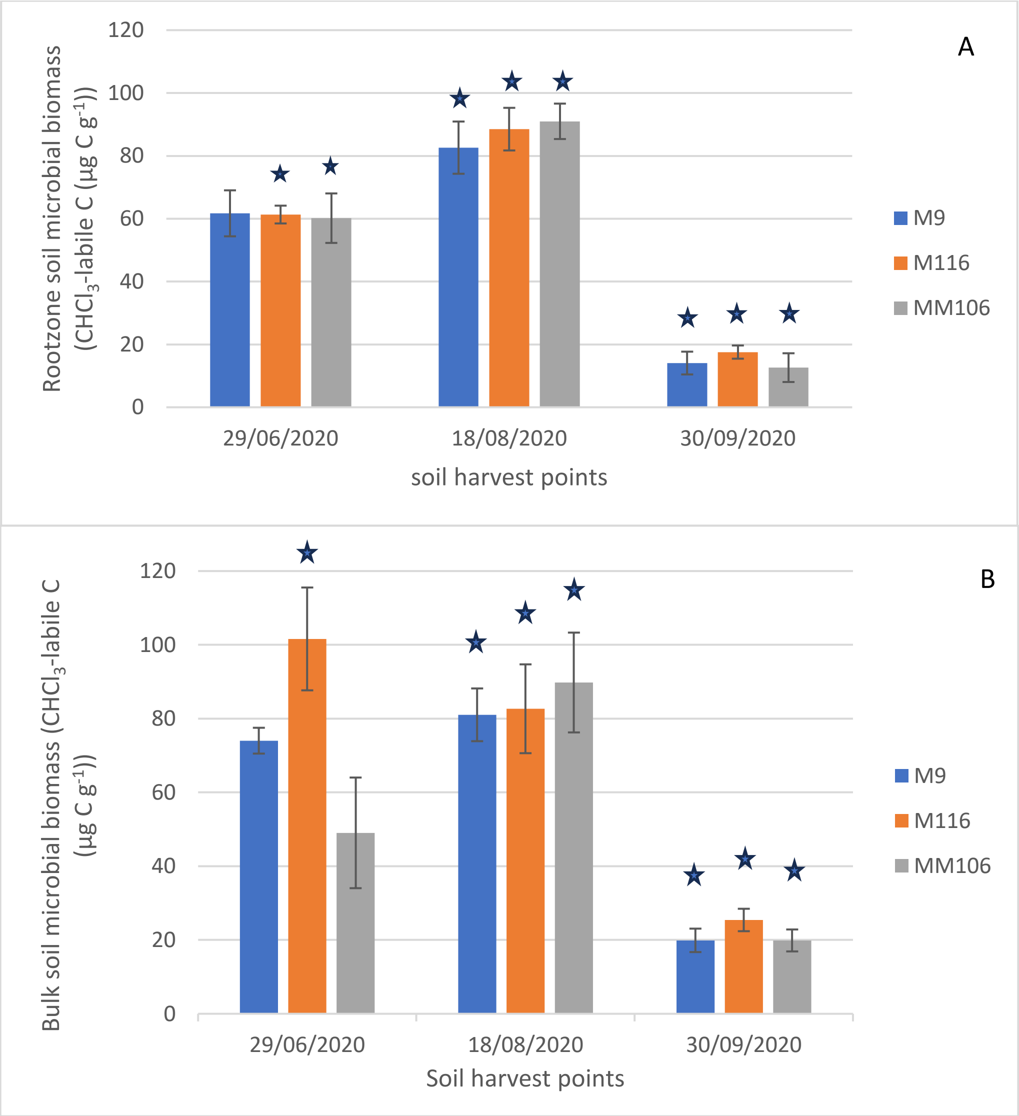


Fig 2.6 A and B. Microbial biomass carbon content across the three harvest points. A) rootzone B) bulk soils. Significant changes across harvest points are indicated with an asterisk (*).

The soils active C (POXC) levels prior to planting showed a mean of 451.74 (± 41.04) mg kg^{-1}. By 13 weeks post planting a significant difference between the bulk and rootzone regions were observed ($P = 0.001$), with the rootzone having the highest concentrations of POXC. There was a high amount of variance within both soil regions. M.9 and M.M.106 both had significant differences in POXC between the soil regions ($P = 0.02$ and $P = 0.01$ respectively) with rootzone being higher than the bulk soil. By the final harvest (**Fig 2.7**) at 19 weeks, the POXC of the blank rhizotrons compared to the soil assessed prior to planting was significantly reduced ($P = 0.002$). There was no significant difference between the three rootstocks. M.116 was the only rootstock to have a significant difference between the two soil regions ($P = 0.02$) with the rootzone having higher levels of active C than the bulk soil. The levels of POXC in both soil regions under the three rootstocks at the final harvest were all significantly lower than the soil samples prior to planting.

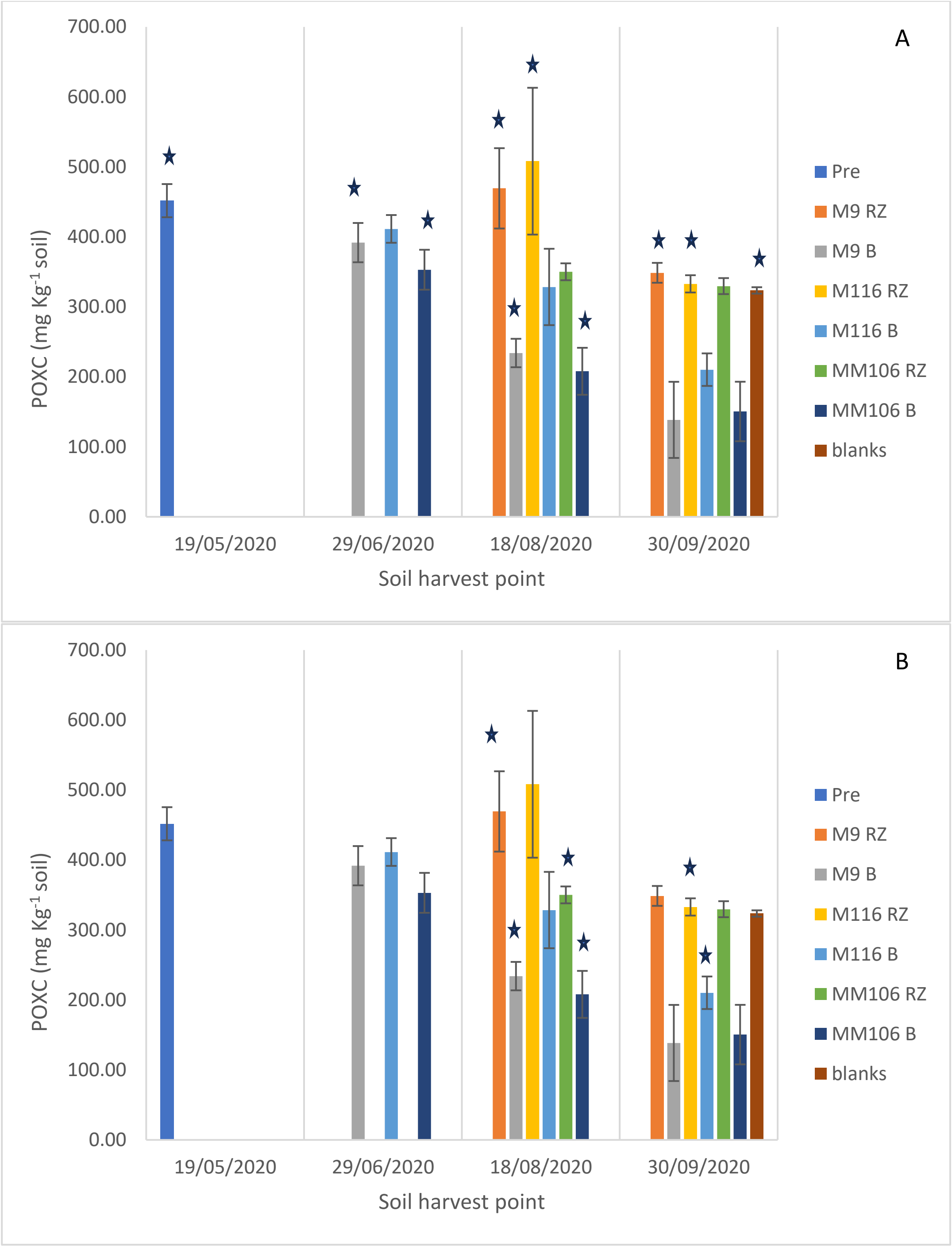


Fig 2.7 A and B. POXC of soil collected pre planting, at the three harvest points (harvest 1 and 2 n=4 and the final harvest n=6) and the four blank rhizotrons. **A,** show significant changes in POXC over time, and **B**, shows differences between soil regions in POXC concentrations. Significant differences indicated by stars (P = <0.05).

2.4.3. Soil nitrogen and nitrate

The soil total % nitrogen (TN) from pre-planting to the final harvest at 19 weeks within the fertigated blank rhizotrons significantly increased ($P = 0.03$), and the soil under M.116 and M.M.106 had also significantly increased from before planting ($P = 0.03$ and $P= 0.01$ respectively). The soil under the rootstock M.M.106 showed a significant increase between harvest 2 and 3 ($P = 0.01$) and M.116 was close to being significant ($P = 0.056$) (**Fig 2.8**).

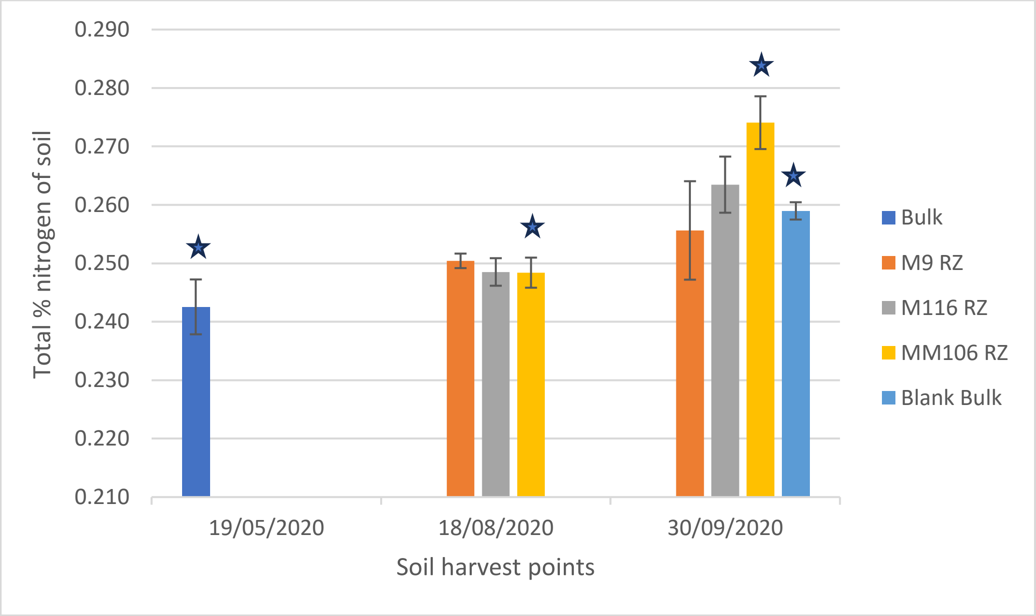
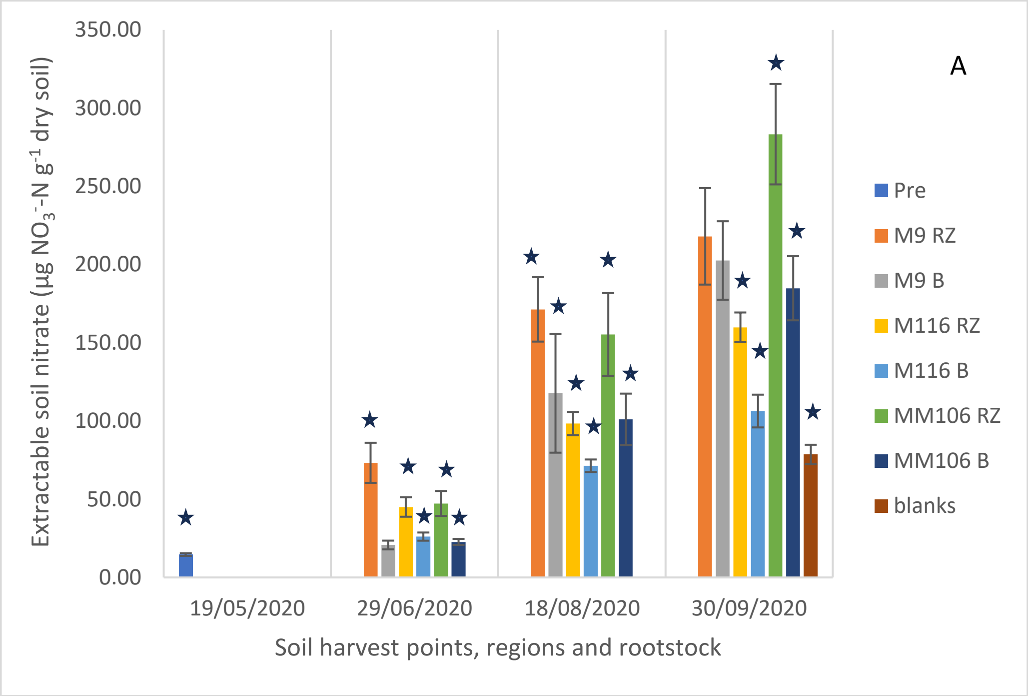
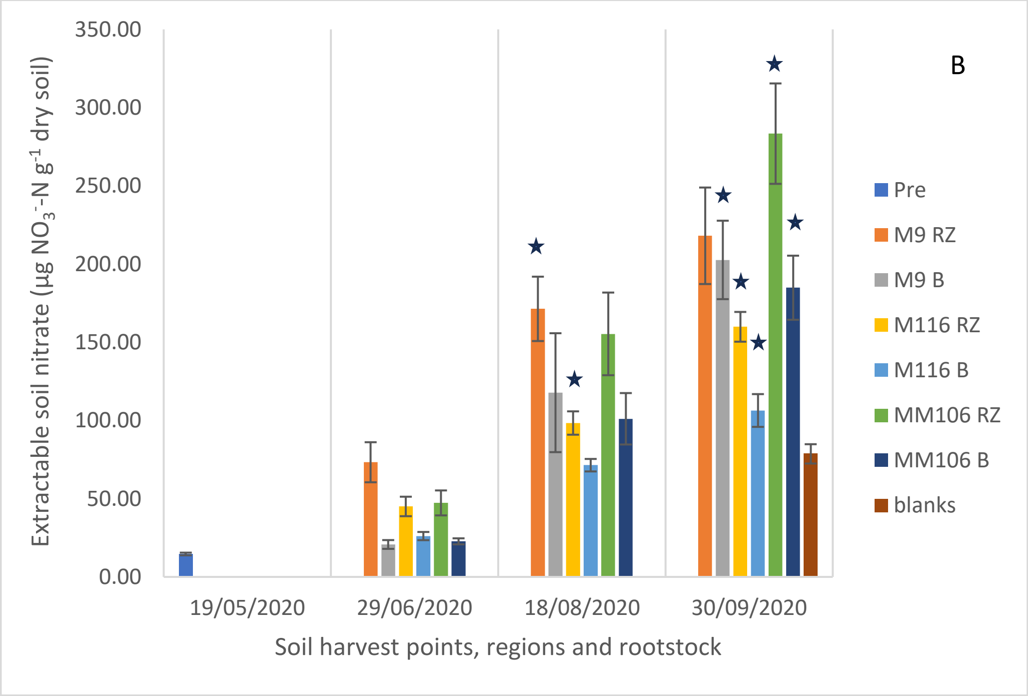


Fig 2.8. Soil total % nitrogen across rootstocks, harvest points, and soil regions. Significant increases in N indicated by stars ($P = < 0.05$) from pre to harvest 2 (H2) and final harvest (end).

Soil nitrate prior to planting, had a mean concentration of 14.66 (±1.47 SD) µg NO_3^--N g^{-1} dry soil (**Fig 2.9**). Six weeks post planting M.9, M.116 and M.M.106 rootzone soils were significantly higher than the nitrate concentration found in the bulk soil ($P = 0.01$, $P = 0.03$ and $P = 0.02$ respectively). Thirteen weeks post planting M.9 had significantly higher soil concentrations of nitrate within the rootzone soil than M.116 ($P = 0.02$), but only M.116 had a significant difference between the two soil regions ($P = 0.02$), the rootzone having the higher concentration. By nineteen-weeks nitrate concentrations in the rootzone and bulk soils of both

M.116 and M.M.106 had both significantly increased (M.116 rootzone P = 0.02 and bulk P = 0.03 and P = 0.02 for M.M.106). M.9 and M.M.106 bulk soils were significantly higher in concentration than M.116 (P = 0.05 and 0.01 respectively), and only M.M.106 rootzone soil was significantly higher in nitrate than M.116 (P = 0.003). Both M.116 and M.M.106 soil regions were significantly different from each other (P = 0.004 and P = 0.03 respectively).





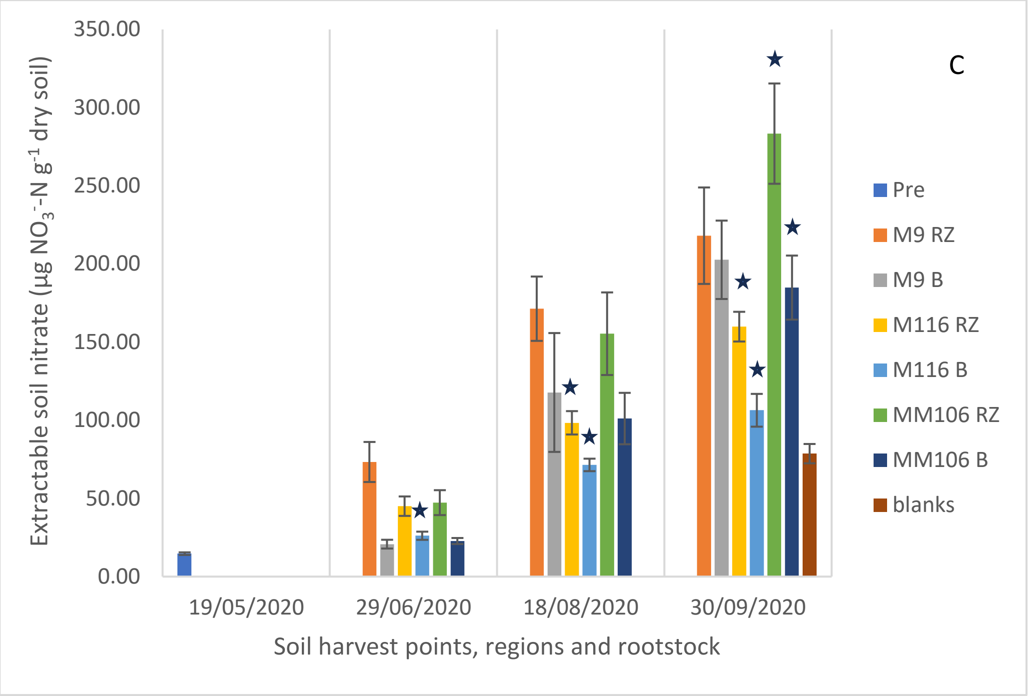


Fig 2.9 A, B, and C. Extractable soil nitrate concentrations over the course of 19 weeks including samples collected prior to planting and their standard errors. **A** shows differences between harvest points, **B** shows differences between rootstocks and **C,** significant differences between bulk and rootzone soil samples. Significant differences indicated by a star, (P = 0.05).

2.4.4. Biomass carbon contents.

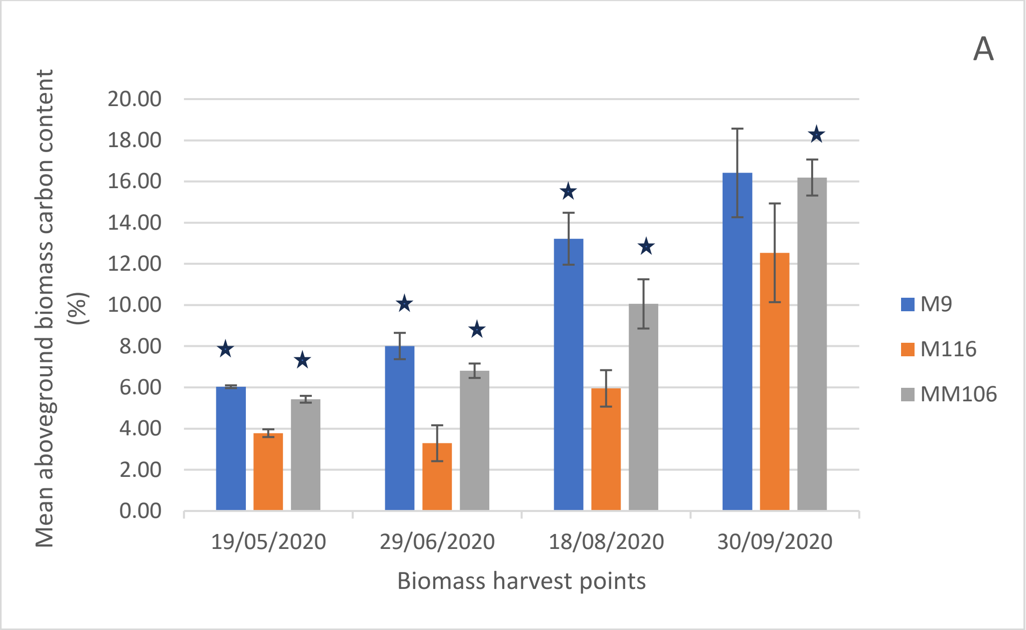
Biomass carbon of the belowground (BG) and aboveground (AG) showed significant changes between rootstocks and harvest points (**Table 2.2** including the total biomass C). The aboveground biomass included some of the rootstock that was above the top of the rhizotron as this enabled equal harvesting (**Fig 2.4**). The AG biomass C (**Fig 2.10 A**) of the trees harvested prior to planting showed that the scions grafted on to M.9 had significantly higher concentrations than either M.116 (P = <0.00001) or M.M.106 (P = 0.02). M.M.106 also had significantly higher C concentration than M.116 (P = 0.001). By six weeks post planting M.116 grown trees were still significantly lower in biomass C than either M.9 (P = 0.01) and M.M.106 (P =0.01). At the thirteen-week destructive harvest the trees grown on both M.9 and M.M.106

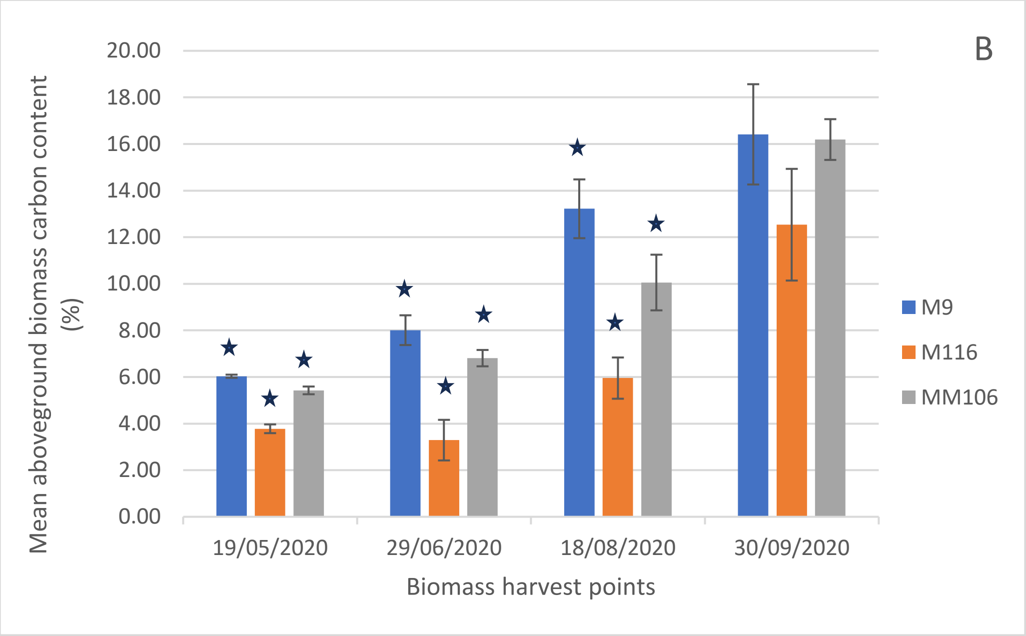
had significantly higher C content than those on the M.116 rootstock (M.9 v M.116 P = <0.01 and M.116 V M.M.106 P = 0.03). By the final destructive harvest, the was no significant difference between the AG biomass C content of the trees grown on the three rootstocks.

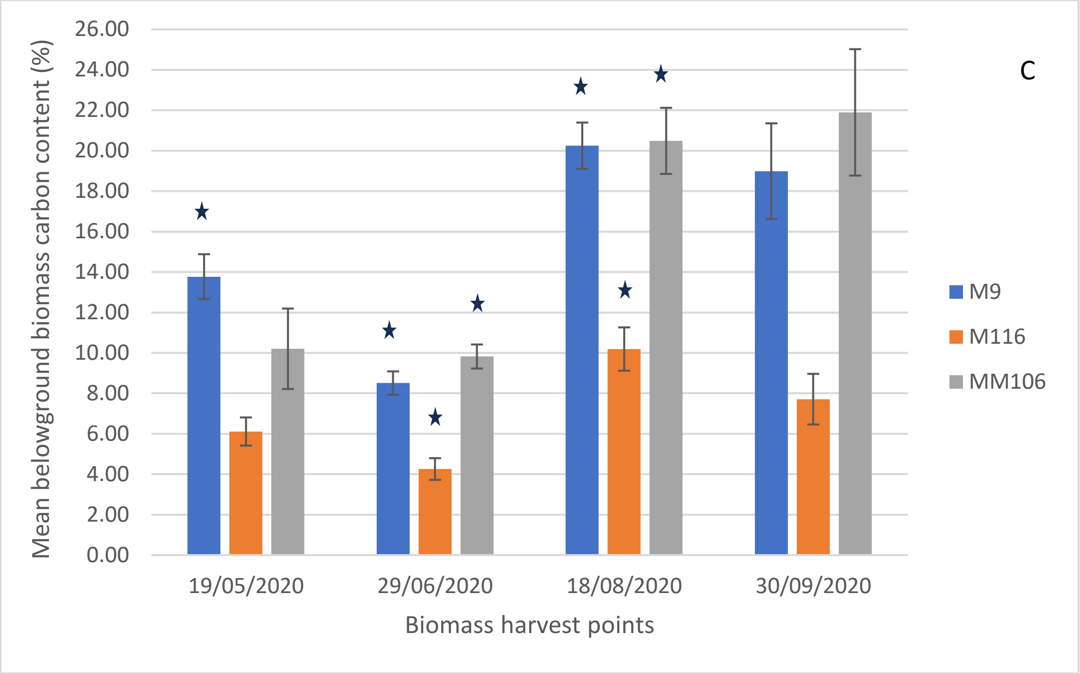
The BG biomass C of the trees harvested before planting showed that M.9 rootstock trees had significantly higher C Content than M.116 (P = 0.001). At the six-week destructive harvest the C content was found to be lower than those that had not been planted. M.116 had significantly lower concentrations of C than either M.9 (P = <0.001) and M.M.106 (P = 0.001). By thirteen weeks post planting M.9 and M.M.106 continued to be significantly higher in biomass C content than M.116 (P = 0.001 and P = <0.01 respectively). The BG biomass C (**Fig 2.10 B**) of M.9, M.116 and M.M.106 were all significantly higher at the second harvest compared to the first destructive harvest (P = 0.0001, P = < 0.01 and P = <0.001 respectively post planting). At the final harvest (nineteen weeks) the belowground biomass C of the trees on the various rootstocks showed that M.116 still had significantly lower C content than both M.9 (P = <0.01) and M.M.106 (P = <0.01).

Table 2.2. Mean biomass C for trees (Total, AG, and BG) across the experiment for each of the three rootstocks under investigation. Asterisk indicating the significant difference at each of the harvests between rootstocks (P = 0.05*, 0.01 **, 0.001 ***, 0.0001****).

Rootstock/ harvest point	**Mean Total Biomass C (g)**	**Mean AG Biomass C (g)**	**Mean BG Biomass C (g)**
Pre planting			
M.9	19.80 **	6.03 ***	13.77 ***
M.116	9.89	3.78	6.11
M.M.106	15.63 *	5.43 ***	10.21
Harvest 1 (6 weeks)			
M.9	16.51 ***	8.01 **	8.51 **
M.116	7.54	3.29	4.26
M.M.106	16.62 ****	6.81 **	9.82 ***
Harvest 2 (13 Weeks)			
M.9	33.47 ****	13.22 **	20.25 ***
M.116	16.14	5.95	10.19
M.M.106	30.54 ***	10.06 *	20.49 **
Harvest 3 (19 weeks)			
M.9	35.40 ***	16.42	18.99 **
M.116	18.28	12.54	7.71
M.M.106	38.08 ***	16.19	21.90 **


A
Mean aboveground biomass carbon content (%)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
19/05/2020
29/06/2020
18/08/2020
30/09/2020
Biomass harvest points
M9
M116
MM106


B
Mean aboveground biomass carbon content (%)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
19/05/2020
29/06/2020
18/08/2020
30/09/2020
Biomass harvest points
M9
M116
MM106



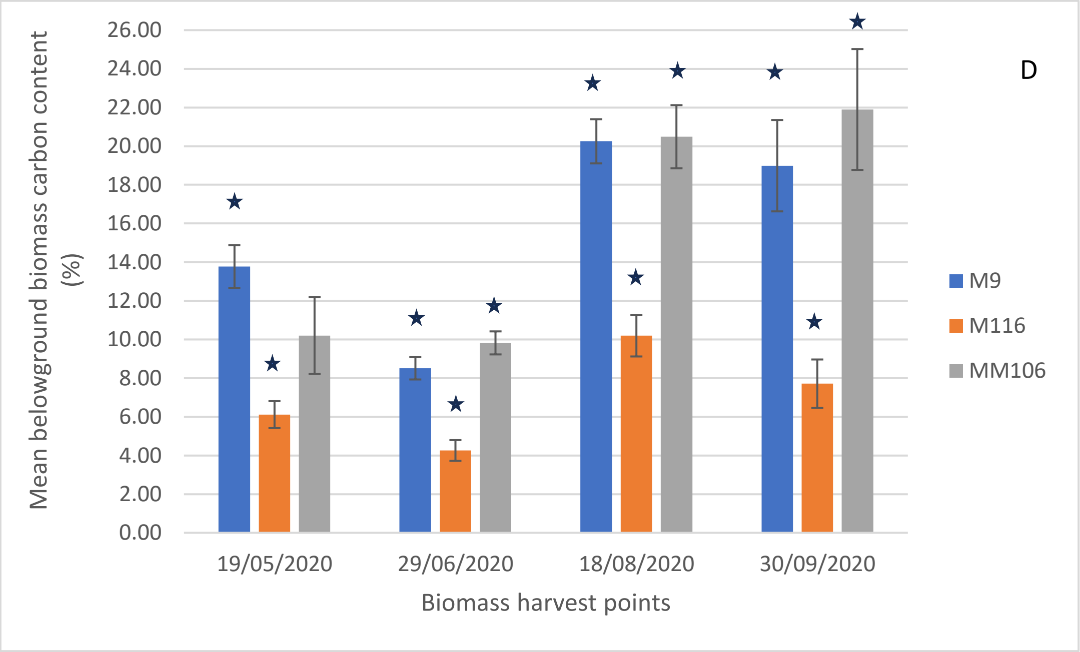


Fig 2.10 A, B, C, and D. Biomass carbon of every tree across the experiment showing the variability of C between individual trees and rootstocks **A and B**) aboveground biomass carbon and **C and D**) belowground biomass carbon. **A** and **C** shows significant changes over time and **B** and **D** show significant differences between rootstocks. The stars shows where significant changes between destructive harvest occurred (P = <0.05).

The total C and N (%) of dry mass was determined for the roots of the six trees of each rootstock harvested at the end of the experiment (19 weeks post planting). There were no significant differences between the rootstocks for either C or N content (**Fig 2.11**).

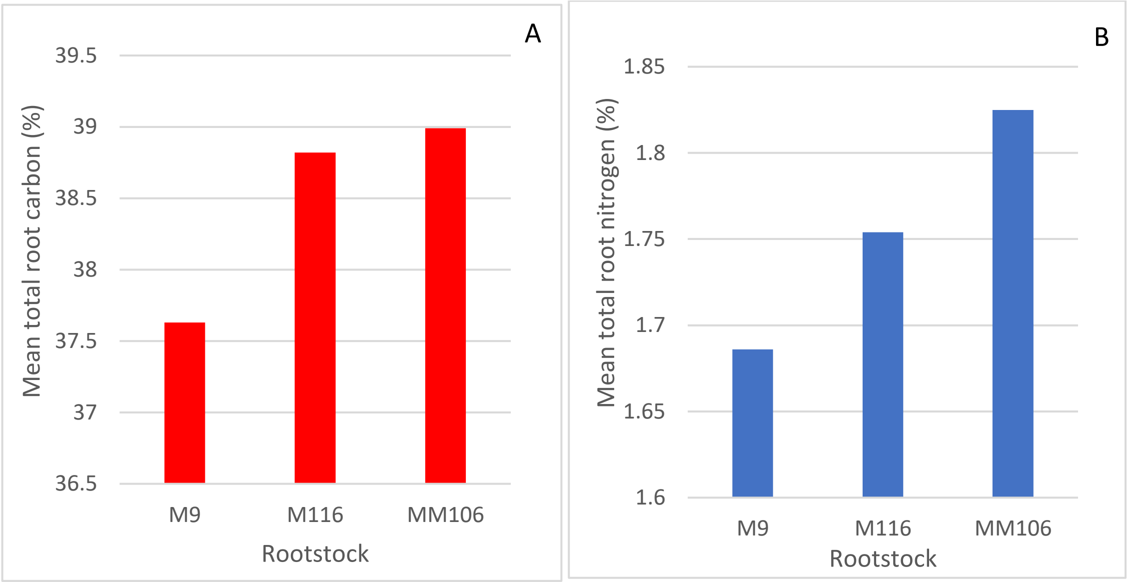
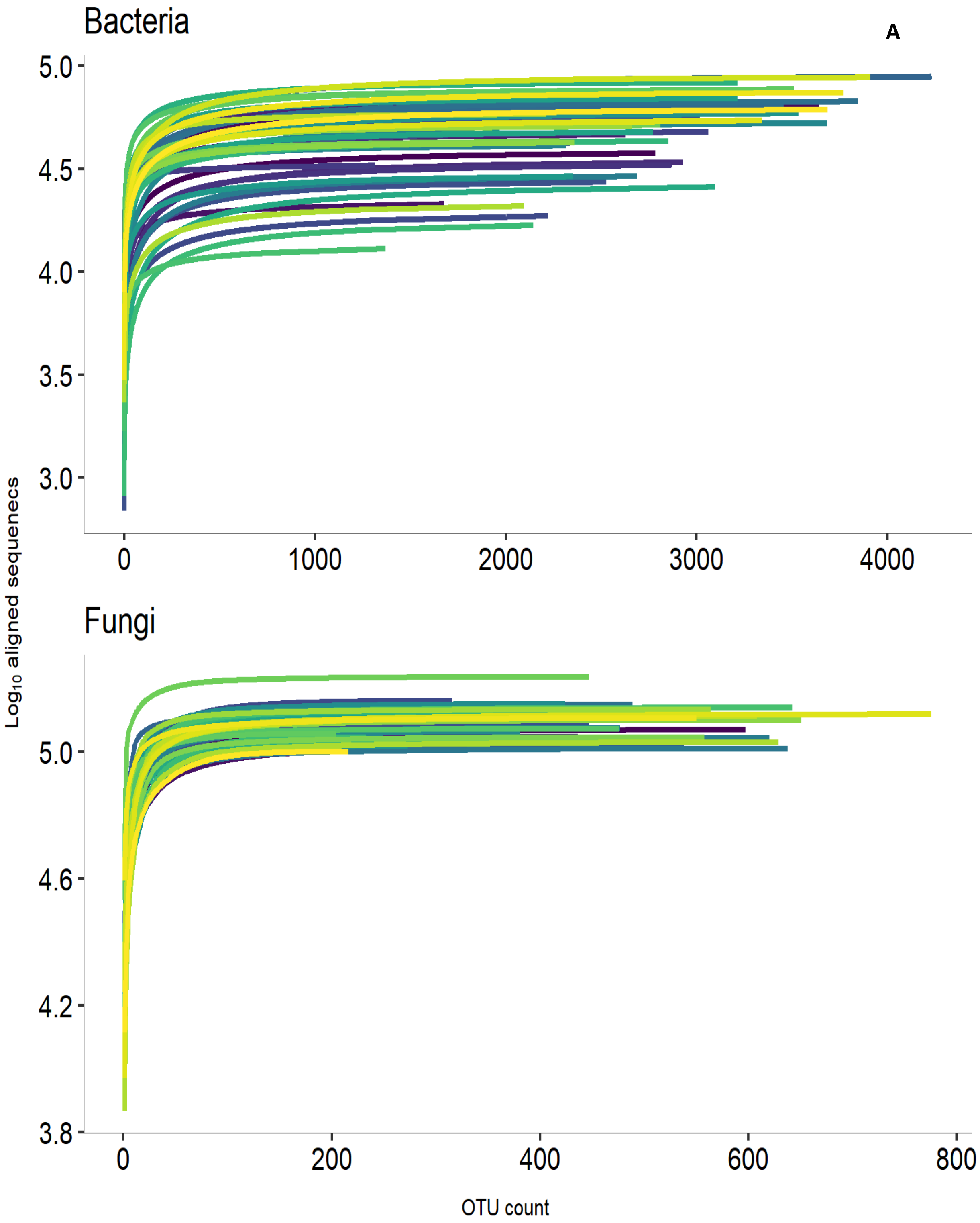


Fig 2.11 A and B. **A** Root total C and **B** root total N (%) at 19 weeks post planting, with standard error bars marked, no significant differences were found between rootstocks.

2.4.5. DNA fungi and bacteria

The DNA data for the rootzone (RZ) collected from 1 cm around the roots and the rhizosphere (RS) soil directly brushed off the roots, showed a large difference in the amount of operational taxonomic units (OTUs) present within the soil. Both soil regions having lower abundance of bacteria (total raw reads RS with 1984542 and RZ 2078445 OTUs), than fungi (total raw reads RS with 4447912 and RZ 5176759 OTUs) (**Fig 2.12**). Following normalisation of the OTUs the rootzone had a total number of 1803 with a mean read of 3036.994 per OTU for fungi and the bacteria had a total of 6278 with the mean reads of 310.3859. In the rhizosphere the total OTUs were 1433 with a mean number of read 3248.917 for fungi and for bacteria total number was 6235 with a mean read per OTU of 261.9811.


Bacteria
A
5.0
4.5
4.0
3.5
3.0
0
1000
2000
3000
4000
Fungi
5.0
4.6
4.2
3.8
0
200
400
600
800
Log10 aligned sequenecs
OTU count

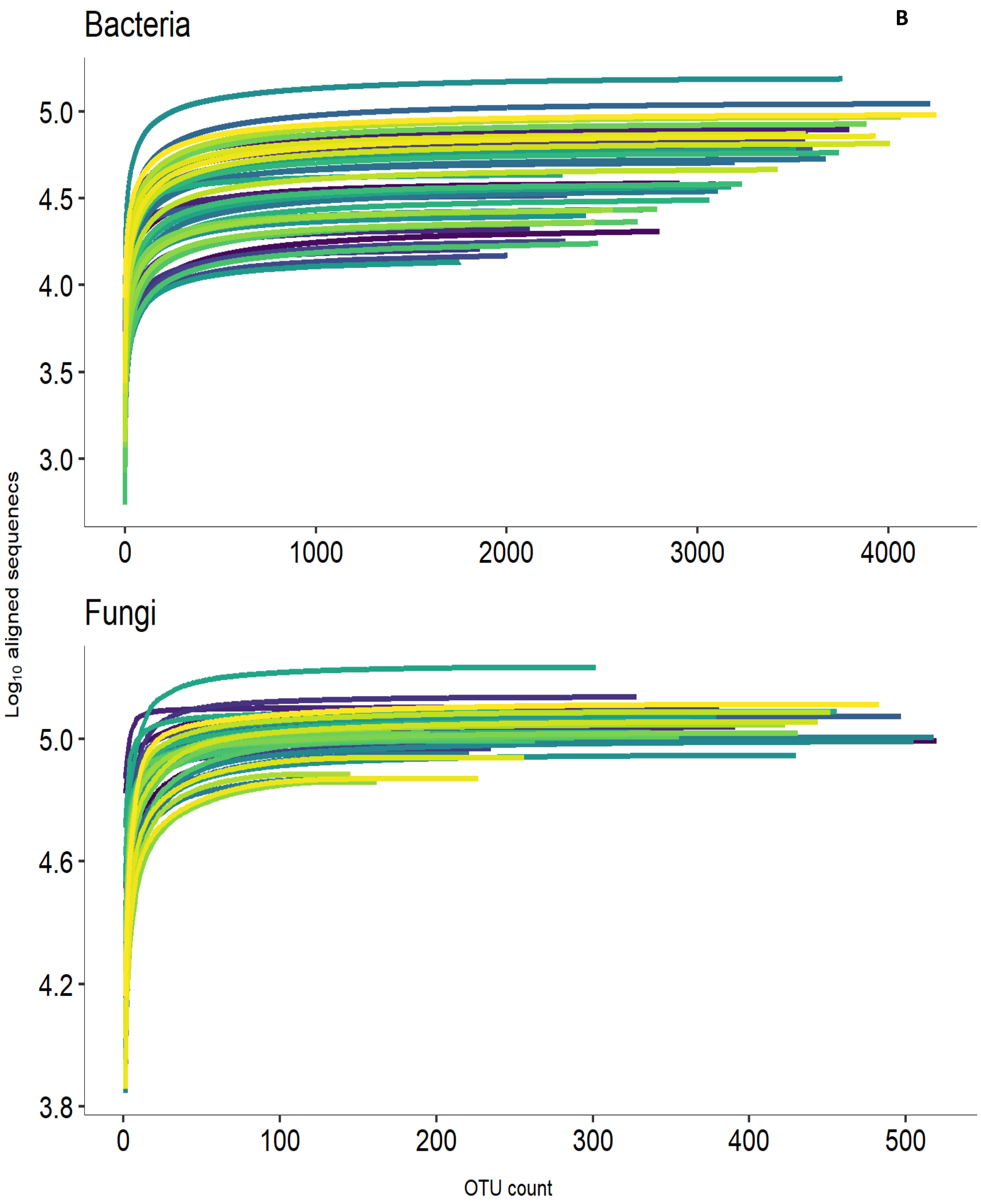


Fig 2.12 A and B. OTU counts for bacteria and fungi. **A**) within the rootzone soil and **B**) with the rhizosphere.

2.4.4.1. Fungi

The soil fungi at the phylum level, Ascomycota was the most abundant fungi within both soil regions and across all rootstocks for the first two harvests. At the final harvest point within the rhizosphere soils Mucoromycota had become the most abundant fungi present. At a class level Sordariomycetes are the most abundant in the rootzone across harvest points and rootstocks. The rhizosphere for the first two harvests and all rootstocks Sordariomycetes were the most abundant, but by the final harvest M.116 and M.M.106 mortierellomycetes were the most abundant but for M.9 it was Mucoromycetes.

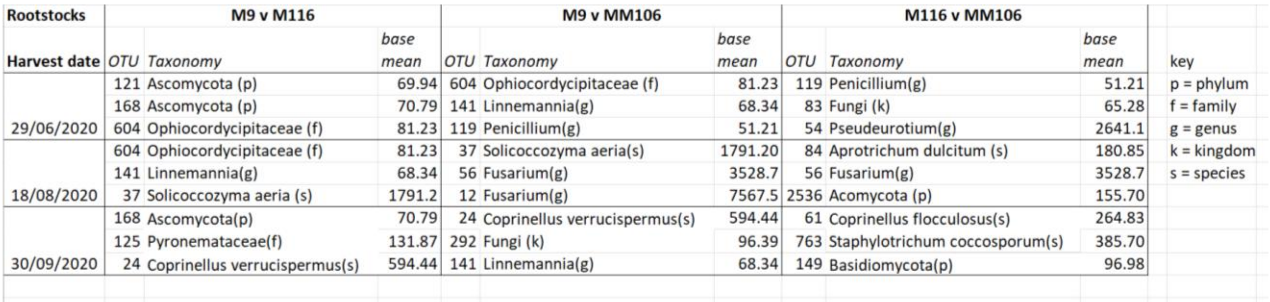
The alpha diversity showed that the rootzone across all three rootstocks under Chao1, harvest date (P = 0.001) was the only significant effect on fungi diversity, whereas in the rhizosphere soil, the harvest date was the only significant factor across all diversity plots (Chao1, Shannon, and Simpson). Rootstocks showed no significant effect on the diversity of fungi species present in the soil regions. The differential analysis was carried out for each harvest point between each of the rootstocks to see if there were differences in fungi present, under the two different soil regions – rootzone and rhizosphere.

Rootzone

Overall, the numbers of different fungi fluctuated between the harvest points, between the first and second harvest the numbers of differences declined but by the third and final harvest in September 2020 the number of different OTUs had increased. Between the different rootstocks at each of the three destructive harvest the number of OTUs varied, the top three OTU's between the three rootstocks are listed below (**Table 2.3**). At the first harvest point in June 2020 there were significant differences in the fungi present between the different rootstocks, M.9 v M.116 28 rows of different OTUs were found. M.9 v M.M.106 and M.116 v M.M.106 had 15 rows of OTUs that were significantly different in abundance. By August 2020

(2^{nd} harvest) M.9 v M.116 and M.116 v M.M.106 both had 7 OTU lines and M.9 v M.M.106 had 11 rows. The final harvest in the September, M.9 v M.116 16 rows, M.9 v M.M.106 19 rows and M.116 v M.M.106 13 rows of different fungi present.

Table 2.3. Harvest point and rootstocks difference in fungi found within the rootzone; OTU number, Taxonomy, and base means of the top three.

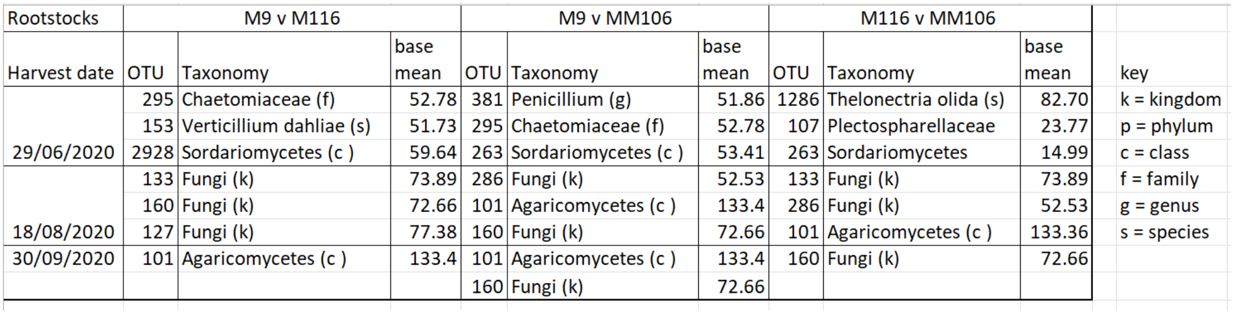


Rootstocks	M9 v M116			M9 v MM106			M116 v MM106			
Harvest date	*OTU*	*Taxonomy*	*base mean*	*OTU*	*Taxonomy*	*base mean*	*OTU*	*Taxonomy*	*base mean*	key
29/06/2020	121	Ascomycota (p)	69.94	604	Ophiocordycipitaceae (f)	81.23	119	Penicillium(g)	51.21	p = phylum
	168	Ascomycota (p)	70.79	141	Linnemannia(g)	68.34	83	Fungi (k)	65.28	f = family
	604	Ophiocordycipitaceae (f)	81.23	119	Penicillium(g)	51.21	54	Pseudeurotium(g)	2641.1	g = genus
18/08/2020	604	Ophiocordycipitaceae (f)	81.23	37	Solicoccozyma aeria(s)	1791.20	84	Aprotrichum dulcitum (s)	180.85	k = kingdom
	141	Linnemannia(g)	68.34	56	Fusarium(g)	3528.7	56	Fusarium(g)	3528.7	s = species
	37	Solicoccozyma aeria (s)	1791.2	12	Fusarium(g)	7567.5	2536	Acomycota (p)	155.70	
30/09/2020	168	Ascomycota(p)	70.79	24	Coprinellus verrucispermus(s)	594.44	61	Coprinellus flocculosus(s)	264.83	
	125	Pyronemataceae(f)	131.87	292	Fungi (k)	96.39	763	Staphylotrichum coccosporum(s)	385.70	
	24	Coprinellus verrucispermus(s)	594.44	141	Linnemannia(g)	68.34	149	Basidiomycota(p)	96.98	

Rhizosphere

Across the three harvests and rootstocks, the number of the OTUs under each of the combinations of rootstocks declined, with the most differences being lost between the first and second harvest points. Between the different rootstocks at each of the three destructive harvest the number of OTUs changed, the top three are shown below (**Table 2.4**). The first harvest in June M.9 v M.116 13 rows of OTUs, M.9 v M.M.106 19 rows and M.116 v M.M.106 11 rows. In August (13 weeks post planting) M.9 v M.116 and M.116 v M.M.106 both had 3 rows and M.9 v M.M.106 had 4 OTU rows that were different between the rootstocks. The September harvest M.9 v M.116 and M.116 v M.M.106 had 1 different OTU whereas M.9 v M.M.106 had two different OTUs.

Table 2.4. Harvest point and rootstocks difference in fungi found within the rhizosphere with OTU number, Taxonomy, and base means of the top three.



Rootstocks	M9 v M116			M9 v MM106			M116 v MM106			
Harvest date	OTU	Taxonomy	base mean	OTU	Taxonomy	base mean	OTU	Taxonomy	base mean	key
29/06/2020	295	Chaetomiaceae (f)	52.78	381	Penicillium (g)	51.86	1286	Thelonectria olida (s)	82.70	k = kingdom
	153	Verticillium dahliae (s)	51.73	295	Chaetomiaceae (f)	52.78	107	Plectosphaerellaceae	23.77	p = phylum
	2928	Sordariomycetes (c)	59.64	263	Sordariomycetes (c)	53.41	263	Sordariomycetes	14.99	c = class
18/08/2020	133	Fungi (k)	73.89	286	Fungi (k)	52.53	133	Fungi (k)	73.89	f = family
	160	Fungi (k)	72.66	101	Agaricomycetes (c)	133.4	286	Fungi (k)	52.53	g = genus
	127	Fungi (k)	77.38	160	Fungi (k)	72.66	101	Agaricomycetes (c)	133.36	s = species
30/09/2020	101	Agaricomycetes (c)	133.4	101	Agaricomycetes (c)	133.4	160	Fungi (k)	72.66	
				160	Fungi (k)	72.66				

2.4.4.2. Bacteria

The bacteria showed changes and some differences between rootstocks and harvest points. At the Phylum level, for both soil regions and rootstocks proteobacteria were the most abundant. The Alpha diversity (**Fig 2.13**) analysis showed that for Shannon and Simpson that the harvest date was important to the levels and varieties of bacteria that were detected within both soil regions under investigation (rootzone and rhizosphere). Beta diversity analysis showed harvest dates had the greatest influence on the soil bacterial communities.

Differential analysis showed that the first harvest date (end of June 2020), for the rootzone soil *Pseudomonas* concentrations were significantly different between M.9 and M.116. By the second harvest point there were no significant differences in the bacterial communities found between any of the three rootstocks. At the final harvest date (end of September 2020) M.9 v M.116 there were 25 rows of OTUs, M.9 v M.M.106 30 rows of OTUs and for M.116 v M.M.106 28 rows of OTUs of different bacterial communities (**Table 2.5**).

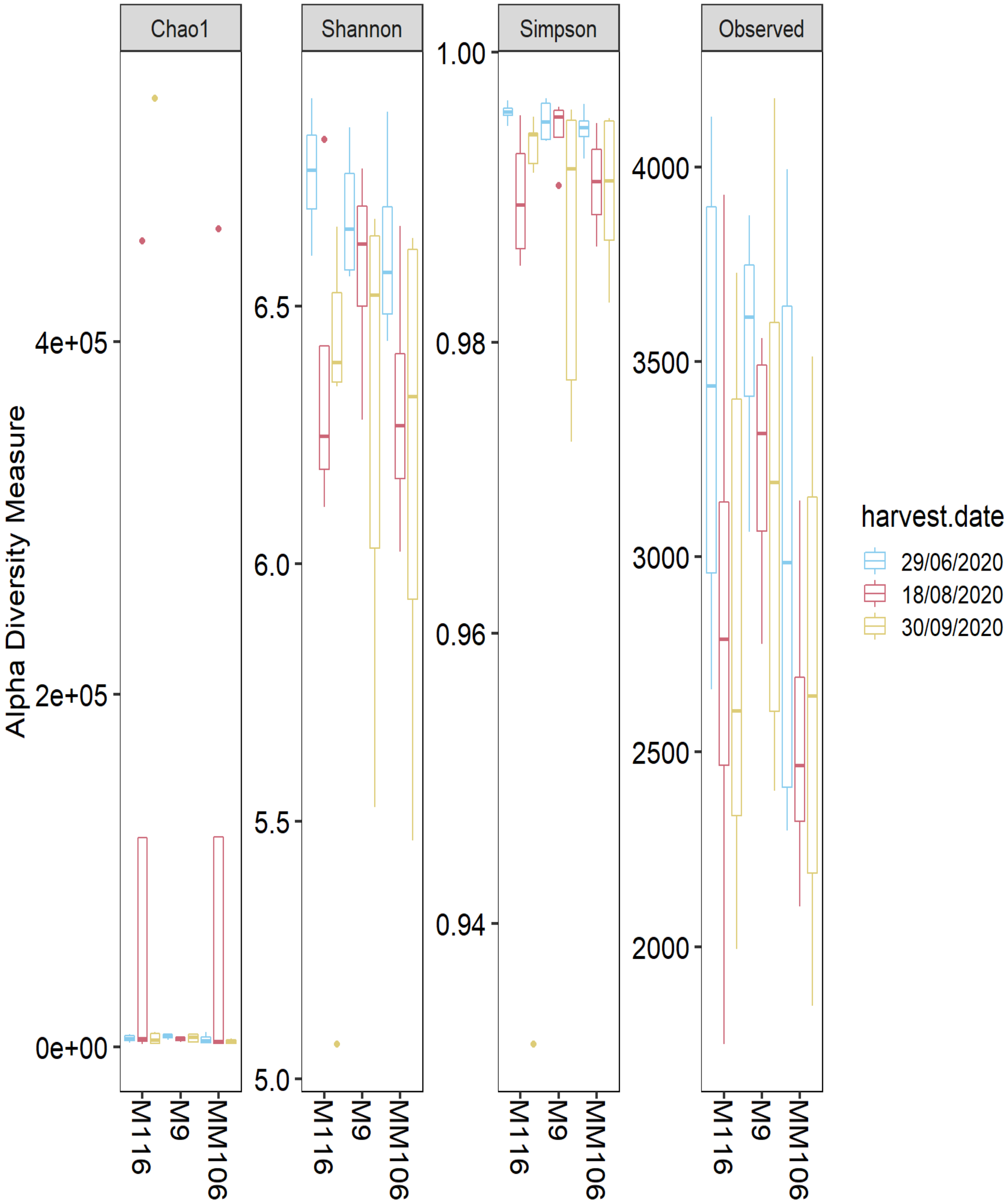
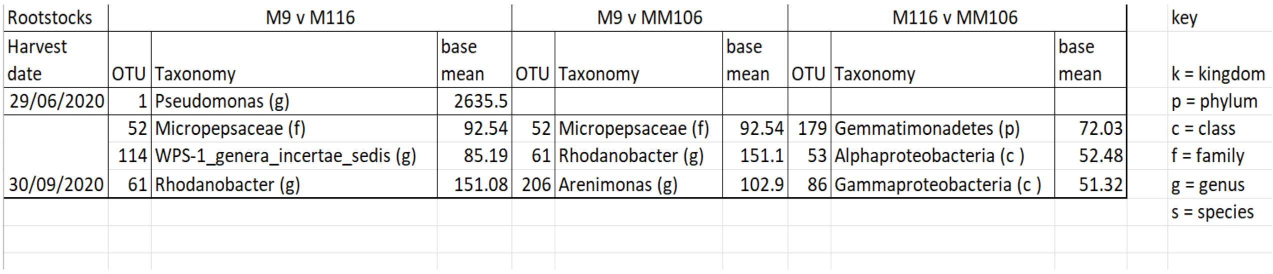


Fig 2.13. Alpha diversity of bacteria found within the soil for the rhizosphere soils.

Table 2.5. Bacterial differences in the rootzone soil under the three different rootstocks.



Rootstocks	M9 v M116			M9 v MM106			M116 v MM106			key
Harvest date	OTU	Taxonomy	base mean	OTU	Taxonomy	base mean	OTU	Taxonomy	base mean	k = kingdom
29/06/2020	1	Pseudomonas (g)	2635.5							p = phylum
	52	Micropepsaceae (f)	92.54	52	Micropepsaceae (f)	92.54	179	Gemmatimonadetes (p)	72.03	c = class
	114	WPS-1_genera_incertae_sedis (g)	85.19	61	Rhodanobacter (g)	151.1	53	Alphaproteobacteria (c)	52.48	f = family
30/09/2020	61	Rhodanobacter (g)	151.08	206	Arenimonas (g)	102.9	86	Gammaproteobacteria (c)	51.32	g = genus
										s = species

The rhizosphere soil at the first harvest (6 weeks post planting), showed one significant bacterial difference between M.9, M.M.106 and M.116 which was for OTU8 -Micrococcaceae. The destructive harvests in August and September had no significant differences in bacterial communities between any of the three rootstocks under investigation.

The correlation analysis showed that the laboratory analysis was not consistently significant across or between the three rootstocks or destructive harvest points. At the six- and thirteen-weeks destructive harvest only M.9 and M.M.106 had any correlations, but by the final harvest all rootstocks had correlations (Appendix 1).

2.5. Discussion

The intentions of this experiment were to 1) Investigate whether different commercial rootstocks influenced the amount of belowground C sequestration. 2) Determine if there were differences in C sequestration between bulk and rootzone soil regions. 3) Examine if microbial communities varied between rootstocks in the two soil regions closest to the root system (rootzone and rhizosphere). It must be kept in mind that the trees were destructively harvested, and so repeated measurements were not possible, and all trees were fertigated with a N rich feed.

Throughout the experiment the daily fertigation levels were increased to keep up with the trees' requirement and increased the soil moisture content. Soil moisture content has been shown to limit the ability of the soil to store C, limit microbial activity, limit oxygen within the soil as the pore spaces are filled with water (Bouma and Bryla, 2000; Zhang *et al.*, 2021). Several studies have assessed the effect that low soil moisture contents on tree growth (Talluto *et al.*, 2008; Toselli *et al.*, 2014), but only the study by Talluto et al. (2008) showed that a lack of water can affect vegetive growth but not fruit yield. This study's limited C sequestration, and biomass growth especially in M.116 could be an impact to possible over fertigation, and the soil becoming waterlogged.

The soil pH_{CaCl2} (**Fig 2.5**) under the two soil regions sampled (bulk and rootzone) all increased towards neutral by the end of the experiment. The bulks soil showed a significant increase in pH across the three rootstocks compared to the rootzone soil. This significant change in the bulk soil pH could be due to the N rich feed, whereas the change in the rootzone soil is more likely to be influenced by root exudations which can help the tree gain nutrients required for growth as described by Wang et al. (2016) and Vives-Persi et al. (2020). Variability between individual trees was observed in all laboratory analysis. These differences between the trees, could be accounted for by variations in the soil before planting, positions within the glasshouse, temperature and light availability, seasonal variations, and the natural differences in the tree growth (vigour).

The rootzone soil total C (**Table 2.1**) did not significantly change over the course of this study, but an increase was seen. The rate at which C was sequestered into the soil may be slow due to the need for the tree to become established, put out roots and aboveground growth which all requires C, so the partitioning of the available C is likely to be an important factor at this point in the tree's life. This can be seen in the increase in both the above and belowground biomass between each destructive harvest. The total soil organic and inorganic C showed a significant change in the soil in the rootzone under M.M.106, but this occurred at different destructive harvest. The soil TOC (**Table 2.1**) by the 19-weeks harvest would be being increased from the root debris as the root grows (Dijkstra, Zhu and Cheng, 2021; Yang *et al.*, 2023) and the increase that had occurred in the soil microbes (**Fig 2.6. A and B**) and their deaths (Sharififar *et al.*, 2023). The decrease in the soil inorganic C levels at thirteen weeks under the M.M.106 could be due to the increase in the percentage of TOC and organic matter, change in the soil pH and moisture content, which could leach the inorganic C out of the soil (Ferdush and Paul, 2021; Sharififar *et al.*, 2023).

The soil organic matter (**Table 2.1**) altered between the final two harvests with M.116 and M.9 soils declining and M.M.106 increasing, and a significant difference was observed between M.9 and M.M.106. This alteration in the organic matter found between the harvests could be due to the increase in the soil moisture content limiting microbial activity by filling up the soils pore spaces for those that lost SOM, whereas the increases could be due to root shedding as they grow and the growth of algae and moss in the rhizotrons. The difference between the semi vigorous M.M.106 and M.9 could be due to M.M.106 having higher levels of root debris as the amount of belowground biomass growth was larger than M.9, but this was not significant.

The rootzone soils active C concentrations were always higher than those found in the bulk soil, which decline throughout the experiment under all three rootstocks (**Fig 2.7**). This difference between soil regions and the increase within the rootzone up to 13 weeks may be attributed to the possible increase in root exudates as the tree grows, which are released into the soil closest to the roots and consumed by microbes. By the destructive harvest at 19-weeks (end of September), the day length and temperatures were reducing, the leaves on the tree were starting to change colour as the tree prepared for dormancy, so reducing their ability to photosynthesize and limiting exudation for microbes to feed upon. The soils moisture could also be having an impact on the active carbon as the soil pores would be filled with water limiting the rate of activity and rate of growth of the soil microbes (Franzluebbers, Hons and Zuberer, 1996; Singh and Kumar, 2021).

Soil microbial C content had increased from six weeks to thirteen weeks post planting for all but M.116 bulk soil which decreased, but by the final harvest (**Fig 2.6.B**) all soil regions under the three rootstocks had significantly decreased from the concentrations found seven weeks earlier (**Fig 2.6.A**). This decline in microbial soil C levels at the final harvest may be due to the changes in the seasons, shortening day length and changes in both atmospheric and soil temperatures. These seasonal changes could be limiting root exudates that the soil microbes feed upon due to the reduction in the tree photosynthesis, as the trees start to prepare for the winter dormancy and leaf senescence. The fertigation of the soil could lead to rapid microbial reproduction but at the same time the soil moisture levels being high this could be increasing soil microbial death as the amount of oxygen for respiration would be reduced due to the soil pores being filled with water. There does not seem to be a link between the levels of the soils microbial C and the tree biomass C content or the soils overall C levels.

The increases in soil nitrate and total N levels across all rootstocks and blanks (non-planted rhizotrons), is most likely due to the high N feed (Universol ® Green, which has a 23% total N content to aid plant growth) in the daily fertigation as well as those from root exudates. The increasing soil N concentrations from the daily fertigation, enables the soil to sequester more C, which in turn supplies the plants and soil microbes with the nutrients required for growth. This relationship between increased soil N and the impact it has on soil C sequestration has been discussed by several teams of researchers (Reay *et al.*, 2008; Bala *et al.*, 2013; Zaehle, 2013), all show that as soil N increases it enables soil C sequestration to increase (discussed in **chapter 1**). The N levels in the roots of the trees sampled at the final harvest reflected the dynamics of the soil's N, increasing as the rootstocks size increased, so the ability to store more N increased.

The rhizotrons left unplanted had shown an increase in C and N over the 19 weeks of the experiment and had also received daily nitrogen-based fertigation to replicate the conditions created for the planted rhizotrons. The C increase could have come from several factors including the feed, microbial activity, and from the moss and algae growth in the rhizotron. The algae had grown where the water had collected at the base of the rhizotrons, as there was limited drainage.

The biomass C for the above and belowground material showed that initially (before planting) M.9 rootstocks had the greatest C content and M.116 had the lowest. Over the 19 weeks of the experiment, the aboveground biomass C on M.9 rootstocks had the highest content of C, whereas the largest rootstock (M.M.106) had the highest levels of belowground biomass C. One possible reason why the M.9 had the highest aboveground biomass increase over the nineteen weeks could be that these have a fast rate of growth to reach full height, matures earlier, which in turn enables earlier crop production, than the other two rootstocks which can have an overall longer economic life span than M.9.

The rootstock with the highest total biomass C was different at each of the destructive harvest changing between M.M.106 and M.9 (**Table 2.2**). This changing in total biomass between each harvest could be due to the way trees partition C, and that fact that the M.9 dwarfing trees need to establish earlier as cropping is encouraged earlier than M.116 and M.M.106. The semi vigorous M.M.106 will have overall enhancement of tree growth, both above and belowground, compared to the other two rootstocks. At 19 weeks post planting, the most vigorous tree (M.M.106 grafted with Cox's Orange Pippen) had the greatest concentration of total, and belowground biomass C, which would be expected from the semi vigorous rootstock (*All About Apple Rootstocks (FS-2022-0638) | University of Maryland Extension*, 2022). The dwarfing M.9 rootstock trees would produce the aboveground biomass quicker than other more vigorous trees for earlier fruit production but would have a smaller root system (below ground biomass C), which allow for denser plantations, than the more vigorous rootstocks that require stronger and deeper root systems. M.116 had the lowest levels of above and belowground biomass C throughout the experiment, which was unexpected due to its intermediate growth habits, but could be due to tree establishment issues, as they could be more sensitive to the soil environmental conditions including moisture content, than the other two rootstocks.

These differences between the above and belowground biomass C could be down to the trees ability to partition the absorbed C for growth and the need for nutrient absorbance by the roots to maintain the tree health. Growing the trees in rhizotrons would impede the root system growth patterns for all rootstocks to some extent, compared to those grown in the field. The root systems in the rhizotrons are unable to grow in all directions, as the boxes were only 5 cm in depth (**Fig 2.2A**), forcing the roots to grow towards the upper clear Perspex sheet and limits the field of natural root growth (Klepper and Kaspar, 1994; Busch *et al.*, 2006). Rhizotrons can be useful to observe root system architecture, but in this study root visibility was poor, as little root growth occurred near the clear Perspex panel, so root architecture was discounted as part of this investigation. Other studies have found them useful tools with different designs and imaging methods used (Busch *et al.*, 2006; Mohamed *et al.*, 2017). The total root C (%) at 19 weeks was uniform across the three rootstocks, unlike those determined by the biomass C analysis which showed M.116 to have smaller C content than the other two rootstocks (**Fig 2.11**).

This study has shown that soil fungi and bacterial communities differ over time, between rootstocks and within the two soil regions under investigation, which supports the work by Deaken et al. (2019). The OTUs showed that there were more fungi present in the soil than bacteria for both soil regions (**Fig 2.12**). The number of fungi found in the soil regions increased with the length of time the trees were grown for, but the numbers of bacteria in the soil regions declined. The decline in the number of bacteria within the soil could be down to hypoxia as a result of the soils moisture content, the change in soil pH towards neutral (Shen *et al.*, 2019), or even environmental changes as the trees were preparing for dormancy so there would be a reduction in photosynthesis and root exudation (Shukla, Vyas and Jha, 2013; Xiao *et al.*, 2021). There were also differences in soil fungal communities between the three rootstocks under investigation, levels and types changed over the nineteen weeks that the trees were grown for (**Tables 2.3 and 2.4**). Bacteria did not always have significant differences between rootstocks over the two soil regions, especially at the first and second destructive harvests (**Table 2.5**). The soil that the trees were planted in had previously been fumigated, so the bacterial and fungal communities would have been limited at the time of planting, but this was not assessed. The DNA of bacteria and fungi that had been detected is most likely to have been introduced on the rootstocks that had been grown at F P Matthews and would need time to develop. Some soil contamination may have occurred during soil preparation, planting and from the environment within the glasshouse.

2.6. Conclusions and future work.

This study has shown that none of the three rootstocks showed any greater potential than the others to sequester C belowground into the soil or into the tree roots, after nineteen weeks of growth. However, there were some significant differences observed in C and N fractions between soil regions, tree biomass C and soil microbiome for the three commercial rootstocks under investigation at each of the destructive harvests. This finding demonstrated that commercial rootstocks have little effect on belowground C sequestration and that the different soil regions will change at different rates. Soil C and N are in a state of constant flux over time, changing as the plant grows and need for nutrients alter throughout the year (seasonal variations). This flux in soil C and N can be seen under the different rootstocks as the content in the soil changes throughout the experiment. Sampling of the soil through the growing season showed that soil microbial and active C changed over time, and by the final harvest in early Autumn the content in the soil decreased significantly from those found earlier in the Summer. The changing of C through the year therefore means that consideration needs to be taken when planning sampling to provide consistent results, to avoid seasonal influence. Rootstock influence on belowground C sequestration, may take longer to show than just 5 months following planting as the trees are trying to establish themselves so a longer-term study would be required to fully answer this question.

Soil bacterial and fungal communities changed throughout the experiment in both soil regions and significant differences were found in the microbial communities under each rootstock. These results further back up previous findings that differences in soil bacteria and fungi occur under different rootstocks, some of which may be associated with soil C sequestration, but this would need further investigations as to which soil microorganisms aid this. The soil used for this experiment had previously been fumigated which would have diminished the abundance of both beneficial and non-beneficial microbes, but this was not analysed. The microbial communities need time to enter and multiply within the soil before differences may occur. Differences were seen between the two soil regions, the rootzone soil (1 cm round the roots) having the greater amount of bacterial and fungal across the three rootstock varieties. Bacteria in both soil regions showed a decline over the three destructive harvests.

Soil regions did exhibit significant differences in the levels of soil C and N. The rootzone soil consistently exhibited higher concentrations of soil C and N compared to bulk soils, for all the rootstocks and harvest points. Total soil N was not a limiting factor to the sequestering of C into the soil due to the constant supply of N and other nutrients through fertigation. Soil fertigation could be a significant factor in the result of this experiment, as the application of a daily N based feed could enable the soil to sequester more C, but this would need further investigation and comparison studies with trees that did not receive daily fertigation (Reay *et al.*, 2008).

2.6.1 Limitations

There were several limitations to this study. Firstly, the length of time the trees had to grow was reduced by 2 months due to the first covid lockdown restrictions pushing back the planting date and reducing the time between destructive harvests, so limiting C sequestration. Secondly, each grafted scion and rootstock were slightly different in size at the time of planting, which could affect the amount of C at the destructive harvests. Thirdly, with the trees being destructively harvested the soil data does not show continuous C and N change but may suggest a trend. Finally, this study was not repeated, so only shows a single year’s pattern of sequestration but this may not be an accurate picture as different years may vary but could not be determined in this study.

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Appendix 1. Table of significant correlations

collection point	rootstock	test 1	test 2	P value	R-squared Values.
Pre planted	soil	soil moisture	POXC	0.05	0.99
Pre planted	soil	Total C	TOC	0.001	0.99
Pre planted	soil	Total C	organic matter	0.001	0.99
Harvest 1	M.9	nitrate (B)	MBC (RZ)	0.05	0.90
Harvest 1	M.9	nitrate (B)	POXC (B)	0.05	0.90
Harvest 1	M.116	nitrate (RZ)	MBC (RZ)	0.05	0.90
Harvest 1	M.M.106	nitrate (RZ)	BG biomass	0.03	0.94
Harvest 1	M.M.106	MBC (B)	AG biomass	0.02	0.97
Harvest 2	overall	nitrate (RZ)	AG biomass	0.002	0.77
Harvest 2	overall	Soil TC (RZ)	organic matter	0.0001	0.91
Harvest 2	overall	Soil TC (RZ)	TOC	0.0001	0.91
Harvest 2	overall	TOC	organic matter	<0.00001	1
Harvest 2	M.9	total soil carbon	BG biomass	0.04	1
Harvest 2	M.9	total organic C	BG biomass	0.02	1
Harvest 2	M.9	total organic C	organic matter	<0.00001	1
Harvest 2	M.9	organic matter	BG biomass	0.02	1
Harvest 2	M.116	soil moisture (RZ)	BG biomass	0.05	0.99
Harvest 2	M.M.106	POCX (B)	POXC (RZ)	0.02	1
Harvest 2	M.M.106	POCX (B)	AG biomass	0.03	1
Harvest 2	M.M.106	POXC (RZ)	AG biomass	0.05	0.99
Harvest 2	M.M.106	total organic C	organic matter	<0.00001	1
Harvest 3	Overall	total soil carbon	Total organic C	0.00001	0.85
Harvest 3	M.9	nitrate (RZ)	POXC (B)	0.01	0.97
Harvest 3	M.9	nitrate (RZ)	total soil N	0.03	0.94
Harvest 3	M.9	total soil carbon	Total organic C	0.03	0.95
Harvest 3	M.9	total soil carbon	root TN	0.02	0.95
Harvest 3	M.9	soil TOC	root TN	0.03	0.94
Harvest 3	M.116	soil moisture (B)	MBC (B)	0.02	0.97

Harvest 3	M.116	soil moisture (B)	total inorganic C	0.04	0.92
Harvest 3	M.116	MBC (B)	TIC	0.01	0.99
Harvest 3	M.116	soil TC	TOC	0.03	0.94
Harvest 3	M.116	BG biomass	root TN	0.05	0.90
Harvest 3	M.116	soil pH (RZ)	soil TC	0.02	0.95
Harvest 3	M.M.106	nitrate (RZ)	MBC (B)	0.02	0.95
Harvest 3	M.M.106	nitrate (RZ)	soil TN	0.01	0.98
Harvest 3	M.M.106	nitrate (RZ)	soil TC	0.01	0.98
Harvest 3	M.M.106	nitrate (RZ)	soil TIC	0.05	0.89
Harvest 3	M.M.106	MBC (B)	soil TC	0.02	0.96
Harvest 3	M.M.106	MBC (RZ)	soil TIC	0.02	0.96
Harvest 3	M.M.106	POXC (B)	soil TIC	0.04	0.92
Harvest 3	M.M.106	soil TOC	organic matter	0.02	0.96

Chapter 3. Scion mediated effects on belowground carbon sequestration

3.1. Abstract

Fruit trees comprise two distinct sections, the rootstock, and the scion, joined at a graft union, allowing for varietal and height selection. Research has shown that scions can influence the roots and nutrient uptake, but little has been done on the effects of scions on soil C sequestration. This investigation aimed to determine whether different scions affected the C sequestration of the M9 rootstock. The results from this investigation have revealed that after eighteen months of growing, most scions had significant differences between them in the concentration of soils total C. At six or eighteen months, no significant differences in the amount of active (POXC), or microbial biomass C were found between the five scions. Still, at 12 months POXC in the bulk soil under the Gala trees, concentrations were significantly higher than Dabinett and Cox's Orange Pippin. At 12 and 18 months, the total N concentrations showed significant differences between most scions. In conclusion, some observed differences exist between the five scions under investigation in soil C and N. Yet, these were inconsistent between each soil collection point or region.

3.2. Introduction

Grafting of fruit trees has been practised for centuries, dating back to the 1st Millennium BCE (Mudge *et al*., 2009; Harrison *et al*., 2016). Grafting involves the joining of a shoot of a desired cultivar (scion- the fruiting section of a tree) onto a selected rootstock. This process of clonal propagation allows for the production of identical fruiting trees with an appropriate level of vigour, removing the genetic variation found when trees are grown from seeds (Mudge *et al.*, 2009).

Another grafting method is interstemming, which involves placing a small piece of dwarfing rootstock between a vigorous rootstock and the desired scion (Mudge *et al.*, 2009; *Interstem Apple Trees – Apples*, 2019) (**Fig 3.1**). Interstemming is therefore a means of controlling the vigour of a tree and can be used to provide resistance, such as to certain pests and diseases (Di Vaio *et al.*, 2009). Dwarfing can be inferred using intersterms with polar auxin transporter genes MdPIN1a and MdPIN1b on roots and scions (Gan *et al.*, 2018). Allelic differences were found between rootstocks (Gan et al., 2018). These can reduce the expression of MdPIN1b (a gene that is needed for the expression of the auxin hormone that controls plant development) in M9 interstem. This MdPIN1B gene affects the auxin transport in the phloem and cambial tissues in the stem and roots, limiting plant and root growth which could limit the C sequestration ability due to a limited tree canopy and root exudations, but this was not studied by Gan et al.

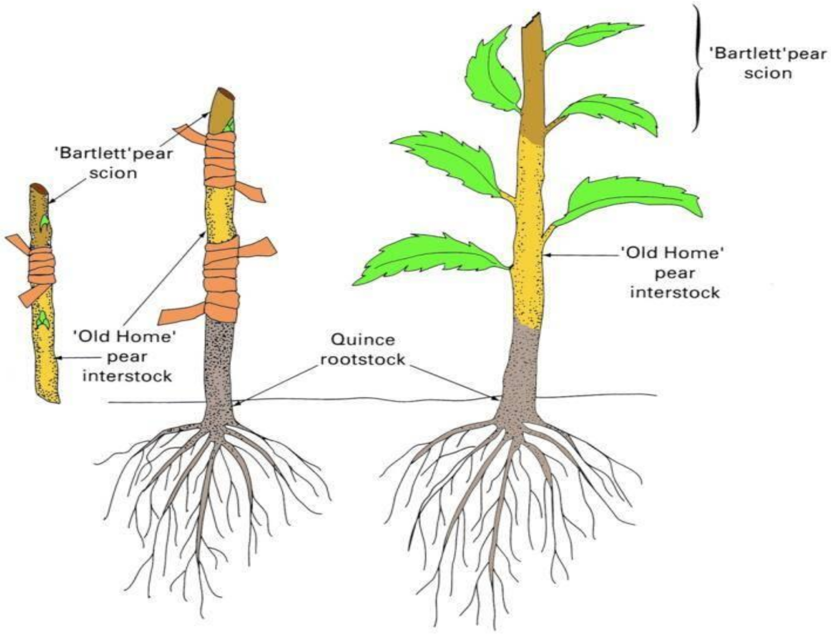


Fig 3.1. Interstem grafting of a fruit tree, showing how the three separate parts are joined together to form one tree (taken from *Interstemor Interstock*, n.d. accessed on 4th October 2022).

Very little work has been done to investigate the effect grafted scion could have on the rootstock concerning sequestering C into the soil; most research has focused on the effect of rootstock on scions, considering just one side of this source-sink relationship. The previous research on rootstocks and scions has focused on their influence on scion growth, productivity, and pest control. Rootstocks and scions are genetically different, so can introduce different characteristics into the whole tree, such as vigour and susceptibility to pests and pathogens which have been extensively studied (Ma *et al.*, 2013; Gan *et al.*, 2018; Spornberger *et al.*, 2018; Valverdi, Cheng and Kalcsits, 2019), but the mechanism of how rootstocks influence scions or vice versa is still not fully understood (Gautier *et al.*, 2019).

Genotypic and phenotypic traits that infer dwarfing, pest and pathogen resistance or even tolerances to various environmental or soil conditions have been widely studied (Tworkoski, Fazio and Glenn, 2016; Gan *et al.*, 2018; Valverdi, Cheng and Kalcsits, 2019). The effect of water and temperature on plant productivity and nutrient uptake in the roots, stems, and leaves (either side of the graft union) was investigated by Valverdi, Cheng, and Kalcsits (2019). They found that the lack of water affected the amount of biomass both above and belowground with the aboveground biomass being the most affected. Their research also showed that the Gala scions, affected the amount of root biomass and nutrient uptake, under different soil conditions than trees grafted with Honey Crisp.

Tietel et al. (2020) investigated the effect of both rootstock and scion on the metabolic profile of the juice of mandarins and the sap in the rootstocks. Their work showed that certain compounds were transferred between the two sections of the tree, whereas others remained in either the roots or scions, and that different gene expressions within the rootstock and scions controlled this. Previous studies have not investigated the transference of photosynthetic C-based metabolites within the tree or the allocation of C into the soil. Instead, they have focused on genetics to enhance crop production and protection.

The investigation aimed to determine whether different scions and their interactions with the M9 rootstocks affected levels of C being sequestered into the roots and the surrounding soil regions (root zone and bulk soils). M9 rootstocks were used as they are the most commercially used rootstock for growing dessert apples in the UK, and in the previous experiment, no rootstocks showed any significant difference in the levels of C sequestered in 4.5 months (**see Chapter 2**).

3.3. Materials and Methods

This experiment was conducted at NIAB at East Malling, Kent UK, (51.286359, 0.452572) over two growing seasons (March 2021 to September 2022). The soil (clay loam confirmed by laser diffraction by NRM, UK, with a pH_{CaCl2} 6.93) was collected from a field at East Malling, sieved using a 2mm sieve, and homogenised before planting.

This experiment used 90 individual M9 rootstocks which were grafted with five different scions (n=18), three of which were dessert varieties (Cox's Orange Pippin (COP), Braeburn and Gala) and two cider varieties (Dabinett and Michelin) (**Table 3.1**). The cider scions are typically grown on M116 or MM106, but for this experiment they were grafted onto M9 so that any effect of the scions could be detected. COP was used as a control to ensure consistency across experiments.

Thirty trees (six per scion) were planted in rhizotrons, placed into a glasshouse and fertigated daily using Universal green (nitrogen-based feed at a rate of 1.2 L per hour for a maximum of 5 minutes three times a day). The remaining 60 trees were planted in 3L pots, placed in a polytunnel, and watered daily by hand, without additional fertilisation. The last 30 trees were given a general foliar feed in July 2022 as the leaves were starting to show signs of nutrient deficiency. This division of planting was because rhizotrons are unsuitable for growing trees for two years, there was a lack of available rhizotrons, and limited space within the glasshouse for all 90 plants. Rhizotrons were used as part of this study to allow for a possible investigation into root architecture. Still the same issues occurred as in the previous experiment (**Chapter 2**), and so were not studied.

The rhizotrons and pots were arranged in a randomised block design in the glasshouse compartment and in the polytunnel to account for any environmental variations. Trees were colour coded with labels to differentiate species and their harvest dates. Six trees of each scion-rootstock combination were destructively harvested every six months (September 2021 for the trees grown in rhizotrons, and March and September 2022 for pot-grown trees) to allow for soil sampling and biomass measurements.

In September 2021, all 30 plants from the rhizotrons were destructively harvested, and soil was collected from both soil regions (bulk and root zone). Three of the potted trees were discarded in June 2021, due to damage at the graft unions and had died; two were due to be harvested in March 2022 (Gala and Braeburn n=5) and one in September 2022 (COP n=5). The trees in the pots were also becoming root-restricted, and only root zone soil could be collected, all trees in September 2022 and one Gala tree from the March 2022 harvest.

Table 3.1. Scions selected for the study investigating their effect on belowground C sequestration all grafted on to M9 rootstock. (‘Flowering times of apples RHS Pollination Groups’, 2007; *Apple trees for sweet cider and hard cider*, accessed 2019).

Scion	Scion information
Cox’s Orange Pippin	A dessert apple discovered in 1825 in the UK and thought to be a cross between Ribston Pippin and Blenheim Orange. This variety can be self-fertile and has a mid-season flowering time (pollination group 3) and mid to late season for harvest. This variety is commercially grown in the UK, but numbers are in decline.
Gala	A dessert apple developed in New Zealand in 1934 by J H Kidd from a cross between Golden Delicious and Kidd’s Orange Red. It can be grown in temperate and warmer climates, requiring only eight hundred hours of winter chill. This variety can be self-fertile (pollination group 4) and is a mid to late season for harvest.

Braeburn	A dessert apple discovered in New Zealand in the 1950's, parentage is unknown but is a possible cross between Granny Smith and Lady Hamilton. Better suited to warmer climates and only requires seven hundred hours of winter chill. A late season flowering variety and later harvesting. It has been a parent to other varieties.
Dabinett	A cider apple discovered in the UK in the 1900's, parentage is unknown but possibly Chisel Jersey mix. Generally self- fertile, late flowering (group 6) and late harvesting (October).
Michelin	A traditional French cider apple discovered in Normandy in the 18th century and introduced to the UK in the 19th century. It is semi self-fertile, flowering group 4 and late harvesting.

3.3.1 Plant and soil analysis

At each destructive harvest of the trees, two regions of soil were gathered, bulk and root zone (1 cm around the roots), as well as the above and belowground biomass, which was cut at the top edge of either the rhizotrons or pots to ensure equal delineation of biomass, and 5 g of the finer roots were cut following drying for 48 hours at 80°C for further analysis.

The total C and N content of soil and roots samples and root % protein was measured at the University of Reading, UK, using the LECO CHN628 analyser, following the AOAC official analysis method (1990) via combustion in pure oxygen environment. Soil texture analysis was conducted by NRM (Reading, UK) using the laser diffraction method. The remaining soil analysis was conducted at NIAB East Malling, Kent, UK.

Soil moisture content was determined using the gravimetric method described by Forster (Forster, 1995) and soil pH was measured using the calcium chloride method (Schofield and Taylor, 1955). The potassium permanganate method was used to determine soil active C (POXC) following the methods described by Weil et al. (2003) and Culman et al. (2014). Soil microbial biomass C was estimated using the ninhydrin-reactive N content assay following chloroform fumigation and extraction of soil samples described by Amato and Ladd (1988) and Vance et al. (1987). Soil extractable nitrate content was measured using the method described by Cataldo et al. (1975). Some modifications were made to ninhydrin and nitrate assays to collect all data (as described in **Chapter 2**). Aboveground and belowground biomass C was measured following the method and calculations described by De Oliveira et al. (2019), Petersson et al. (2012), and Manickam et al. (2014).

3.3.2 Statistical analysis

The data was analysed in Microsoft Excel 365 using Pairwise one-way ANOVA, following normalisation of the data to ensure assumptions were met. These were used to determine significant differences between the five scions under investigation, differences between two the soil regions (rootzone and bulk soils) and changes over time for the soil and tree biomass harvests. Pearsons's correlation coefficient to determine relationships between the various soil and plant analysis. Statistically significant differences were indicated by p- values of <0.05.

3.4. Results

The soil pH of the potted trees significantly increased between planting (pH $_{CaCl2}$ 6.93), and the final destructive harvest in September 2022 (pH $_{CaCl2}$ 7.5-7.6) under each of the five cultivars (P = < 0.001) (**Fig 3.2)**. After the first six months (trees grown in rhizotrons), bulk (B) soil pH under Cox's Orange Pippin (COP), Dabinett's, and Dabinett's root zone (RZ), had significantly increased from the soil sampled before planting (COP (B) P = <0.01, Dabinett (B) P = 0.04, and (RZ) P = 0.02). The trees at harvest two (those grown in pots) in both soil regions had significantly increased in soil pH from the levels found before planting. The root zone soil pH was significantly higher under Dabinett trees than either Gala (P = 0.04) or Braeburn (P = 0.02). By destructive harvest 3 (18 months after planting and pot-grown), all the root zone soils had significantly increased from harvest 2 (six months earlier).

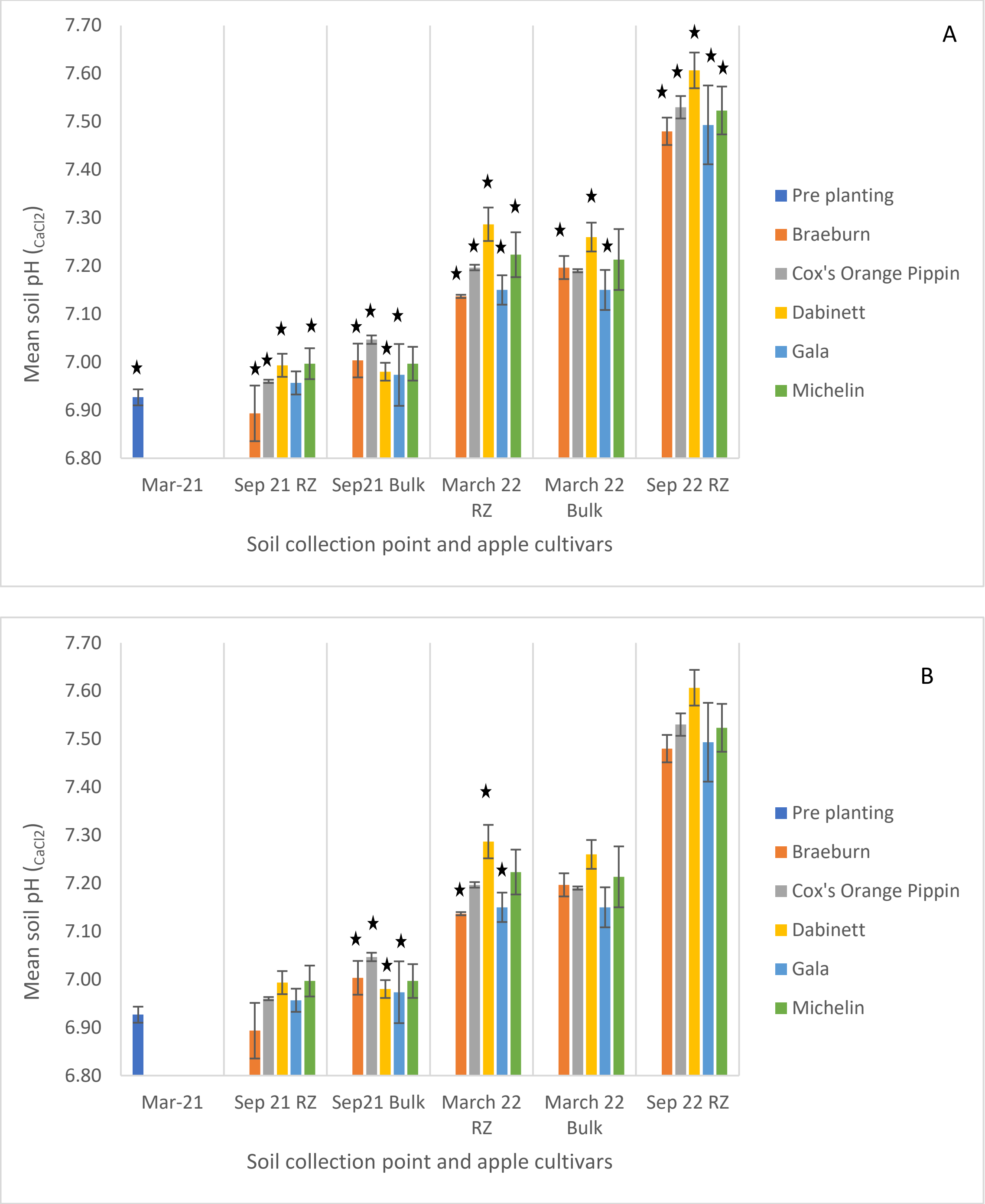


Fig 3.2 A and B. Mean soil pH ($CaCl_2$), over the experiment across the two soil regions (root zone -RZ and Bulk zone) under investigation for the five apple cultivars. **A,** shows significant changes in soil pH over time and **B,** shows significant differences between rootstocks. Significant differences indicated by the Stars ($P = <0.05$).

The rhizotrons harvested in September 2021 six months after planting, were all fertigated daily and amounts given were altered as the trees grew and demands increased. The pot-grown trees (March and September 2022 destructive harvests) were watered once a day by hand by a member of the glasshouse team and water volumes were not measured. By September 2021, soil moisture, except for the bulk soil under Michelin trees and both soil regions under COP, had declined significantly, from the samples measured before planting (significance ranged from P = 0.03 to P = <0.00001) (**Fig 3.3**). Cox's Orange Pippen had significantly higher soil moisture content in both soil regions compared to Braeburn (root zone) and Dabinett (bulk) (P = 0.04 and P = 0.05 respectively), and in the bulk soil, Michelin was also significantly wetter than Dabinett (P = 0.05).

A year after planting, soil moisture content in March 2022 had significantly increased across both bulk and root zone (RZ) soils (P ranged from 0.01 to <0.00001), but there was no significant difference between the five scions. After eighteen months of being pot-grown the soil moisture content (%) had significantly decreased across the five scions from March 2022 (P ranged from 0.004 to < 0.00001) compared to those six months prior. Gala's root zone soil moisture was significantly wetter than Michelin's in September 2022 (P = 0.05).

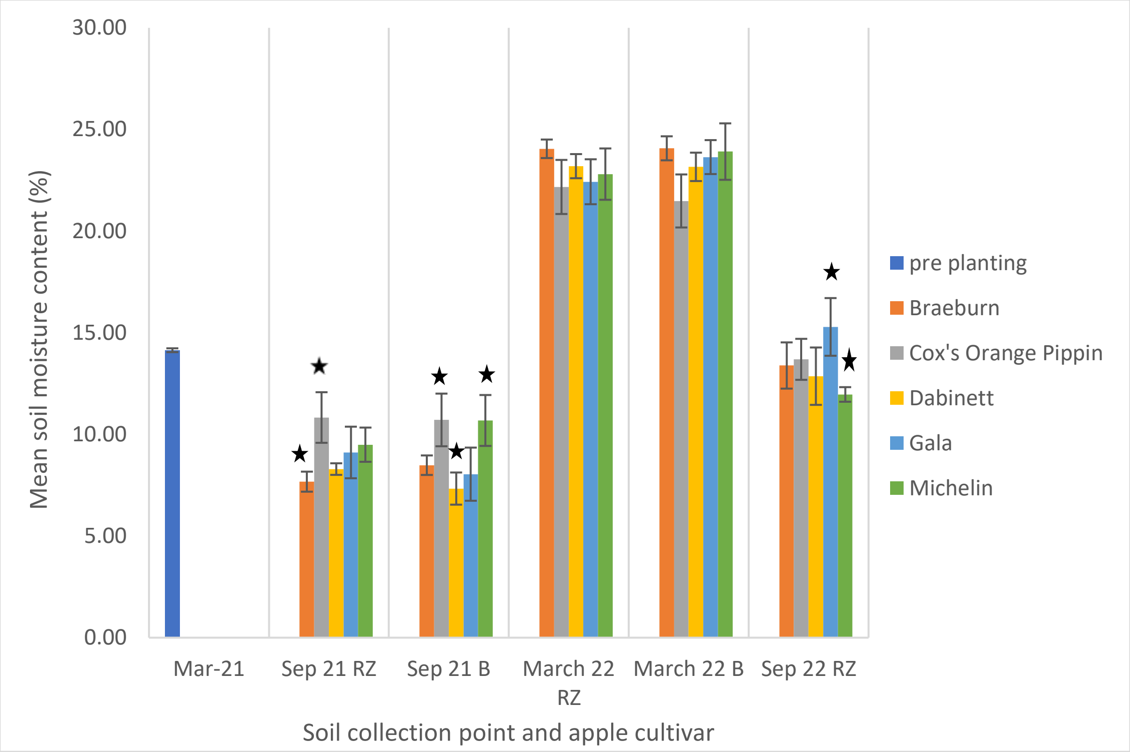


Fig 3.3. Mean soil moisture content over the 18 months with standard error bars, from soil collected before planting occurred, and then at each of the three harvest points for the five scions (n=6) and the bulk (B) and root zone (RZ) soil regions. September 2022 only had soil from the root zone due to the roots being pot-bound, with stars indicating significant differences between cultivars at each harvest point.

3.4.1 Soil C analysis

Soil total C (%) before planting trees in March 2021 had a mean of 3.36% (**Fig 3.4**). The soil total C in both soil regions of all the trees grown in rhizotrons (harvested in September 2021) had increased from before planting, but this increase was not significant. September 2021, the soil total C found in the root zone soil under the Gala trees was significantly higher than COP (P = 0.04).

The soil total % C at twelve months after planting, collected from the potted trees in March 2022 had significantly increased in both bulk (B) and root zone (RZ) soils under Michelin (P = 0.01 (B) and P = 0.05 (RZ), Dabinett (both regions P = 0.01), and COP (both regions P = <0.01).

In the root zone and bulk soil Dabinett, Michelin and COP had significantly higher concentrations of TC (%) than Gala and Braeburn. Eighteen months post planting, the soil total % C (root zone only) had significantly increased under COP (P = 0.03) and Dabinett (P = 0.01) from soil collected prior to planting. Still, there was no significant change since the March 2022 destructive harvest. At the final harvest, differences between most scions were significant, except for four combinations of scions. The four scion combinations, which were not significantly different were COP and Dabinett, Dabinett and Michelin, Braeburn and Gala, and COP and Michelin.

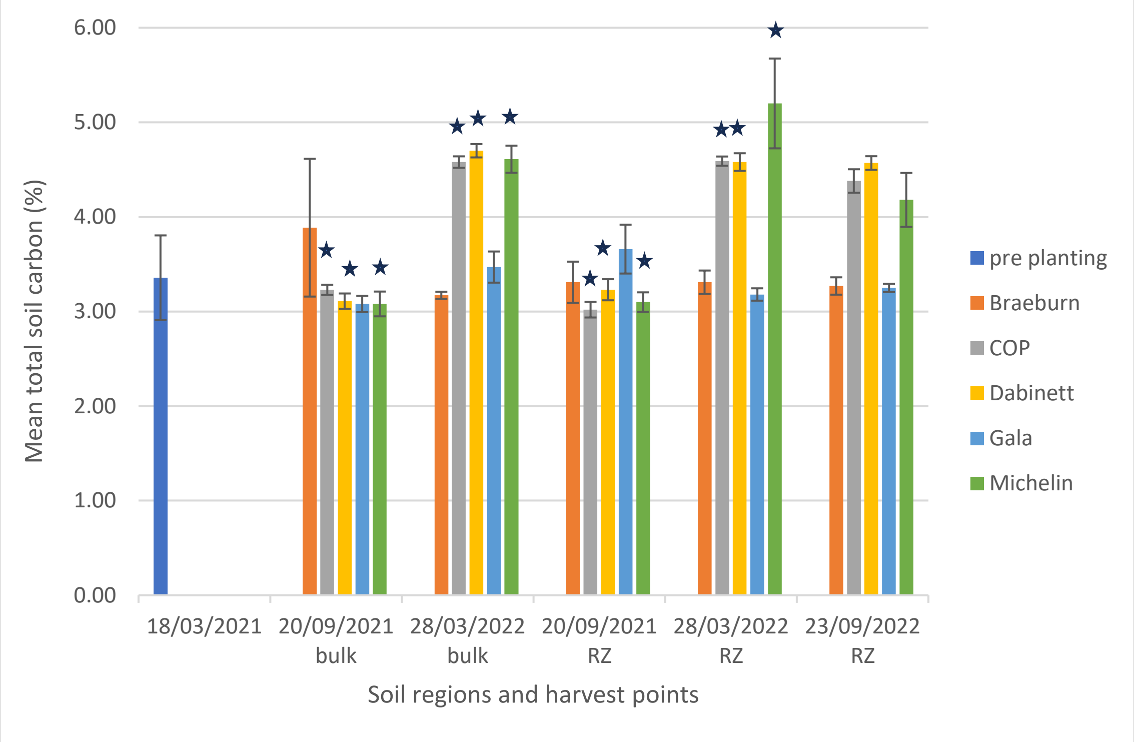


Fig 3.4. The mean total % soil C across the experiment for the five scions and two soil regions under investigation with standard error bars (n =6 with exceptions of Braeburn, Gala in March 2022 and COP September 2022 where n= 5). Significant differences over time are indicated by stars (P = <0.05)

Prior to planting (March 2021), soil POXC had a mean value of 344.40 POXC mg Kg^{-1} soil (n=3), but the soil concentrations of POXC across the five cultivars varied at each destructive harvest. By September 2021, the levels of POXC found in both soil regions had increased across all scions but were only significant in the bulk soil for four of the five scions, from concentrations found before planting. Braeburn's bulk soil was the only one that had not significantly increased from March 2021 (P = 0.07). There were no significant differences in soil POXC between the soil regions under any of the five scions.

A year after planting (March 2022), the POXC concentrations in the pot-grown trees had declined within both soil regions across all five scions from prior to planting. Gala, Dabinett, Michelin and COP bulk soils had significantly decreased since planting a year earlier (P = <0.01, P = 0.001, P = <0.001, and P = 0.01 respectively). In the root zone soil Braeburn, Dabinett, and Michelin were the only scions that significantly declined in POXC concentrations (P = 0.01, P = <0.01, and P = <0.001, respectively). Bulk soils POXC concentrations under Gala were significantly higher than Dabinett (P = 0.03) and COP (P = 0.05). The root zone soils showed no significant differences between any of the scions.

Eighteen months after planting, no significant differences in the root zone soil POXC concentrations were seen between any of the five scions under investigation (**Fig 3.5**). The root zone soils POXC concentrations had not significantly changed across the five scions since the previous destructive harvest in March 2022 or the soil prior to planting (March 2021).

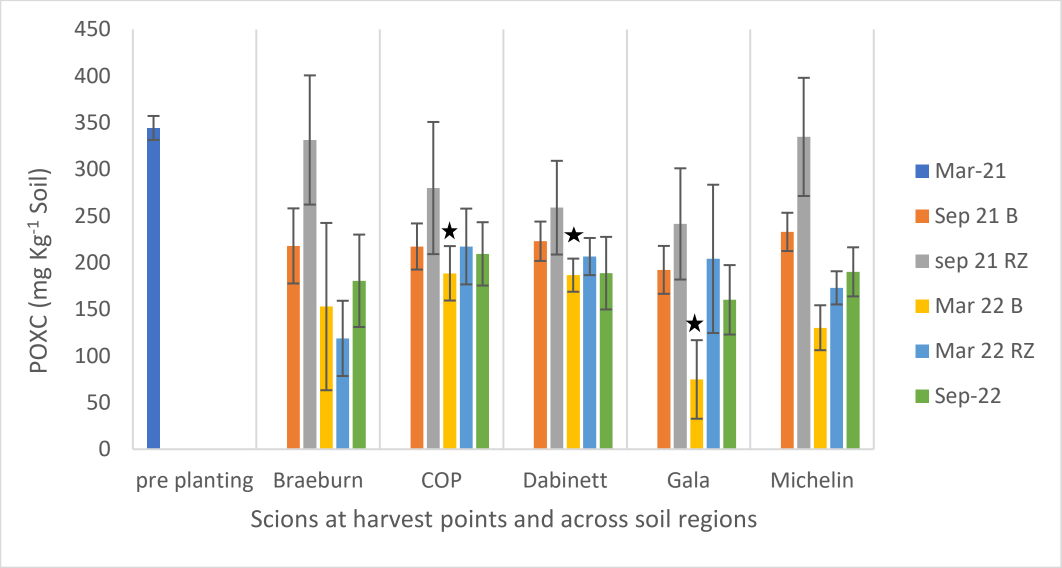


Fig 3.5. Mean soil permanganate oxidisable C and standard error bars in the two soil regions under the five scions across the experiment. Significant differences between scions are marked with a star.

Soil microbial biomass C, before planting, had a mean of 37.56 $CHCl_3$-labile C (µg C g^{-1} soil). At six months post-planting (September 2021), the trees in the rhizotrons MBC content of the root zone soil under COP had significantly increased since planting (P = 0.05). No significant differences existed between the scions found in either soil regions (bulk or root zone) at this harvest.

During the first harvest of the pot-grown trees in March 2022, all the root zone soil MBC concentrations significantly increased from those of the pre-planted soil samples. The bulks soils of Braeburn, COP, and Gala (P = 0.02, P = 0.01, and P = 0.02, respectively) had also significantly increased in MBC from before planting. There were no significant differences in March 2022 between the five scions or the two soil regions. For most of the scions, the soil MBC was higher in the bulk soil regions except for Gala, where the root zone soil had the higher concentration of MBC. At this stage, two trees had died (one Gala and one Braeburn), and one Gala tree had also become pot-bound, so no bulk soil was available for testing. The MBC in the root zone soil 18 months after planting (September 2022) showed no significant differences between any of the five scions (**Fig 3.6**), or from the concentrations before planting. Compared to the soil samples collected in March 2022, the levels of MBC had decreased significantly across all but one scion, COP (P = 0.07). One of the COP trees died in June 2021 due to damage at the graft union.

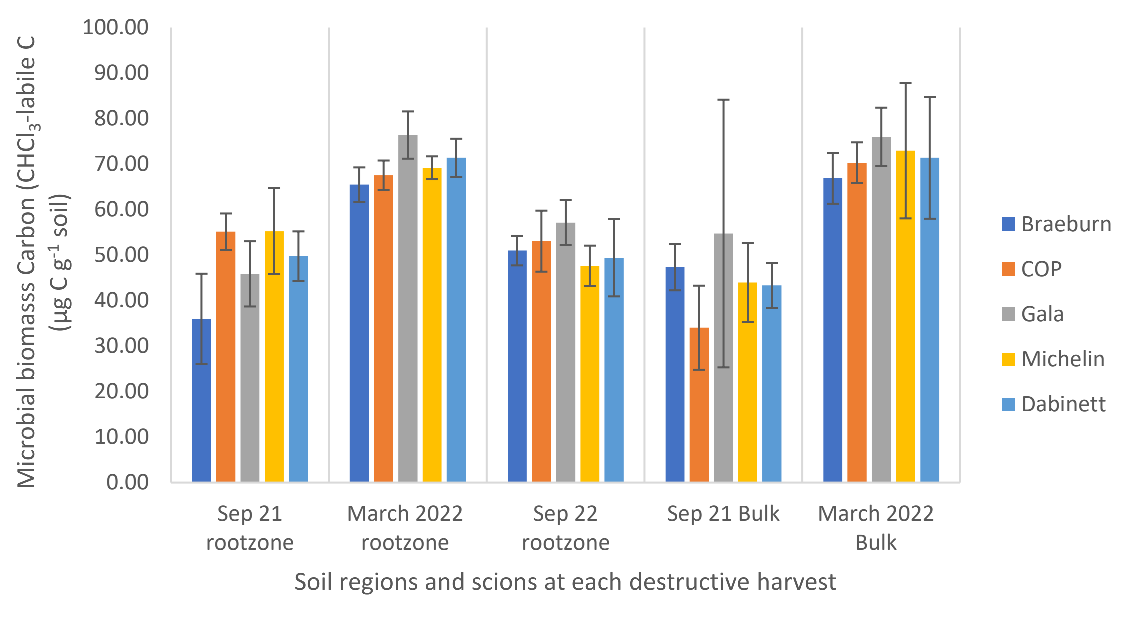


Fig 3.6. Mean microbial biomass C with error bars showing the changes under each scion (n=6) at the three destructive harvests over the course of the 18-month experiment.

3.4.2. Soil N analysis

Soil total % N before planting (March 2021) had a mean of 0.23% (n=3). September 2021 soil total % N in the rhizotrons, had no significant differences between either the soil regions or scions. In March 2022, the total % N in the bulk soil showed significant differences between scions, except for Gala v Braeburn, COP v Michelin, and Dabinett v Michelin. In the root zone soils, two combinations of scions, Braeburn v COP (P = <0.001) and Braeburn v Dabinett (P = 0.001), showed significant differences, with Braeburn being lower in total % N than the other scions.

September 2022, Braeburn (P = 0.02) and Dabinett (P = <0.01) had significantly increased in total % N from March 2022. Four combinations of scions were not significantly different from each other for the root zone soil for total % N; COP v Dabinett (P = 0.2), Dabinett v Michelin (P = 0.5) with Dabinett having higher concentrations of soil N. Whereas, Braeburn and Gala had very similar values (P = 0.9). Michelin had higher soil N than COP (P = 0.7). Michelin had the widest variation of values for the total % N at this harvest point (**Fig 3.7**).

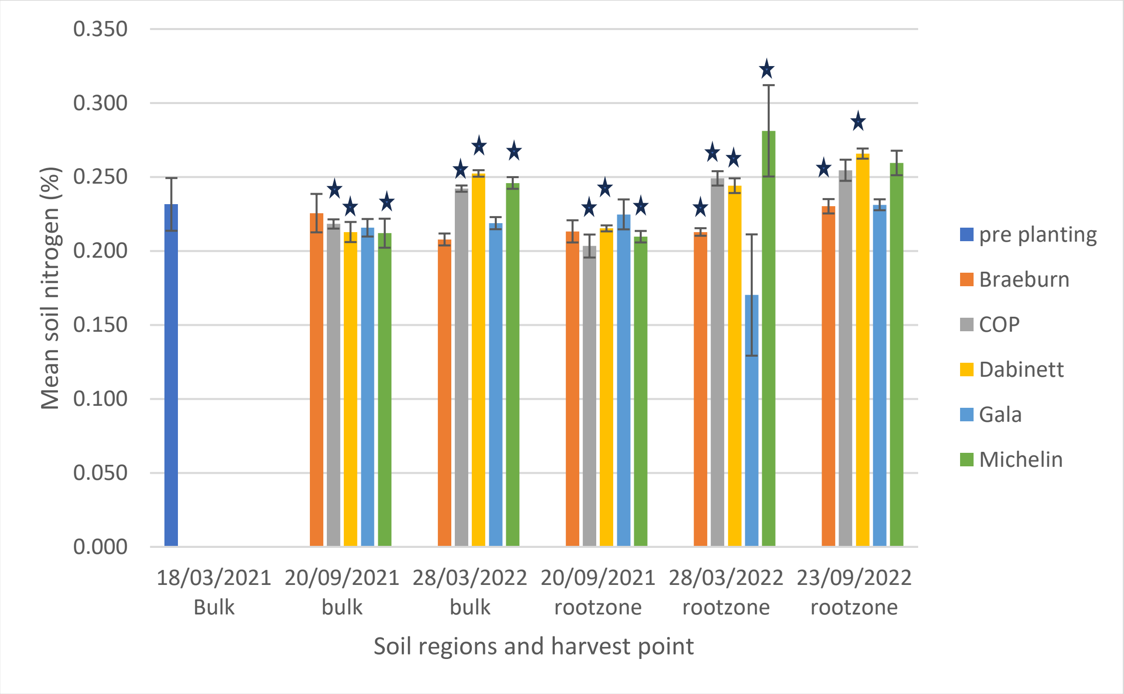


Fig 3.7. Mean total soil nitrogen (%) across the experiment for the five scions and two soil regions under investigation with standard error bars (n =6 with exceptions of Braeburn and Gala in March 2022 and COP September 2022 where n= 5). Stars indicate significant differences between sampling dates (P = <0.05).

Extractable nitrate levels in the soil collected before planting had a mean of 18.56 µg NO_3^- -N g^{-1} soil. At six months (rhizotron harvest) only Dabinett had a significant difference between the two soil regions, with the bulk soil having the higher concentration (P = 0.02). All soil regions had significantly increased in nitrate concentration at six months (**Fig 3.8**).

A year after planting trees into pots (March 2022), the root zone soil showed Michelin had a significantly higher concentration of soil nitrate than Dabinett (P = 0.05), COP (P = 0.04) and Braeburn (P = 0.04) (**Fig 3.8**). September 2022, eighteen months after planting, the soil nitrate levels in the root zone soils had no significant differences. Between March and September 2022, Michelin showed a significant decrease in soil nitrate (P = 0.04). From before planting to both March and September 2022 harvests, the soil nitrate had significantly decreased across all five scions and in both soil regions.

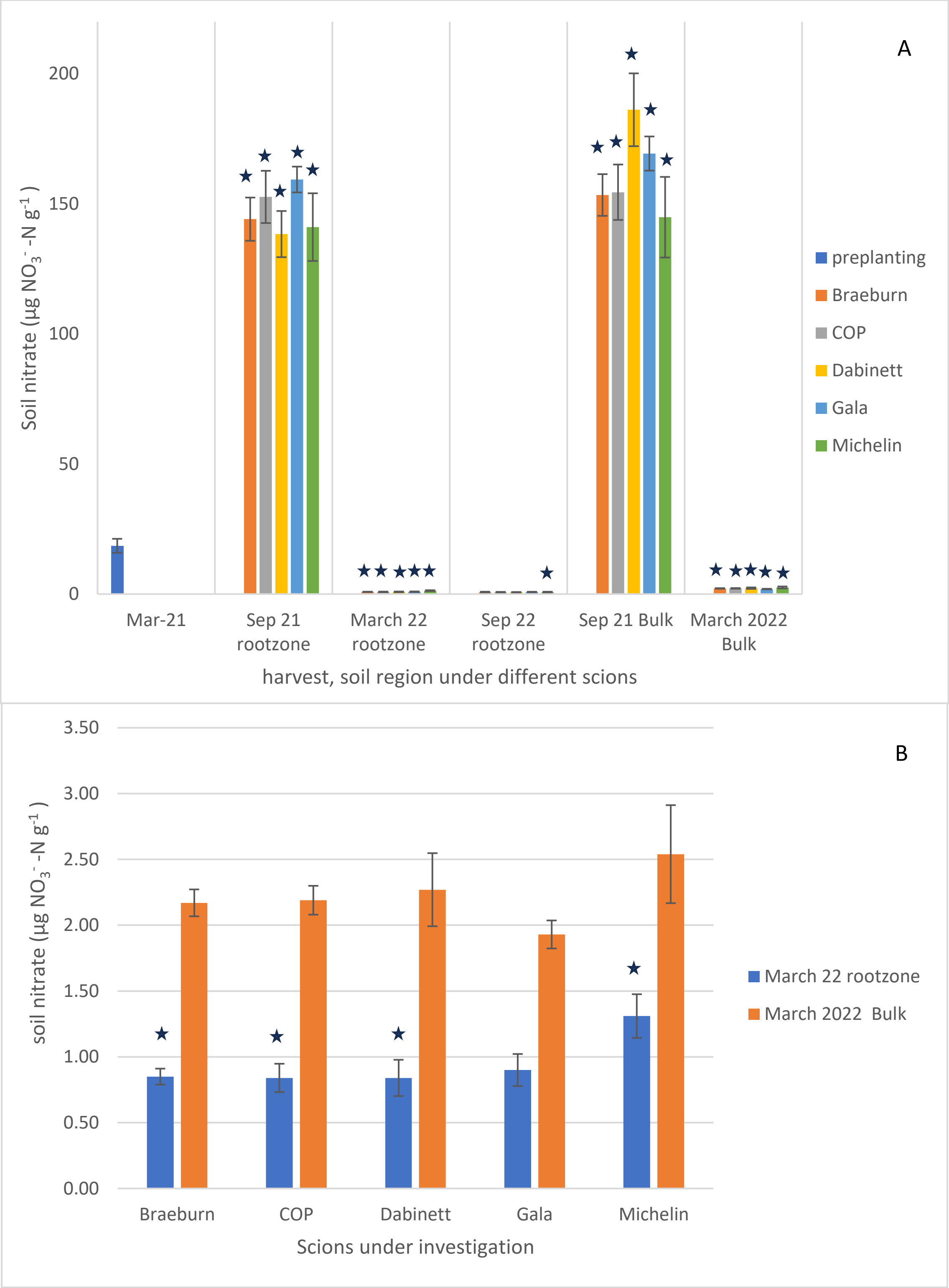
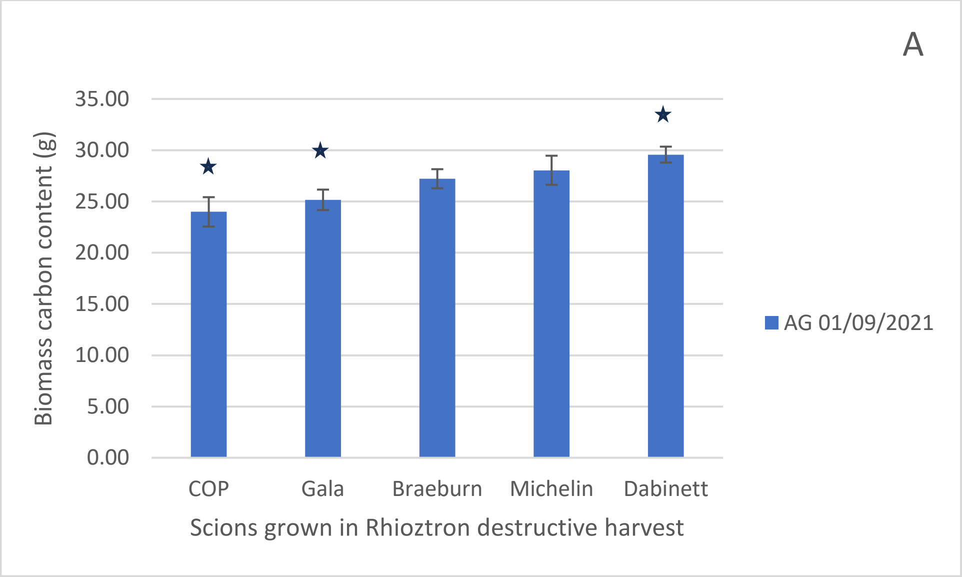


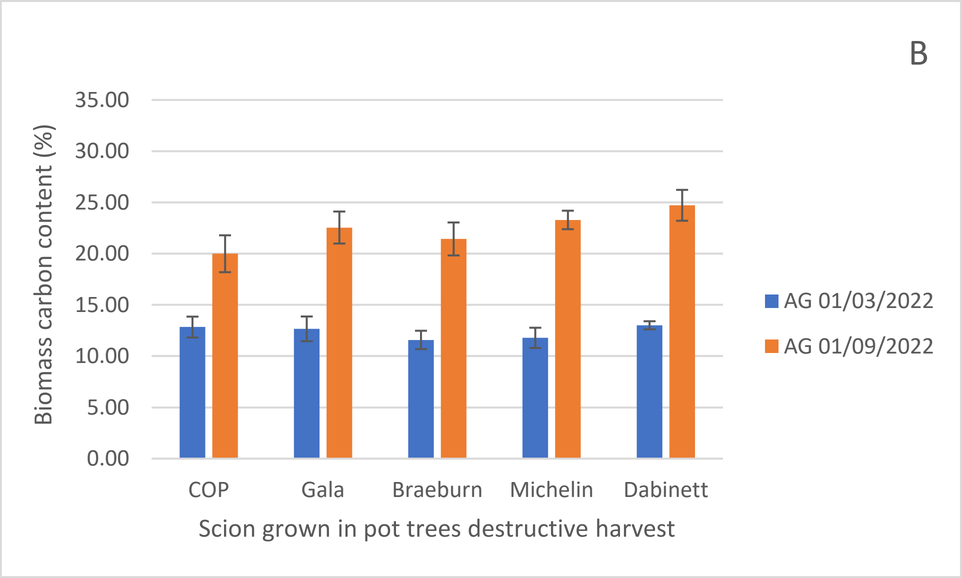
Fig 3.8 A and B. Mean soil nitrate concentration and standard error bars across all soil collection points, soil regions and scions under investigation. **A**, indicates changes over time and **B**, shows differences between the scions at the March 2022 harvest (1st of the pot grown trees), stars indicate significant differences (P = <0.05).

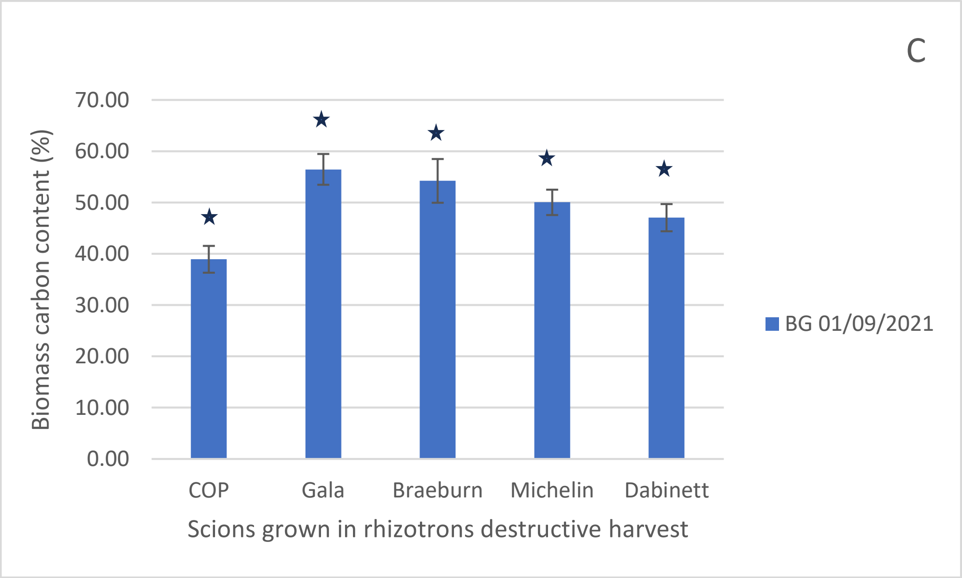
3.4.3 Biomass C analysis

Aboveground biomass C in September 2021 COP had the lowest concentration of C, and Dabinett had the highest concentration of C (**Fig 3.9 A**). COP and Gala were significantly lower in biomass C than Dabinett (P = 0.01 and P = 0.01, respectively). The belowground biomass in September 2021, Gala, had the highest levels of C, with COP being significantly lower than the other four species (**Fig 3.9 B**). Dabinett grafted rootstocks were also significantly lower in belowground biomass C than those grafted with a Gala scion (P =0.02).

In March 2022, the pot-grown trees had no significant differences between the varieties for the above or belowground biomass C content. At the final destructive harvest in September 2022, all the aboveground biomass C had significantly increased from March to September 2022 (**Fig 3.9 A**), but there was no significant difference between scions. The belowground biomass C of the Gala grafted trees were significantly higher than COP (P =0.01), Braeburn (P = 0.01), Michelin (P = 0.01), and Dabinett (P = 0.05).


A
Biomass carbon content (g)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
★
★
★
COP
Gala
Braeburn
Michelin
Dabinett
Scions grown in Rhioztron destructive harvest
AG 01/09/2021


B
Biomass carbon content (%)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
COP
Gala
Braeburn
Michelin
Dabinett
Scion grown in pot trees destructive harvest
AG 01/03/2022
AG 01/09/2022


C
Biomass carbon content (%)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
★
★
★
★
★
COP
Gala
Braeburn
Michelin
Dabinett
Scions grown in rhizotrons destructive harvest
BG 01/09/2021

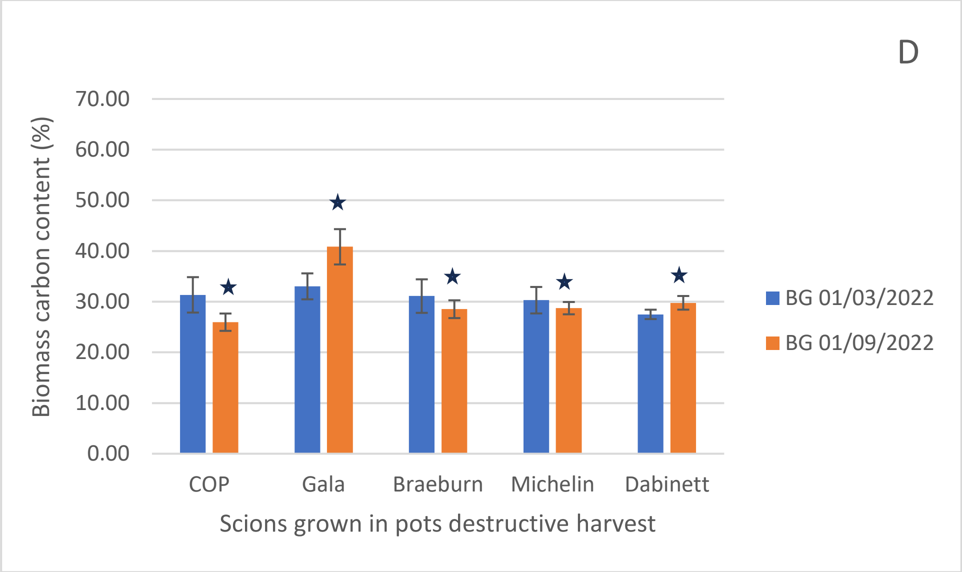


Fig 3.9 A, B, C, and D. **A and B**) Aboveground biomass carbon, **C and D**) Belowground biomass carbon at each destructive harvest. **A and C** represent trees grown in rhizotrons and harvested September 2021, **B and D** represents the trees grown in pots and March, and September 2022. Stars indicate significant differences between the scions (P = <0.05).

3.4.4. Root total C, N, and Protein

Roots TN, TC, and protein were assessed in September 2021 and 2022. No significant differences were found in the roots of the M9 tree between any of the five scions (**Fig 3.10 A, B, and C**). By September 2022, TN % and Protein % within the roots across all five scions had decreased relative to the September 2021 samples, Dabinett (nitrogen P = 0.0001 and protein P =0.0001), Gala (TN P = <0.001 and protein P = <0.001) and Michelin (nitrogen P = 0.001 and protein P = 0.001) were significant decreases.

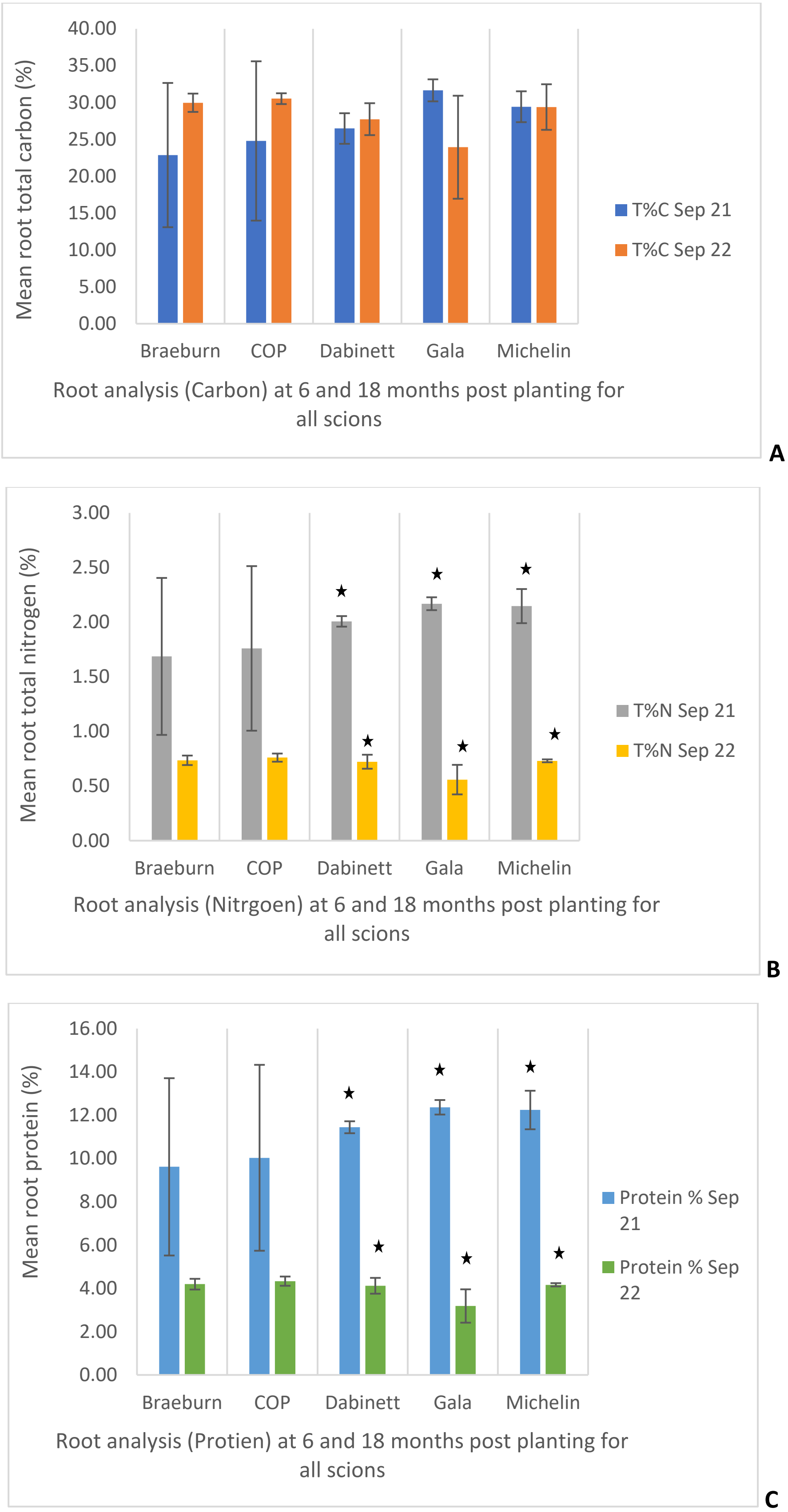


Fig 3.10 A, B, and C. Mean values with standard errors for the roots **A)** total % C, **B)** total % N and **C)** % proteins (n=3), at 6- and 18-months following planting, significant differences (stars) indicating changes over time.

3.5. Discussion

This study demonstrated that different apple cultivars influence the ability of M9 rootstock to sequester C belowground and had different effects in each soil regions within the rhizotrons (September 2021) or pots (March and September 2022). Results gathered across the five scions and the two soil regions (bulk and root zone) at the three destructive harvests revealed some significant indications of possible effects of scions on C sequestration, such as COP, Dabinett and Michelin, but these were not consistent across the eighteen months (two growing seasons) of the experiment.

Soil pH increased across both soil regions with significant differences between the five scions at six months in the rhizotrons and for the pot-grown trees over the eighteen months. Dabinett and COP could have more influence on the types of root exudate that change the soil pH than the other scions in the earlier stages of establishment, as by eighteen months, no scions had significant differences in the soil pH. The soil increase from acid to neutral, which occurs after planting due to the excretion of metabolites from the root system, as described by Vives-Peris et al. (2020). The change in the soil pH for the rhizotron-grown trees could be due to the use of the daily N-based fertigation in conjunction with root exudation. The changes in the soil pH in the pots by the end of the experiment could be the trees exuding more exudates due to the need to extract any nutrients from the soil (Leisso, Rudell and Mazzola, 2018) as the trees were becoming pot-bound and had limited nutrients within the soil, that they had been planted in. The range of pH found within the soil throughout the experiment showed a suitable environment for the growth of soil microbes (Liao, Wu and Zhu, 2016; Malik *et al.*, 2018), which would build up the MBC concentration and the increase in root exudation would bring more C from the tree into the soil.

Soil moisture content in the pots had significantly increased from the soil sampled before planting, which could be due to the inaccuracy of hand watering compared to the regulated fertigation that the trees in the rhizotrons received. Humphrey et al. (2021) showed that soil moisture can be a limiting factor for C sequestration due to its effect on plant photosynthesis (Humphrey *et al.*, 2021). The increase soil moisture could limit pores spaces (oxygen) within the soil, limit microbial growth due to hypoxia, affect C aggregation on soil particles, and cause C to leach in pot-grown trees.

Soil total C in the pot-grown trees had significantly increased over twelve and eighteen months. The possible explanation for this significant increase could be root exudation, the increased root biomass and debris lost as roots grow, and root turn over (death). There would also be C from both living and dead soil microbes that would show in overall TC, although by eighteen months, the amount of MBC had declined. The increase in the bulk soil TC in March 2022 could be due to the transference and leaching of C from the root zone into the bulk soil, as the trees were still watered over the winter months. Also, the decrease in the aboveground biomass C shows the possible transference of C from the leaves to other storage bodies, including the soil at senescence before winter dormancy (Smart, 1994). Although there was an increase in total C for the rhizotron-grown trees some of this could be due to the daily N fertigation, which can encourage more significant soil C sequestration and enhance biomass growth both above and belowground (Bala *et al.*, 2013; Lu *et al.*, 2021; Kowalczyk, Wrona and Przybyłko, 2022). The significant differences in soil TC between the five scions at the final harvest (September 2022) could be due to the differing amounts of biomass C and tree sizes both above and belowground, impacting photosynthesis and root exudates' production. There is likely to be an impact from the trees becoming pot-bound, and the limited availability of nutrients for tree growth.

The aboveground biomass C showed seasonal variations between September and March harvest points, which would be expected as apple trees are deciduous and drop their leaves in late autumn. Another factor that could have affected the aboveground biomass C between March and September 2022 was the limited nutrient supply in the pots. Research by Valverdi, Cheng, and Kalcsits (2019) showed that soil moisture and nutrients can affect both the rootstock and scions and vary between cultivars; this backs up the finding of this study.

The highest values of belowground biomass at six months could be due to the high availability of nutrients in the soil from the fertigation, and from the trees trying to establish and anchor themselves into the soil. The trees in the pots had a much smaller volume for root growth in the search for the limited nutrient availability that would help the tree establish and grow, so this is most likely why the biomass C of these trees were lower. The partitioning of C between each scion favoured the belowground biomass in the eighteen months of this experiment (Génard *et al.*, 2008). Therefore, the biomass C of a tree will continually change over time as the tree grows and natural root turnover occurs (Psarras *et al.*, 2000; Baddeley and Watson, 2005). The soil used in this experiment was a clay loam, and this could impact the C sequestration because of the ability of clays to bond with C, therefore helping C sequestration, promoting long-term storage and protecting it from microbial turnover (Blanco-Canqui and Lal, 2004).

The total C found in the roots was comparable across all five rootstocks at both harvest points, but the C found in the roots did not match the levels of biomass C at the two corresponding harvest points. This difference between the C found in the roots and biomass could be due to the variations in the root systems of the individual trees and that the root C was tested on an equal sample size.

Both total % N and the protein % in the roots significantly decreased between sampling points for Gala, Michelin and Dabinett. These changes could be due to the demands of the tree’s growth, so N and protein were being moved into other biomass material or the limited nutrients found in the soil required for aboveground growth rather than being partitioned into the roots. This would compare to the lower values of belowground biomass C for both Michelin and Dabinett in September 2022. Still, Gala trees had the highest belowground biomass C content than any of the other four scions, which suggests that cultivar partitioning of nutrients could be influencing root N and protein content, but this would require further study to determine if this is correct.

Active soil C (POXC) varied between each tree's soil regions, and by the first harvest (rhizotrons) had significantly increased. However, trees grown in pots declined across both soil regions and all scions from the levels found in the soil prior to planting. A year after planting, Gala was the only scion to be significantly different in the amount of active C, but this significant difference was not visible at eighteen months. This could indicate that the tree and the soil microbes are using the C that is easily accessible at slightly different rates throughout the year and recycling the C back into the atmosphere, so C in these soil regions is not being sequestered. The trees collected from the rhizotrons in September 2021 were being regularly fed, which could be providing soil microbes with an alternative source of food, rather than using those naturally found in the soil, which allowed for the growth of microbes, which were of limited supply in the soil for the trees grown in the 3 L pots.

Microbial C levels across both soil regions had increased over the first two destructive harvests, with the bulk soil showing the greatest increase. These increases in MBC concentrations by the first harvest could be due to the fertigation providing extra nutrients for soil microbial growth. The increase in pot-grown trees harvested in March 2022 could be due to natural growth of soil microbes, although this would have been limited during the winter due to the colder atmospheric and soil temperatures. The MBC significantly declined between March and September 2022 across four of the five scions. This decline in MBC by the final destructive harvest could be down to the lack of nutrients and root exudates available, thus limiting the growth of soil microbial communities as the tree became pot-bound (McNear Jr., 2013; Liang *et al.*, 2019).

Soil total N changed across the experiment and varied between soil regions under the different conditions; these changes were insignificant. The significant changes could be due to the plants taking the nutrients for leaf, root growth, and photosynthesis. Soil N has a role in aiding in the sequestration of C into the soil, as research has shown that a higher N enables the soil to sequester more C (Bala *et al.*, 2013; Zaehle, 2013), so the addition of N feed in the rhizotrons would have encouraged C sequestration. The changing N concentration in the pot-grown trees could be due to N-rich root exudates, but this would need to be investigated. The nitrate levels in the soil over the 18-months of this experiment, had no significant differences between the five scions, but differences were found between soil regions and over time. By the end of the experiment, the soil nitrate levels had decreased, which could be due to nitrates being used by the tree, leaching from the soil, and the lack of any fertigation.

Carbon, N, and other essential nutrients required for tree growth and fruit production comes from various sources – either from the soil in the case of N and other nutrient and photosynthesis for C. Limitations on sources of essential nutrients, such as N including nitrates from the soil (the trees becoming pot bound in this experiment) can affect concentrations being stored in various sink- for N the tree’s leaves. Limited N has been shown to impact the ability of the leaves to photosynthesise, as N is essential to produce proteins that are essential for photosynthesis and therefore on the ability to source C, this is known as the source- sink relationship. This relationship between source and sink can change as the plant grows, as leaves age, seasonally (especially in deciduous trees like apple), and can also be affected by abiotic stress (in this case nutrient limitation as the trees became pot bound) (Smith, Rao and Merchant, 2018; Burnett, 2019; Bera, Sow and Ranjan, 2022). This relationship is still an area of active research as the need to understand transportation and partitioning of the nutrients to sink sites to improve overall crop yield and potential CO_2 mitigation.

For two reasons, direct comparisons between the scion/rootstock interaction of the trees under investigation cannot be easily made. Firstly, trees were destructively harvested, so these results are not of continuous growth by the same tree but can show possible trends. Secondly, each grafted scion and rootstock will have been slightly different in size at the time of planting.

3.6. Conclusion

The results of this 18-month experiment show that, in this period of growth, no scion had a greater influence on the C sequestration across the different fractions of C in the soil or roots. Some significant differences between scion varieties regarding total C in the soil were observed at the end of the experiment. The amounts of active C were found to have decreased over the experimental period, but in contrast, the amount of microbial C in the soil increased, and no scions showed significant effects on this. The decrease in POXC showed that C was consumed by the soil microbial communities or recycled back into the atmosphere. Overall, the concentrations of C found in the root zone soil generally were higher than those of the bulk soils. The timings of soil collection did show some effect on the C concentration found in the soil, which would need to be accounted for in future studies. As expected, the trees' biomass C and the C present in the roots increased over time, but biomass growth had possibly been limited in the final harvest due to the lack of available nutrients as the trees had become pot bound.

3.6.1 Limitations and future study

There were several limitations to this study. Firstly, the containers the trees were grown in were not standardised, with some trees being grown in pots and others in rhizotrons of different capacities, which caused the roots to become pot bound. Secondly, there was no coherent feeding plan for all the trees, as only those grown in the rhizotrons had regular feeds. This would have allowed for more direct comparisons across sampling points. Thirdly, this study has not been repeated, so these results indicate the impact different scion varieties may have on M9 rootstocks. Fourthly, it does not show the effects that scions may have on other rootstocks, under different climatic conditions or soil types. Finally, with the trees being destructively harvested the soil data does not show continuous C and N change but is a single point in time.

Further studies would need to be conducted into the scion influence over rootstocks on C sequestration to remove some of the limitations that affected this study. These studies would need to be carried out over a longer period to determine if any of the observed significant differences were seen as trees aged. It would also be useful to see how different soil types may impact C sequestration under different scions. If this study was to be repeated, all growing containers would need to be the same size with sufficient capacity to reduce the likelihood of tree roots becoming pot-bound and tree growth being limited by the lack of available nutrients. Also, a suitable fertigation pattern must be implemented across all trees.

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Chapter 4. Incremental increases in ambient temperature effects on belowground carbon sequestration of apple trees

4.1. Abstract

With global atmospheric temperatures increasing and changing weather patterns due to rising CO_2 levels, this could impact food security for the increasing human population. Soil management and soil C sequestration are important in the fight against climate change. This investigation tried to determine what effects increased atmospheric temperature had on soils ability to sequester C under different varieties of apple trees across three temperature ranges (ambient, 2°C and 4°C above ambient), using the climate change tunnels at Brogdale Farm, Kent, UK. The results showed that across the four seasonal collection points soil TC and TN concentrations in the 2°C above ambient tunnel were significantly higher than the other two tunnels and with the most significant change happening between September and December. Soil POXC and microbial biomass C showed more variability in concentrations and seasonal changes, POXC significantly declined between September and December, whereas MBC significantly increased between March and September's soil collections. In conclusion a rise in atmospheric temperature by 2°C will increase the total soil concentration of both C and N, whereas further increases to 4°C above the current ambient temperatures soil C is more likely to be lost from the soil.

4.2. Introduction

Temperatures are predicted to rise between 1°C and 2°C over the next 20-30 years from pre-industrial levels, which is likely to affect plants and soils (Olesen and Bindi, 2002; Valverdi, Cheng and Kalcsits, 2019; Organización Mundial de la Meteorología, 2020). With the increasing winter temperatures due to climatic changes, food security is important with the expanding population. Warmer winters are likely to affect how plants grow and, especially, the formation of leaf buds and flower production (Greer *et al.*, 2006). Apple trees require a certain amount of winter chill to induce leaf and flower buds to form and break (open) in the spring, which produces the crop. The winter chill is related to trees in dormancy during the winter, and the temperatures generally drop below 12°C *(*Fishman, Erez and Couvillon, 1987; Heide and Prestrud, 2005; Guak and Neilsen, 2013). The amount of winter chill required varies between apple varieties, some requiring longer periods and others as few as 100 hours, such as Tropical Beauty (*Pomiferous, the worlds biggest apple resource, 2021;* Carter*, 2007*). With temperatures increasing globally, the amount of winter chill trees receive could be diminished, which may affect the leaf bud numbers, reducing photosynthetic activity, and earlier leaf senescence, by inducing plants to move through their seasonal cycles faster and therefore, the amount of available C that can be sequestered into the soil (Zani *et al.*, 2020).

Soil management and C sequestration have become important in the fight against climate change (warming temperatures and changes to rainfall patterns) and key enablers of global food security (Tiefenbacher et al., 2021; Wang et al., 2023). Tiefenbacher et al. (2021), stated that higher temperatures and similar rainfall patterns to those currently received are likely to increase the turnover of stored soil C, releasing more carbon dioxide (CO_2) back into the atmosphere. The loss of C from soil mineralisation needs to be limited, in combination with enhanced organic matter inputs. Soil organic C and soil erosion are linked and are affected by climatic conditions (Wang *et al.*, 2023). Wang et al.'s, investigation showed that atmospheric temperature was an important factor in the storage and loss of soil organic C in cropland through the erosion and subsequent burial of soil in different areas of land. It showed that organic matter decomposition increased with temperature and was sufficient to replace soil C lost through local erosion. In contrast, the amount of soil C decreased as temperatures increased.

Other studies have shown a greater vulnerability to the loss of soil C stocks as temperatures increase, especially in unprotected pools. Research by Hartley et al. (2021) has shown that soil C stocks would be reduced overall as temperatures increase. They concluded that soil texture, especially the clay content in the soil, affects soil mineralisation and C storage ability, where soil with a coarser texture released more C than finer textured soil. Hartley et al, also concluded that latitude was an important factor in how temperatures would affect the C turnover in stable C (soil in tropical latitudes was less vulnerable to increasing atmospheric temperatures). Models are currently unable to accurately predict soil C pool vulnerability to climate change or locations with the greatest losses, we still need further refinements. Devi (2021) showed that soil organic C (SOC) under a range of different types of trees was strongly influenced by atmospheric temperature (lower temperatures elevated SOC). This study did not specifically investigate the effect of temperature but a range of variables on SOC.

Researchers have also investigated the effect of increasing soil temperatures on photosynthesis and mineral uptake (Gur, Bravdo and Mizrahi, 1972; Valverdi, Cheng and Kalcsits, 2019; Moore *et al.*, 2021). Increasing atmospheric temperatures have been shown to impact the photosynthetic ability of leaves (Moore *et al.*, 2021). The review by Moore et al. stated that the higher temperature does not increase the leaf's ability to reach "thermal optimum" but, in fact, reduces the rate at which photosynthesis can occur, even in elevated atmospheric CO_2. This reduces the plants' ability to process CO_2 and produce and transfer C-based exudates into the soil. The work focuses on cereal crops, but it does discuss that temperatures over 35°C can affect plant yield and shorten the lifespan of plants, as it induces plant maturation, which would reduce plant photosynthesis. A study into how elevated soil temperatures affected nutrient uptake and its distribution within an apple tree was conducted by Valverdi et al. (2019). Their study showed that increasing soil temperatures will affect the trees’ ability to take up and partition nutrients under various combinations of apple rootstock and scions, due to their differences in genetics. This therefore is an important factor when planning how to future proof apple production in increasing global temperatures.

There is insufficient research on the effect of increasing atmospheric temperature on soil C sequestration, specifically under apple trees. The current study aimed to investigate whether increasing atmospheric temperatures influenced the amounts of belowground C sequestration of different scions across three different temperature ranges.

4.3. Materials and Methods

This experiment was conducted at the National Fruit Collection at Brogdale farm in Kent, UK (Tunnel location 51°17'45.5"N 0°52'54.6"E). This site had an established long-term climate change study being conducted in conjunction with the University of Reading and the Fruit Advisory Service Team (FAST), investigating the effects of changes in temperature and rainfall on aboveground growth, fruit production and quality, but not belowground C sequestration.

The temperature and rainfall experiment at Brogdale was made up of three large interconnected polytunnels per temperature (**Fig 4.1 A and B**); each of these tunnels had a different temperature range, ambient (which had open sides) 2°C and 4°C above ambient in the other two tunnels. The two warmer tunnels were fully enclosed and had automated ventilation to help maintain the higher temperatures. Each temperature tunnel was divided into three rows of apple trees under contrasting irrigated treatments. The irrigation treatments aimed to replicate possible climatic changes in the rainfall levels, one treatment representing the rainfall occurring outside of the tunnels and the other two representing either a 20% reduction or increase in rainfall. Irrigation across the three temperature tunnels was not controlled with a good degree of accuracy due to leaks in the polytunnel roofs. This study will only focus on the effects of temperature on belowground C sequestration.

The soil type was silty clay loam (determined by laser diffraction (Faé *et al.*, 2019)), with a mean pH$_{\mathrm{CaCl2}}$ 5.5. The climate change experiment utilised in this study comprised twenty-two different apple varieties growing on M9 rootstocks planted in the polytunnels in 2013. Eight scion varieties (shown in **Table 4.1**) were chosen to be investigated for soil C content, with six replicates in each of the three temperature treatments across each tunnel to account for the site's slope and environmental variations within the tunnels.

Fig 4.1 A and B. A, Exterior and **B**, interior of the interconnected polytunnels used at Brogdale for FAST and the University of Reading climate change experiment at the National Fruit Collection Brogdale Farm, Kent, UK. Images were taken by Catherine Chapman on 10th March 2021.

Cox’s Orange Pippin was used to provide continuity following previous experiments investigating the effects of rootstocks and different scions on C sequestration, a benchmark variety that can be used to measure others against. Scions were selected to include dessert, cider, and culinary apples.

Table 4.1. Scions under investigation at Brogdale (*'Flowering times of apples RHS Pollination Groups', accessed 2021; Pomiferous, the worlds biggest apple resource, 2021*).

Scion	Scion information
Cox's Orange Pippin	A dessert apple discovered in 1825 in the UK and is a cross between Ribston Pippin and possibly Blenheim Orange. This variety can be self-fertile and has a mid-season flowering time (pollination group 3) and mid to late season for harvest. This variety is commercially grown in the UK, but numbers are in decline.
Gala	A dessert apple developed in New Zealand in 1934 by J H Kidd and is a cross between Golden Delicious and Kidd's Orange Red. It can be grown in temperate and warmer climates, requiring only eight hundred hours of winter chill. This variety can be self-fertile (pollination group 4) and is a mid to late season for harvest.
Braeburn	A dessert apple discovered in New Zealand in the 1950's, parentage is unknown but is a possible cross between Granny Smith and Lady Hamilton. It is better suited to warmer climates and only requires seven hundred hours of winter chill. A late-season flowering variety and later harvesting. It has been a parent to other varieties.
Tropical Beauty	A dessert apple from South Africa in 1930, but parentage is unknown. It requires as little as one hundred hours of winter chill, is partially self-fertile, has a mid to late flowering period, and is a later harvested fruit. This is not grown commercially in the UK.
Bramley's seedling	A multipurpose apple (culinary, cider and dessert) discovered in Nottingham in the UK and originally planted in 1809, but not brought out commercially until the 1850's. It can be self-fertile with a mid-flowering season (pollination group 3) and is a late fruit harvest. It is a triploid variety of apple.
Winter Pearmain	A culinary and dessert apple known by several names including Winter White Pearmain, Winter Queening, one of the oldest UK grown apple varieties from around 1200AD. It is a self-fertile tree, flowers early in the season and is a late

	harvest.
Discovery	A dessert apple was introduced in 1949, and only one of its parents is known, Worcester Pearmain. It is self-sterile, requires pollination partners to produce fruit, a mid-season flowering point (pollination group 3), and an early cropping variety.
George Cave	A dessert apple, which can be used for juice and cider, was introduced in Essex UK in 1923 and has unknown parentage. It prefers to be grown in temperate climates, has a mid-season flowering point and is an early harvester. This is not grown commercially in the UK.

Soil samples were collected every three months (from March 2021 to December 2021) at a depth of 20-30 cm, and within 30 cm of the tree trunk to ensure the soil was within the rootzone (**Fig 4.2**). Soil samples were put into labelled bags and markers were placed to prevent repeat sampling at the same site. The sampling sites were backfilled immediately to avoid water pooling and cold spot development in the soil. The upper 20 cm of soil was not investigated, as this soil layer could contain roots from grass or weeds growing around the tree and leaf litter, which may influence soil C levels.

Fig 4.2. The location of the soil coring in relation to the tree in the ambient tunnel at the National Fruit Collection, Brogdale Farm, Kent, UK. Image was taken by Catherine Chapman 9th March 2021.

4.3.1. Soil and statistical analysis

Various laboratory methods were used to analyse the different fractions of the soil C content. The methods used for analysis included total carbon (TC) and nitrogen (TN), which were conducted by the University of Reading using the LECO CHN628 analyser, following the AOAC official method of analysis (1990). NRM (Reading UK) analysed soil texture using the laser diffraction method.

At NIAB East Malling soil moisture content was determined using the gravimetric method described by Forster (1995), and pH was determined using the calcium chloride method of

Schofield and Taylor (1955). The potassium permanganate method was used to determine soil active C (POXC) following the procedures described by Weil et al. (2003) and Culman et al. (2014). The ninhydrin assay described by Amato and Ladd (1988) was used to determine both the microbial biomass N and C (MBN/MBC) within the soil, following soil fumigations as described by Vance et al. (1987), but this will be focusing on MBC results. The nitrate concentration in the soil was determined using the method described by Cataldo et al. (1975).

Some alterations were made to ninhydrin (MBN/C) and nitrate assay methods to collect all data points. MBC was only investigated at two of the four soil collections, just before the trees came out of dormancy (March 2021) and in late summer (September 2021). The analysis was done on three of each scion collected at these two points, estimating MBN and MBC due to time and cost restraints. The standard solutions were decreased in concentrations from 10 to 6 ml (or 1000 - 600 µm NH_2-N L^{-1}) with an increase of 1 ml. Also, the amount of sample used was decreased from 1 ml to 0.6 ml and the citric acid increased from 1 ml to 1.4 ml, the remainder of the process was unchanged.

The nitrate standards were increased from 0-10 mL (µg NO_3^- -N mL^{-1}) to 14 mls in of 2 and 4 mls increments.

Several modifications were made to the original plan for this experiment due to soil conditions and weather factors. The number of scion varieties sampled in June and September 2021, was reduced by half due to the soil being baked solid, thus making it exceedingly difficult to gather samples. The second alteration to the original plan was that the experiment was cut short by a year due to storms in early 2022 damaging the polytunnels, making them unsafe to work in, and not maintaining temperatures.

The data was analysed in Microsoft Excel 365 using Pairwise one-way ANOVA after checking that the assumptions were met. Pearsons's correlation analysis calculated significant differences between each data sets collected. This was carried out to determine if there were any significant effects on soil C and N between the three temperature tunnels and the eight selected scions within each temperature tunnel as well as between the three tunnels at each and across the four-sampling point across 2021. P values of <0.05 indicate significant differences.

4.4. Results

4.4.1 Tunnel temperature

The temperatures between the three experimental tunnels were not always maintained at the +2°C and + 4°C above that of the ambient tunnels as the experimental design of this existing experiment had planned for (**Table 4.2** data provide by Adam Peter). The differences in temperature between the tunnels varied day by day, monthly and whether you were working from the mean minimum or maximum temperatures. But when looking at the mean maximum temperatures of the two warmer tunnels they do show that over the year (2021) they were approximately +2°C and +4°C above those found within the ambient tunnel.

Table 4.2. Temperature of the three tunnels for the year 2021 showing the mean monthly minimum and maximum temperatures, temperature differences between the above ambient tunnels for minimum and maximum temperatures as well as the yearly mean of each tunnel.

Tunnels	**Ambient**		**2°C +**				**4°C +**			
Month (2021)	**Mean min**	**Mean Max**	**Mean min**	**difference from ambient**	**Mean Max**	**difference from ambient**	**Mean min**	**difference from ambient**	**Mean Max**	**difference from ambient**
January*	2.1	6.1	1.8	-0.3	7.1	1.0	2.0	-0.1	7.9	1.8
February*	3.4	7.9	3.2	-0.2	9.7	1.8	3.5	0.1	10.2	2.3
March	3.5	11.3	3.4	-0.1	13.9	2.6	3.7	0.2	15.2	3.9
April	2.1	11.8	2.4	0.3	14.7	2.9	2.8	0.7	17.2	5.4
May	7.1	16.4	7.5	0.4	19.5	3.1	7.6	0.5	21.0	4.6
June	12.6	22.0	13.0	0.4	25.0	3.0	13.1	0.5	26.0	4.0
July	13.7	23.0	13.9	0.2	26.1	3.1	15.2	1.5	28.7	5.7
August	13.3	21.5	13.3	0.0	24.4	2.9	14.0	0.7	26.6	5.1
September	12.3	21.9	12.2	-0.1	24.8	2.9	12.7	0.4	26.8	4.9
October	9.0	15.8	8.8	-0.2	18.0	2.2	9.0	0.0	18.9	3.1
November	5.6	10.8	5.3	-0.3	12.5	1.7	5.5	-0.1	13.0	2.2
December	5.0	9.4	4.7	-0.3	9.7	0.3	4.9	-0.1	10.2	0.8
Yearly means	7.5	14.8	7.5	0.0	17.1	2.3	7.8	0.4	18.5	3.7

4.4.2 Soil pH

The average soil pH_{CaCl2} in March 2021 was 5.5, but there were variations between scions under each of the three temperature treatments, ranging from a pH_{CaCl2} 4.8 in the +2°C tunnel under the Gala trees to pH_{CaCl2} of 6.0 in the +4°C tunnel under Braeburn trees. There was no overall difference in soil pH between the three temperature treatments, but five scions showed significant differences between two tunnels (**Fig 4.3**). The soil moisture content showed no significant differences between the three temperature tunnels.

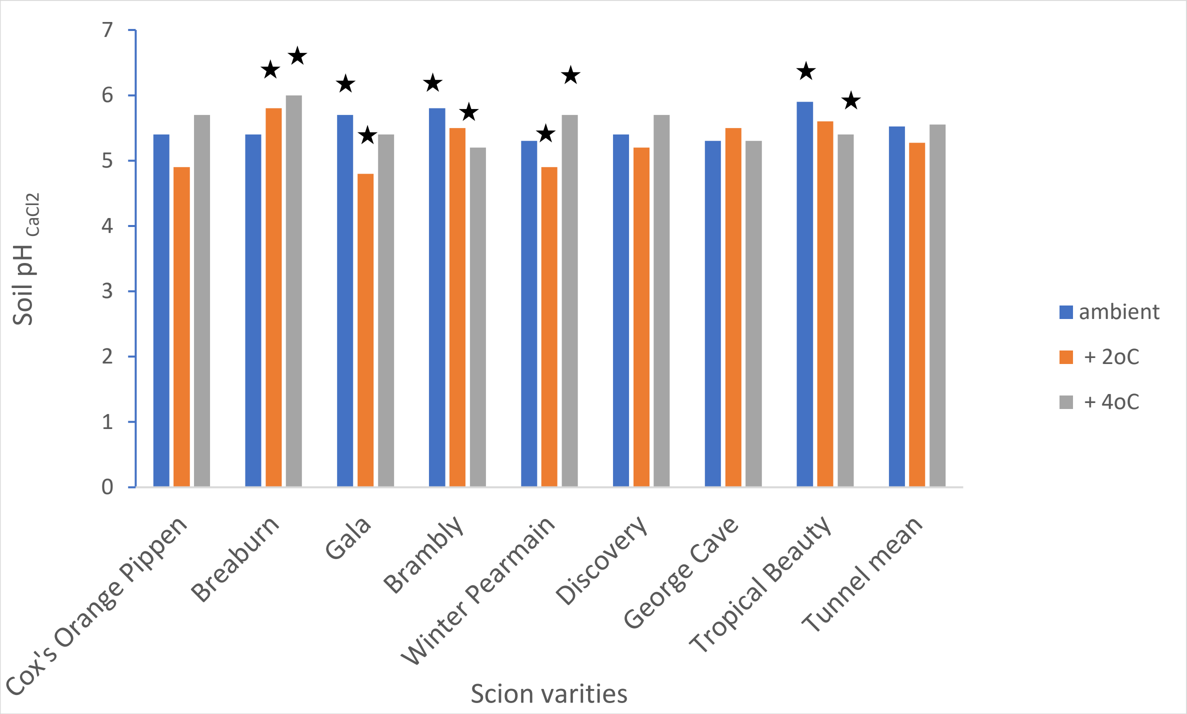

Fig 4.3. Mean soil pH for the eight scions and alleyways across the three temperature tunnels. Significant differences (P = <0.05) between temperature tunnels are indicated by asterisks (*).

4.4.3. Soil carbon

Soil TC (%) significantly increased between September to December (P = <0.01) in the ambient tunnel. Across the three different temperature treatments the +2°C tunnel had a significantly higher content of TC than the ambient and +4°C tunnels at each soil collection point. In December, the ambient tunnel was also significantly higher in TC content than the +4°C tunnel (**Table 4.3**). In the ambient tunnel Gala and Alleyways showed a significant increase in the TC between September and December 2021 (P = 0.02 and P = 0.02, respectively), the two warmer tunnels showed no scion had significant changes over the four timepoints.

Table 4.3. Overall P values of soil total carbon comparing tunnels at the four collection points in 2021. Significant differences have a P = <0.05 and are indicated with an asterisk (*).

Collection point	Tunnel 1 (mean % value)	Tunnel 2 (mean % value)	P value
March 2021	Ambient (1.36)	+2°C (1.61)	P = <0.001 *
	Ambient (1.36)	+4°C (1.41)	P = 0.4
	+2°C (1.61)	+4°C (1.41)	P = <0.01*
June 2021	Ambient (1.33)	+2°C (1.62)	P = <0.001 *
	Ambient (1.33)	+4°C (1.38)	P = 0.5
	+2°C (1.62)	+4°C (1.38)	P = 0.001 *
September 2021	Ambient (1.30)	+2°C (1.59)	P = <0.0001 *
	Ambient (1.30)	+4°C (1.37)	P = 0.2
	+2°C (1.59)	+4°C (1.37)	P = 0.001 *
December 2021	Ambient (1.45)	+2°C (1.57)	P = 0.01 *
	Ambient (1.45)	+4°C (1.33)	P = 0.01 *
	+2°C (1.57)	+4°C (1.33)	P = <0.00001 *

Soil active C test (POXC) was conducted on a subsection of the six trees sampled under four out of the eight scions. POXC fluctuated throughout the experiment, the overall value of POXC in the soil had declined significantly between September and December 2021 for the +2°C (P = 0.001) and +4°C tunnel (P = 0.0001) but did not decline in the ambient tunnel. Across the three temperature tunnels for three out of the four soil sampling points, the +2°C tunnel had the highest concentrations of POXC. Still, it was only significant in June (ambient V +2°C P = 0.01 and +2°C V +4°C P = 0.01) and September (ambient V +2°C P = 0.02). In June, the POXC found in the soils of the alleyways in the +4°C tunnel was significantly higher than in the ambient tunnel (P = 0.02). Also, in September, the alleyways and Braeburn showed significant differences between the temperature tunnels (ambient V +4°C P = 0.02 (+4°C highest concentration)) and ambient V +2°C P = 0.02 (ambient was highest), respectively). Several scions and alleyways showed significant changes in their soil's POXC concentrations within the same temperature tunnels between soil collection points. These changes in POXC concentrations under the trees or within the alleyways mainly occurred between September and December 2021 (**Fig 4.4**).

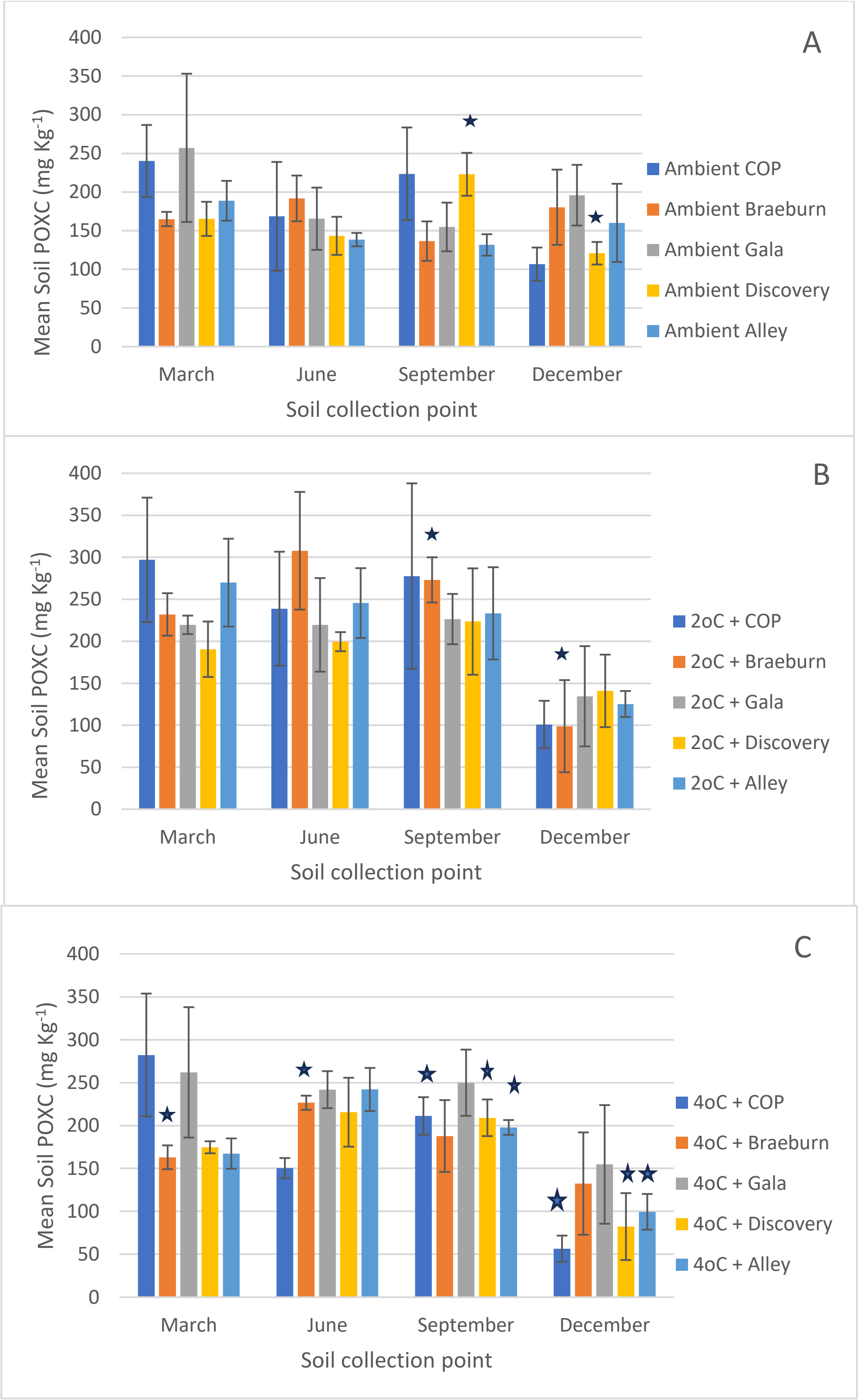

Fig 4.4 A, B, and C. Mean soil POXC (mg KG^{-1} soil) under four different scions (n=3) and alleyways under the three tunnel temperatures over the seasonal soil collection points, **A** ambient, **B** 2°C above ambient, and **C** 4°C above ambient, with the most significant changes occurring between September and December, stars indicate significant differences.

Soil MBC and MBN concentrations were measured in March and September to show soil under trees at the end of winter dormancy and summer. In March, the MBC and MBN showed no significant difference in the soil concentrations between the three temperature tunnels. In September, however, all tunnels were significantly different for both MBC and MBN, with the soil concentration in the +4°C tunnel highest and ambient the lowest (MBC and MBN: Ambient V +2°C P = 0.02, Ambient V +4°C P = 0.00001 and +2°C V +4°C P = 0.03). The soils' MBC and MBN concentrations increased between March and September across all three tunnels; both above ambient tunnels showed a significant increase (for both MBC and MBN +2°C P= 0.01 and +4°C P = <0.00001). Braeburn and Discovery were the only trees that showed significant increases between March and September in MBC/MBN soil concentration (P = <0.01 and P = 0.001, respectively). In September, Braeburn and Discovery were the only trees to show significant differences between the three different temperature tunnels, with +4°C above the ambient, showing the highest soil concentration of MBC. The Braeburn and Discovery trees were significantly lower in the ambient tunnel than the +4°C tunnel (P = <0.01 and P = 0.02, respectively), but for the Discovery trees, the +4°C was only just significantly higher in soil MBC than the +2°C tunnel (P = 0.05).

4.4.2. Soil nitrogen

Soil total N (%) concentrations across the three temperature tunnels at each harvest showed that the +2°C above that of the ambient tunnel was significantly higher than at least one other tunnel (**Table 4.4**).

The soils sampled in June, September, and December 2021, had significant TN differences, between tunnel temperatures for 2 scions and/or alleyways (**Table 4.5**). Also, within two of the temperature tunnels (ambient and +4°C), there were significant differences between soil collections: ambient tunnel (March to June ($P = 0.02$) and September to December ($P = <0.01$), and +4°C tunnel, September to December ($P = 0.05$). Within each temperature tunnel at each soil sampling point, several scions also showed significant differences. In the ambient tunnel in September, the soil under Discovery was significantly higher in TN than Gala ($P = 0.02$). In December, the soil under George Cave was significantly higher in TN than the alleyways ($P = 0.03$). In the +2°C tunnel in December, George Cave had significantly higher levels of soil TN than Braeburn ($P = 0.04$). In contrast, in the +4°C tunnel in March, Gala was significantly higher in soil TN than Braeburn ($P = 0.05$).

Table 4.4. Mean soil total nitrogen (%) concentrations and their significant difference (P = <0.05) found between different temperature treatments across the four soil collection points.

Soil collection point	Tunnel with the highest TN (mean value)	Tunnel with the lowest TN (mean value)	P value
March 2021	+2°C above ambient (0.18)	+4°C above ambient (0.16)	P = <0.01
June 2021	+2°C above ambient (0.18)	Ambient (0.16)	P = 0.0001
June 2021	+2°C above ambient (0.18)	+4°C above ambient (0.16)	P = 0.01
September 2021	+2°C above ambient (0.18)	Ambient (0.16)	P = 0.001
September 2021	+4°C above ambient (0.17)	Ambient (0.16)	P = 0.05
December 2021	+2°C above ambient (0.18)	+4°C above ambient (0.16)	P = <0.0001
December 2021	Ambient (0.17)	+4°C above ambient (0.16)	P = 0.001

Table 4.5. Mean soils total nitrogen (%) content between temperature tunnels at different soil collection points

Soil collection point	Scion/alleyway	Highest TN Temperature tunnel (mean values)	Lowest TN Temperature tunnel (mean values)	P value
June	Braeburn	+2°C (0.20)	Ambient (0.16)	P = <0.01
June	Braeburn	+2°C (0.20)	+4°C (0.16)	P = 0.01
June	Gala	+2°C (0.18)	Ambient (0.15)	P = 0.02
June	Alleyway	+4°C (0.18)	Ambient (0.15)	P = 0.05
September	Gala	+2°C (0.18)	Ambient (0.15)	P = <0.01
September	Gala	+4°C (0.17)	Ambient (0.15)	P = 0.02
September	Alleyway	+2°C (0.19)	Ambient (0.15)	P = 0.05
December	Brambly seedling	+2°C (0.19)	+4°C (0.16)	P = 0.02
December	Brambly seedling	Ambient (0.18)	+4°C (0.16)	P = 0.03
December	George Cave	+2°C (0.19)	Ambient (0.16)	P = 0.01
December	George Cave	+2°C (0.19)	+4°C (0.16)	P = <0.01

Soil nitrate concentrations in March showed one significant result of atmospheric warming, + 4°C tunnel having the highest concentration (+4°C V +2°C $P = 0.02$). In the ambient, +2°C and +4°C tunnels, there was a significant decrease in the soil nitrate concentration between September and December 2021 (Ambient $P = 0.001$, +2°C $P = <0.001$ and +4°C $P = 0.0001$). In the +2°C tunnel, soil nitrate concentrations significantly increased from March to June and June to September, ($P = <0.001$ and $P = 0.02$, respectively). Two scions showed significant differences between temperatures at two different soil collection points. In December, Discovery in the ambient tunnel had higher concentrations of nitrate than +4°C tunnel ($P = 0.03$), whereas, in March, Tropical Beauty in the ambient tunnels had higher soil nitrate than +2°C tunnel ($P = <0.001$). At each of the harvests in each temperature tunnel there were significant differences between the different scions and alleyways (**Table 4.6**).

Correlation analysis between the soil collection point and all the different soil tests, within each different temperature tunnel (**Table 4.7**), showed strongly linear relationships between the soils TN and TC (from 75% -95%). Soil nitrate and moisture content had a strong positive relation to each other in June, only in the +4°C tunnel ($P = <0.01$) but mostly any relations of soil nitrates were to either soil total C or N. POXC could not be assessed because only a subsample was used to estimate what was happening in the soil.

Table 4.6. Mean soil nitrate concentration (µg NO_3- -N ml-^{1}) under different scions showed significant differences between and within the same temperature tunnels at each of the four soil collection points.

Harvest point	Tunnel temperature	Scion (highest conc.)	Scion (lowest conc.)	P value
March	Ambient	Tropical Beauty (4.87)	Winter Pearmain (2.40)	P = 0.01
March	Ambient	Tropical Beauty (4.87)	George Cave (3.40)	P =0.05
March	+2°C	Braeburn (3.75)	Cox's Orange Pippen (2.36)	P = <0.01
March	+2°C	Alleyway (3.60)	Cox's Orange Pippen (2.36)	P = 0.02
March	+2°C	Braeburn (3.75)	Discovery (2.02)	P = 0.02
March	+2°C	Braeburn (3.75)	Tropical Beauty (1.85)	P = 0.001
March	+2°C	Alleyway (3.60)	Tropical Beauty (1.85)	P = 0.01
March	+4°C	Alleyway (6.47)	Winter Pearmain (1.95)	P = 0.02
September	+4°C	Gala (10.65)	Discovery (4.64)	P = 0.03
December	Ambient	Gala (2.90)	Cox's Orange Pippen (1.57)	P = 0.02
December	Ambient	Discovery (12.42)	Cox's Orange Pippen (1.57)	P = 0.01
December	Ambient	George Cave (5.38)	Cox's Orange Pippen (1.57)	P = 0.01
December	Ambient	Tropical Beauty (4.59)	Cox's Orange Pippen (1.57)	P = 0.02
December	Ambient	Discovery (12.42)	Braeburn (2.51)	P = 0.02
December	Ambient	Discovery (12.42)	Gala (2.90)	P = 0.01
December	Ambient	George Cave (5.38)	Gala (2.90)	P = 0.05
December	Ambient	Discovery (12.42)	Tropical Beauty (4.59)	P = 0.05
December	Ambient	Discovery (12.42)	Brambly Seedling (4.56)	P = 0.05
December	Ambient	Discovery (12.42)	Winter Pearmain (4.58)	P = 0.05

Table 4.7. December correlation analysis results of only the significant positive relationship between different soil analysis.

Dec-21					
Temp	**scion/other**	**Test 1**	**Test 2**	**R^2 value**	**P value**
Ambient	Alley	Total N	Total C	0.99	0.05
	Braeburn	Total N	Total C	0.96	0.001
	Winter	Total N	Total C	0.97	<0.001
	Discovery	Total N	Total C	0.96	0.001
	George	Total N	Total C	0.85	0.01
	Tropical	Total N	Total C	0.96	0.001
2°C +	Overall	Total N	Total C	0.84	<0.00001
	Alley	Total N	Total C	0.99	0.05
	Braeburn	nitrate	Total N	0.89	0.01
	Braeburn	Total N	Total C	0.81	0.01
	Gala	Total N	Total C	0.94	0.001
	Brambley	Total N	Total C	0.94	0.001
	Discovery	Total N	Total C	0.91	<0.01
	George	Total N	Total C	0.96	0.001
	Tropical	Total N	Total C	0.82	0.01
4°C +	Overall	Total N	Total C	0.87	<0.00001
	COP	Total N	Total C	0.92	<0.01
	Braeburn	Total N	Total C	0.90	<0.01
	Gala	Total N	Total C	0.97	<0.001
	Brambley	Total N	Total C	0.87	0.01
	Discovery	Total N	Total C	0.97	<0.001
	Tropical	Total N	Total C	0.96	0.001

4.5. Discussion

This study investigated whether the trees' ability to sequester C in the soil changed as atmospheric temperatures increased. Overall, increases in atmospheric temperature, did have an impact on soil C and N concentrations; an increase of 2°C increased soil C, but a 4°C increase decreased soil C levels to similar to, or below, that of ambient temperatures. There were also inconsistencies for scions and alleyways that showed significant differences for C and N concentrations, as these altered across temperature treatments and soil collection points.

Soil pH (**Fig 4.3**) and moisture content varied slightly between the different scions, alleyways, and tunnels, none of which were significant. These variabilities could be down to the topography of the tunnels as there was a slight incline across the site (top to bottom), so moisture and nutrients could be leached downhill and form pools. Also, within each tunnel, the different irrigation regimes (even though they were not functioning adequately) would influence the soil moisture content. Soil moisture, rainfall (in this case, irrigation that simulated different rainfall patterns) and soil pH have all been shown to impact soil's ability to sequester C, as discussed in previous chapters. There were seasonal soil moisture changes consistent with the UK rainfall patterns. Soil moisture was not significantly correlated with soil C throughout this experiment, which could be because the trees were well established, and the moisture levels did not significantly change at each soil collection point. The trees were also grown in polytunnels, so water vapour given off by the trees and soil would remain within the polytunnels and would likely create their own microclimatic conditions, which could limit soil moisture loss.

Soil TC (**Table 4.2**) showed that an increase of +2°C above that of the current ambient temperature, increased the soil TC content. Still, when temperature was increased further to +4°C above the current atmospheric temperature, soil TC declined over the four sampling points and was similar to or lower than those found in the ambient tunnel. This result indicated that C was lost from the soil at the highest atmospheric temperature treatment. These results are consistent with those found by Guttières et al (2021), which showed that at higher temperatures the soils mineralisation rates increased, increasing the loss of C from the soil. Guttières study was carried out in a controlled environment, with temperatures not exceeding 20°C, whereas the current experiment was conducted in polytunnels, where temperatures repeatedly reached over 30°C in the summer (in Kent) and changed throughout each day and year. Other studies have also shown that higher temperatures affect soil nutrients and the plants' ability to photosynthesise and impact on the plants' overall health, which could have an impact on C sequestration (Zhao, Ta and Wang, 2017; Valverdi, Cheng and Kalcsits, 2019; Wang *et al.*, 2023). By the final harvest, the TC levels in the ambient tunnel were significantly higher than those in the +4°C tunnel. The reduction in the C sequestration in the +4°C tunnel could also result from the reduction in the trees' ability to photosynthesise and produce C-based exudates (Moore *et al.*, 2021).

Soil TC in the ambient tunnel did show that Gala and the alleyways had significantly increased between the final two harvests, but these were the only two across all tunnels and scions that did. Valverdi et al. (2019), showed that different scions could affect nutrient uptake from the soil and root exudates. This could also explain the increase in soil TC under the Gala trees but not the alleyways, which is more likely to change in content from leaching of TC down the site, exudation from the grass (occurring all year), and possible soil compaction from tractors entering the tunnels to aid in harvesting and pruning (Deurer *et al.*, 2012). There would also be seasonal changes in photosynthetic levels, root exudation, the trees growth needs, and the addition of leaf litter inputs that come from perennial trees every year, all of which would contribute to changes in soil C levels.

Breaking soil TC into the two fractions of active (POXC) (**Fig 4.4**) and MBC, showed greater fluctuations over the soil collection points. These fluctuations in MBC and active C could be due to the seasonality and the changes in atmospheric and soil temperatures (Bardgett *et al.*, 1997; Farrell *et al.*, 2011), as well as increasing rates of replication by soil biota as atmospheric and soil temperatures increased. There were also differences between scions and the alleyways, but these were not the same as those found in TC and differed between POXC and MBC. Again, these were seasonal changes for POXC, with the most significant changes happening when the air temperature between September and December declined. The +4°C tunnel had the most significant changes under the different scions sampled, which suggests that active C (POXC) is being lost during this period and likely to be expelled from the soil as CO_2 back into the atmosphere.

September's MBC was also significantly higher in the +4°C tunnel than the other two tunnels found in March. The higher levels of MBC in September in the highest temperature treatment may suggest a higher activity (number) of microbes, which would be consuming organic matter (which would include C) and could account for the observed decrease in active C in December, as the microbes would be releasing CO_2 through their activity back into the atmosphere. The increase of MBC concentrations between the two soil samplings showed that as the atmospheric temperature increased, the activity of the soil microbiota and C loss from the soil increased.

The soil's total N levels showed (**Table 4.5**) that the +2°C tunnel had significantly higher concentrations than the other two tunnels, similar to those found for TC. This higher level of soil TN will likely aid the increased C sequestrations (Reay *et al.*, 2008; Bala *et al.*, 2013). TN and TC are generally found in a ratio to each other and have been described by many studies and this can be seen in the high amount of positive linear correlation (**Table 4.7**) found in this investigation (Reay *et al.*, 2008; Zaehle, 2013; Cotrufo *et al.*, 2019).

As with all the other soil tests, soil nitrate also showed variations over time, temperature, and under different conditions. Research has shown that soil nitrate changes seasonally with the lowest amounts in the winter as the soil and air temperatures decrease (Sahoo, 2022), and starts to rise again in the spring. These seasonal changes in temperatures impact soil microbial activity (warmer temperatures, higher microbial activity). The increase in temperatures between the tunnels could enhance microbial activity, as seen in the significant levels of nitrate in the tunnel, which was 4°C higher than ambient. The difference between the scions could be again down to the different microbial activity and the types and amounts of root exudates the trees are releasing into the soil, which could be affected by the amount of photosynthesis that occurs and how the atmospheric temperature may be limiting this (Taylor, Myrold and Bottomley, 2019; Sahoo, 2022).

4.6. Conclusions and future work.

This investigation showed that temperature significantly affected the levels of C sequestration. Overall, an increase of 2°C above current temperatures enables greater C and N sequestration, but higher than this (+4°C), soil C is then released from the soil as CO_2. This C loss at +4°C brings it close to, if not slightly lower than, those found in the ambient tunnel. The results also showed that different scion varieties may cope better and sequester C above 2°C higher than the current temperatures.

The C and N concentrations in the soil fluctuated over the four collection points, showing some seasonal changes in the trees' ability to sequester C, but this was not always clear. The active and microbial C showed significant increases in concentrations found during June and September (summer and early autumn soil collections). The soil concentrations varied within each tunnel, between scions, alleyways, and the individual tree, showing that no two trees' C sequestration abilities are the same.

Further and longer-term studies need to be conducted to see if the results found in one year is replicated in other years or if there are yearly variations in soil C sequestration belowground due to temperature differences. This would provide a clear picture of how temperature influences soil C sequestration under the three temperature tunnels and how we can protect apple crops from climate change.

4.6.1. Limitations

This study had several shortcomings. Firstly, internal temperature sensors would have been deployed across the tunnels to get accurate air temperatures rather than relying on incomplete data sets from two local weather stations collected by another student to give an approximate temperature of the site. Secondly, atmospheric CO_2 levels in the tunnels should be measured as this may impact the C levels found in the soil, especially within the closed tunnels. Thirdly, a final data set should be collected the following March to see what happens to the C in the soil over the winter months, as this would show if the C remained or is lost from the soil. Fourthly, the depth of collected samples does not show the entire C profile within the soil or total soil depth at this site or the underlying geology. Finally, if time and cost were not limiting factors, completing the MBC data for all time points and samples would provide a better understanding of the soil microbial TN/C.

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5 Soil carbon content across different age of trees in cider orchards

5.1. Abstract

Apples are an important economic crop for the UK with over 22,000 ha's under cultivation. Cider apples have a long productive lifespan and have been presumed to gradually sequester more C in soil over their lifetime, providing a productive land management tool to mitigate rising atmospheric CO_2 levels. This space-for-time substitution chronosequence study investigated how soil C concentrations changed with age. The results showed that soil C decreased rapidly in the first six years of planting, most likely due to the oxidisation of C from soil amendments added around the time of planting. It also revealed that C was still being lost over the remaining 40 years, but this was at a much-reduced rate. Active C showed high concentrations in the first 6 years, declining as the orchards got older. The extractable nutrients in the soil were inconsistent, and both phosphorus and potassium similarly to C concentrations. The soil magnesium, however, showed a very slight increase in the first six years before declining with the ageing orchards. In conclusion, this study has shown that stored soil C and N are not increased with the ageing of the orchards, which is contrary to the general assumption that soil C concentrations will increase as orchards age and therefore may not help to mitigate against rising atmospheric CO_2 levels. This study also did not show any peak age for which C is stored before any decline is observed, which has also been described in previous studies on different orchard crops.

5.2. Introduction

The UK in 2022 had 22,281 hectares (ha) of land under orchard production. Apple orchards covered the largest area (13,719 ha), dessert apples had 7,419 ha, whereas cider apples and perry pear orchards had 6,300 ha under cultivation. The remaining 8,562 ha of orchards were planted with pears, cherries and plums (*GOV.UK*, 2023). The economic value of UK orchard fruit in 2022 reached £376.8 million, with apples (of all types) and perry pears valued at £311 million. The United Kingdom is one of the major cider apple growing countries globally (*Global growth of the cider market and its implications in agriculture*, June 2022) and is predominately grown in western counties such as Herefordshire and Somerset. Across the EU in 2017, 473,500 ha of dessert apples were grown with 34% of these orchards located in Poland. The land under cultivation of orchard fruits had increased by 23,900 ha since 2012 (*Agricultural production - orchards - Statistics Explained*, 2017).

Cider orchards have a longer lifespan than commercial dessert orchards, ranging from 50 to 80 years, compared to 15 to 20 years for dessert apples. Therefore, it would be assumed that cider orchards would have greater potential to sequester C into the soil over their lifespan than dessert orchards, as cider orchards are in the ground longer. A general assumption that older orchard soils might sequester greater amounts of C needs to be investigated, by understanding how soil C changes under ageing orchards.

Cider orchards are generally grown on larger, more vigorous rootstocks, including M111, and M25, and increasingly on M116 (Merwin, Valois and Padilla-Zakour, 2008). These larger rootstocks enable more robust tree growth with stronger trunks with deeper root systems to access available water and nutrients. Cider orchards are harvested by mechanical shaking, as fruit quality for cider production is less important than the dessert apple industry.

Cider orchards are planted in rows wide enough for machinery to pass between them and the tree stands are generally kept clear of grass and weeds by herbicides, reducing competition for water and nutrients. The effect of grass on SOC in orchards was investigated by Xiang et al. (2022). Their findings showed that grass within the orchard and under the growing trees increased the SOC significantly and was linked to biotic and abiotic influences. They concluded that grass coverage, especially in older orchards, increased the SOC stocks compared to orchards less than 5 years old. The use of wheeled machinery within the alleyways is likely to cause compaction within the vehicle tracks. Research carried out by Deurer et al. (2012) showed that this compaction increased soil C concentrations.

Multiple studies have considered C sequestration under different orchard crops. Still, few have specifically investigated the influence that age has on soil C concentrations or the different fractions of C within the soil. The studies that have been conducted are on different orchard crops, so there is little corroborating evidence for what occurs in the soil for apple trees, and they have limited age ranges.

In a study by Zhang et al. (2021), they investigated what impact different climatic regions had on SOC, TN concentrations, total N:C ratio and soil moisture in apple orchards. Soil samples were taken from across the regions of the Loess Plateau, China, under various ages of orchards (6 -33 years), to a depth of 500 cm. Their results showed SOC, TN, and C:N ratios increased over the different ages of plantations. The sampled semi-humid regions showed higher concentration and stocks of SOC (6.6% and 32.1% respectively) and TN (stock 38.4% and concentration 12.1%) than those in the semi-arid regions. Meanwhile, the semi-arid regions had higher C:N ratios than those in the semi-humid regions. Their study concluded that the precipitation and orchards age significantly impact on SOC, TN, and C:N ratio. Still, the C and N decreased with soil depth. However, their study does not state the variety of apples or rootstocks under cultivation in the plantations, so direct comparisons from their study cannot be easily made. Also, it does not mention any decline in the trees C sequestration ability, unlike Wu et al. (2012) study.

The soil C sequestration of Chinese apple orchards was investigated by Wu et al. (2012). Their study indicated that apple tree C sequestration peaked at 18 years, when the trees were in the mature stage of their life cycle, and then declined as they continued to age. They found apple trees older than 8 years could be classified as carbon sinks. The relationship between age and C sequestration ability was described by a parabolic function and was used to determine the peak age for C sequestration.

In another study based in China, Yang et al. (2021), examined how the soil organic matter (SOM) and soil water content changed across different soil depths (0 -60 cm and 0 – 400 cm, respectively) and tree stand ages (7, 12, and 22 years) of apricot trees. Their results showed that SOM matter increased until the trees were 7 years old, but after this, the levels of SOM started to decline. The soil water content in the upper soil layers was reduced as the trees matured, so roots were extended into deeper soil layers to extract water, which eventually dried out. They determined that the "optimal age" for SOM for apricots was 10 years, after which the trees became weakened by the reduced soil water content, nutrient availability, and the degradation of the SOM due to the reduction in input from leaf litter, fruit, and fertilisers with current management practises. This work supports the study on apple trees by Wu et al. (2012) that orchard trees have a peak in their ability to sequester C belowground.

In a recent study of mixed-aged date palm plantations in Ethiopia by Betemariyam and Kefalew (2022), they wanted to determine the C stocks in both the biomass and the soil (up to 60 cm below the soil surface). Their results indicated that biomass C of the above and belowground increased as the trees aged (5-10, 10-20 and those older than 20 years), similar to the results found in the study by Zhang et al. (2021) on apples. Betemariyam and Kefalew (2022) investigations also showed the SOC was significantly higher in the upper 30 cm of the soil than those at deeper levels and that the total SOC across the 60 cm sampling depth was in line with reported levels for farmland worldwide of between 30-300 Mg C ha $^{-1}$, yet compared to other ecosystem soil such as tropical forest it was much lower.

Reports by Natural England (George, 2010; Gregg *et al.*, 2021) have both stated that orchards are capable of being C sinks in the above and belowground matter, including soil. The assumption is this will increase with age. The systems which orchards are planted means that they are classed more as single trees rather than forests or agroforestry systems, as they do not form a continuous canopy. The two reports acknowledge there is still limited research on this area. Still, the available research shows that traditional orchards may be less sensitive to climate change than intensive systems and that orchards have great potential in CO_2 mitigation.

The aims of the current space-for-time substitution chronosequence field experiment were: -

1, To determine whether orchard age affects the soil C sequestration.

2, To determine if tree rows sequester more C than the grass alleys.

3, To investigate the potential of soil nutrients to influence the orchards' ability to sequester C.

4, To determine if farms, rootstocks, or scions influence soil C sequestrations.

5.3. Materials and methods

Twenty-three different orchard locations (**Fig 5.1.**) were sampled across three farm sites for this investigation. Seventeen orchards were based at Thatcher's Cider Company (TH), Somerset, UK (51.332740, -2.833089), four at Orchard Park Farm (OPF 1-4), Somerset, UK (51.0945, -2.59344) and the remaining two orchards at Stone House Farm (OPF 12 and 14 or SH), Somerset, UK (51.11346, -2.5916). The sampling was conducted over two-weeks in late November and early December 2021.

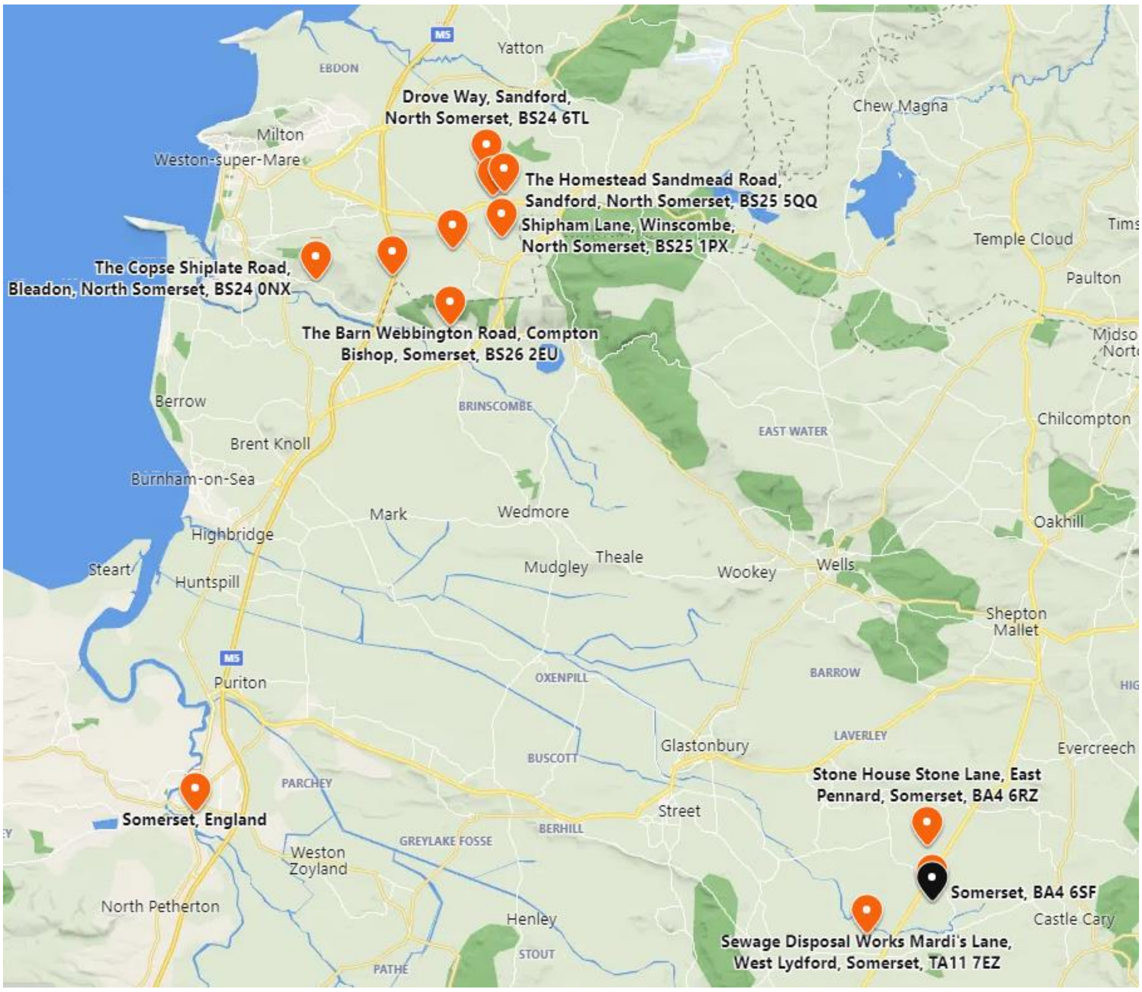


Fig 5.1. Map showing the locations of orchards in Somerset where the soil samples were collected in late November and early December 2021.

GPS location was collected on arrival at each of the orchards. Soil was cored and collected at a depth between 20 to 30 cm below the surface using a hand auger, collecting a minimum of 500 g before cold storage. Eight samples were collected within the herbicide strip under the tree canopy and eight from alleyways per orchard, with 368 soil samples collected across the three farms. The sampling was assigned across the orchard in the standard “W” pattern, taking in orchard edges, middle and ends of the lines to ensure an even spread across each orchard as they varied in size and shape (**Fig 5.2.**).

Fig 5.2. One of the sampled cider orchards shows two rows of trees with herbicide strip, the grass alleyway between, and the downhill slope of the orchard towards a stream. Image taken by Catherine Chapman on 23rd November 2021.

The orchards sampled ranged in age from 2 years up to 46 years of age, the trees being grown on several different rootstocks for intermediate height growth; these included M111, M116, MM106, M25, A2. The rootstocks were grafted with three different scions: Dabinett, Tremlett’s Bitter or Somerset Red Streak. Each orchard contained a single variety and rootstock, not a combination of different rootstocks/scions. The orchards are listed in **Table**

5.1. with their age, rootstock, scion, pH, and the orchard's soil texture. The soil texture analysis (determined by laser diffraction) and pH (using the water analysis method) showed variations across and within the orchards. The newer planted orchards had soil amendments added around the time of planting to help establish the trees (either a mushroom-based compost or PASS compost 100).

Table 5.1. Orchard ages, rootstocks, fruiting variety, mean soil pH (n=6) and textural analysis from the oldest to the youngest orchards**.**

Year of planting	Age	Rootstock	Scion	Soil pH	Soil texture
1975	46	MM106	Tremlett Bitter	7.2	Silty clay
1977	44	MM106	Dabinett	6.9	Sandy silty clay loam
1990	31	MM106	Tremlett Bitter	6.5	Clay
1992	29	MM106	Somerset Red streak	6.3	Heavy/ silty clay loam
1996	25	MM106	Somerset Red streak	6.7	Silty clay
1997	24	M25	Dabinett	7.9	Silty clay
1998	23	MM106	Dabinett	6.5	Silty clay
1998	23	MM106	Somerset Red streak	6.5	Silty clay
2001	20	M111	Dabinett	6.6	Silty clay loam
2010	11	M111	Dabinett	7.1	Silty clay (loam)
2010	11	M111	Tremlett Bitter	7.2	Silty clay loam
2012	9	M111	Dabinett	6.9	Silty clay
2012	9	M111	Somerset Red streak	7.2	Silty clay/clay
2013	8	M111	Dabinett	7.4	Silty clay
2013	8	M111	Tremlett Bitter	7.4	Clay
2015	6	M111	Dabinett	7.3	Silty clay loam
2016	5	M111	Dabinett	7.4	Clay (silty)
2017	4	M111	Dabinett	6.2	Silty clay
2017	4	A2	Dabinett	6.9	Clay
2018	3	M25	Dabinett	7.4	Clay
2018	3	M25	Dabinett	6.4	Clay

2019	2	MM106	Somerset Red streak	7.2	Clay
2019	2	M116	Dabinett	6.8	Clay

5.3.1. Data collection

The 368 soil samples were sent away to NRM in Reading, UK, where several different analyses were conducted. The carbon checker plus package tested for soil TN, TC, Organic and Inorganic C levels determined using the Dumas Combustion method as described by AOAC official analysis methods (1990), BS EN 15936:2012. The fraction of soil active C, was determined by using permanganate oxidisable C as described by Culman et al. (2012) and the following calculation (determined by Weil et al (2003)).

Active C (mg kg±1) = [0.02 mol/l ± (a + b x absorbance)] x (9000 mg C/mol) x (0.02 l solution/0.005 kg soil)

The soil bulk density was measured using the method described by DL Rowell (soil science - methods and applications. D L Rowell. ISBN 0-582-08784-48)

A second round of tests was also conducted by NRM on 142 samples using their Standard Soil Plus package and soil textural classification. The test included soil pH using the water soil mix calibrating the probe using pH 4 and pH 7 solutions. The soil texture classification was determined using Laser diffraction and the UK soil classification. The final set of analyses determined the soil’s available phosphorus (P) using the Olsen’s extraction method and the levels of potassium (K) and magnesium (Mg) by the ammonium nitrate method.

5.3.2. Statistical analysis

The results were statistically analysed in Microsoft 365 Excel to see if the individual orchards significantly differed between row and alleyways using one-way ANOVA’s and ternary plots using the XLSTAT add-in. Following this Minitab 21.2 was used for further analysis. The first stage was normality plots for the entire data set, divided into two age groups (under six and eight and over and further divided into trees and alleyways). Regression analysis was then conducted, under 6 and eight and older and subdivided into trees and alleyways. Following the regression analysis, the percentage-based results were transformed using angular transformation (Acrsin). Regression analysis used on the Acrsins transformed data and untransformed data, on the raw data as well Box-Cox transformations with optimal lambda (λ) was also conducted. Correlation analysis was conducted using Spearman pairwise analysis as the data was not normally distributed. Following this, one-way ANOVA was conducted on the transformed data, and a post hoc Tukey test was used. Graphing was also done in Minitab to allow for split plots and multiple variables to be shown.

5.4 Results.

The results of this study will be described in four sections to answer the four aims of this investigation. Firstly, age of orchards related to soil C, and this will be divided into two: under six years since planting (because of mulching around the time of planting) and over 8 years from planting. Secondly if there were significant differences between rows and alleyways, Thirdly, to discuss if soil nutrients are related to C sequestration. Finally, if farms, rootstocks, or scions have any effect on C sequestration.

The soil pH was slightly acid to neutral across the 23 orchards, with no significant differences between the rows and alleyways (**Table 5.1**). The soil types ranged between clay, silty clay, and silty clay loam (**Tabel 5.1 and Appendix 1**), but there were some variances within the same orchards. The clay content across the 23 orchards ranged from 20 – 73 %.

5.4.1. Relationship to age.

Soil carbon and nitrogen: Soil TC, organic C, active C, and TN all showed a significant decline in the concentration found in the soil over the 23 orchards sampled as the age of the orchards increased. The sharpest declines in soil concentrations of C and N were found in the youngest orchards (orchards 6 years old and under). However, a decline was still observed in the older orchards although at a much slower rate (**Fig 5.3**) or the concentrations had reached an equilibrium.

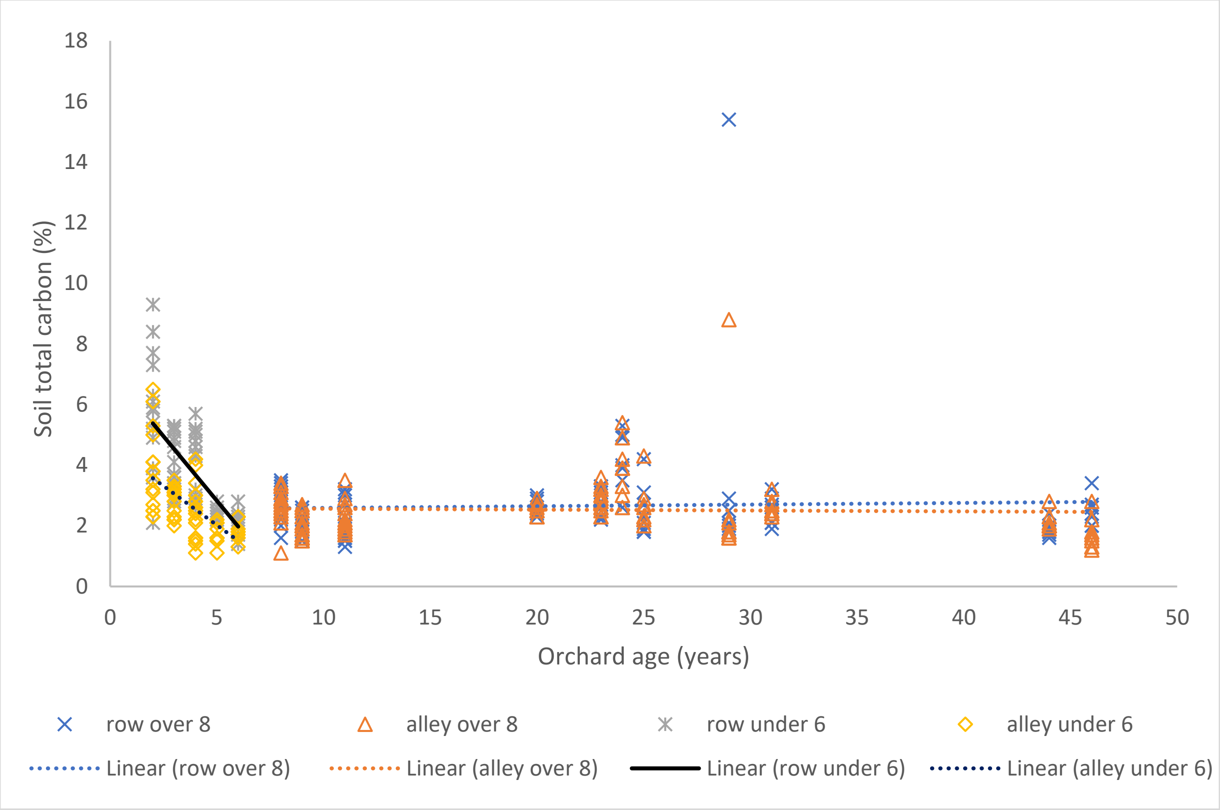


Fig 5.3. Soil total percentage carbon over all orchards, divided into two ages (≤ 6 and ≥ 8) for tree rows and alleyways. Regression analysis showed orchards 6 years and under Alleyways (R^2 0.3842), orchards 6 years and under Tree rows (R^2 0.4586), orchards 8 years and older Alleyways (R^2 0.011) and trees in orchards 8 years and over (R^2 <0.00001).

Soil bulk density and organic matter: The bulk density of the soil found in the alleyways of the orchards under 6 years of age had a moderately strong, significant correlation to age, where the bulk density increased as the orchard aged (P = <0.001). Once the orchards were eight years and older, this correlation between age and bulk density positively correlated to increasing age, but it was no longer significant (**Fig 5.4**). The trees the under six-year and over eight-year categories had positive correlations between age and bulk density. Still, only the trees under six remained to be significantly so (P = 0.03). Bulk density in the under six and over eight-year-old orchards in the tree and alleyways were significantly negatively correlated to the soil's organic matter and the soils TC. The soil organic matter, however, was found to follow the same trend as soil TC, SOC, active C, and TC across the two different age ranges and soil sampled regions in the orchard.

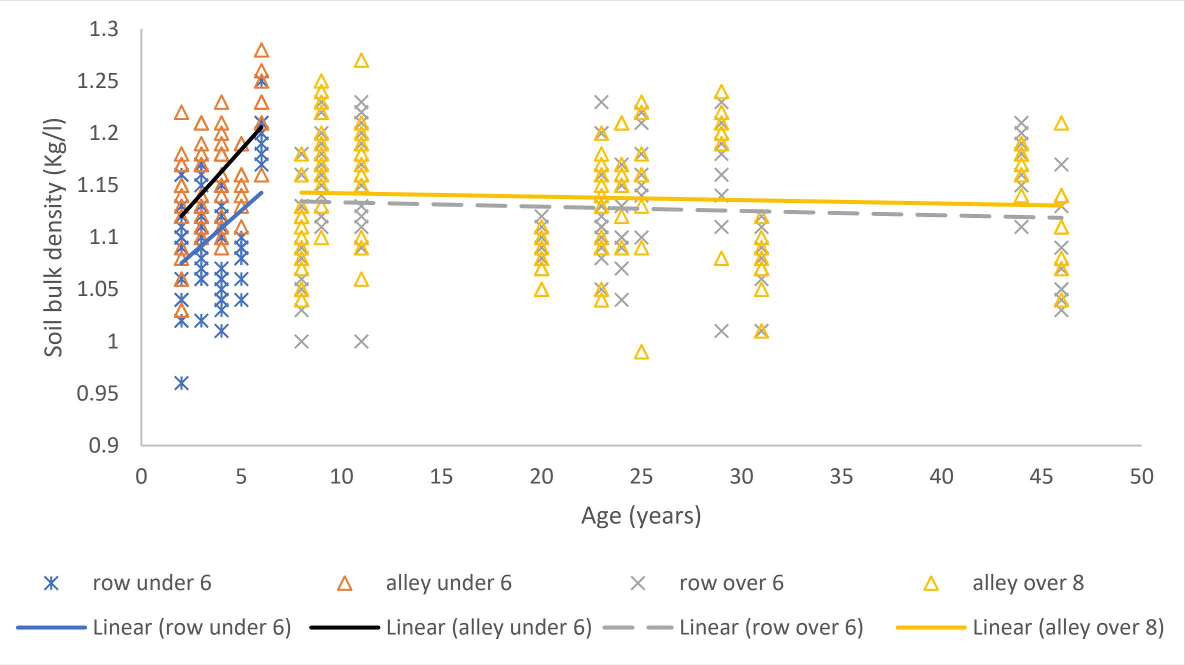


Fig 5.4. Soil bulk density across the 23 orchards, with the correlations between the soils bulk density and the increasing orchard ages. The orchards correlations divided into ages and orchard positions of sampling, orchards of 6 years and under and those 8 years and older, in either the tree rows or in the corresponding grass alleyways.

5.4.2. Relationships between tree rows and alleyways.

The relationship between tree rows and alleyways over all the orchards showed that soil TC (**Fig 5.5**), TOC, active C, TN, and organic matter concentrations were significantly higher in the rows than in the alleyways (**Table 5.2** and **Appendix 2**). The bulk density of the soil was significantly higher in the alleyways compared to the tree rows (P = <0.0001). In the orchards 6 and under, TC, TOC, active C, TN, and organic matter concentrations were significantly higher in the tree rows than in the alleyways (all P = <0.00001). The orchards that were eight years and older for TC, TOC, active C and TN concentrations, were higher in the tree rows than in the alleyways, but these were not significantly different. Bulk soil in the younger orchards the alleyways were significantly higher than the tree rows (P = <0.0001). In contrast, in the older orchards, the sum of the rows and alleyways were almost identical, row 134.33 and alleyways 134.34, so no significant difference was seen.

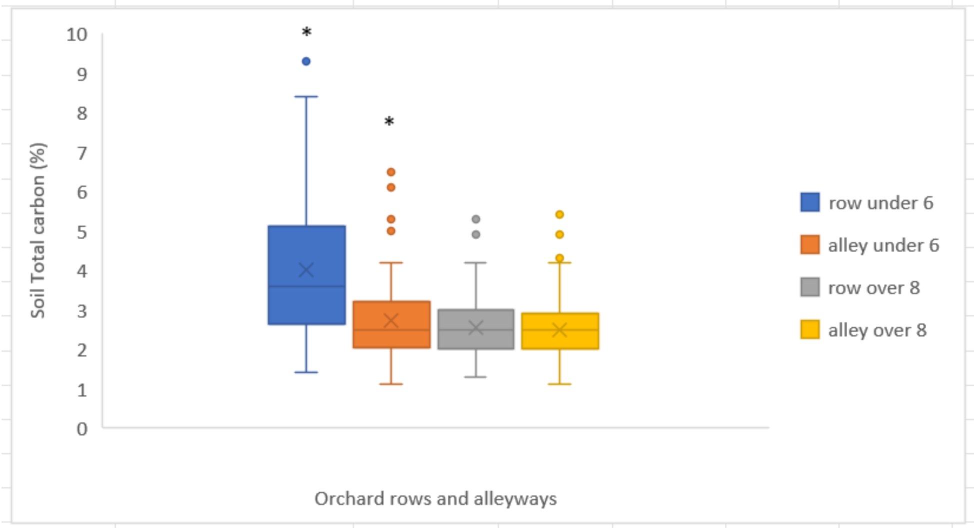
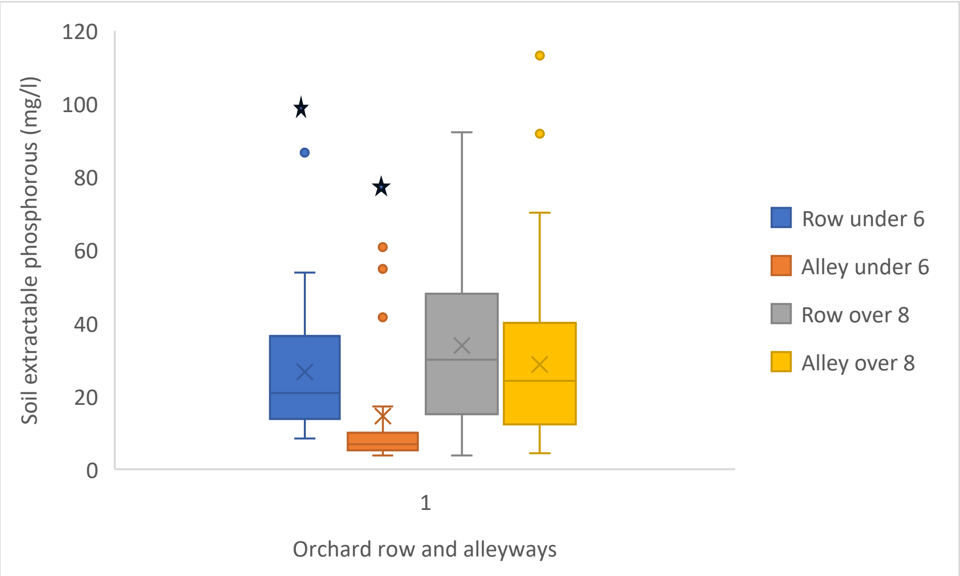


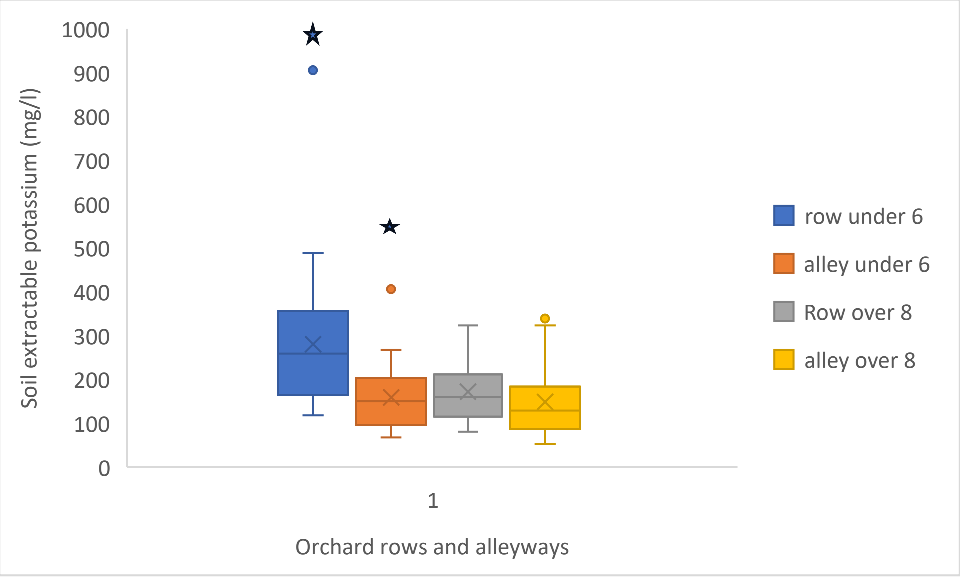
Fig 5.5. The soils total carbon (%) of the rows and alleyways with the outliers indicated by the coloured dots above the error bars, significant differences indicated by an asterisk (*) in the orchards under 6 (P = <0.05).

Table 5.2. Total organic carbon, active carbon, total soil nitrogen and soil organic matter for tree rows and alleyways for all 23 orchards sample as overall values, and subdivisions into the two age groups of 6 and under and 8 years and older. Significant differences (P = <0.05) indicated by asterisk (*).

	Mean total organic carbon		**Mean active carbon**		**Mean total nitrogen**		**Mean organic matter**	
Age	*Tree*	*Alley*	*Tree*	*Alley*	*Tree*	*Alley*	*Tree*	*Alley*
Overall	2.9 *	2.3	724.47 *	617.14	0.31 *	0.26	4.99 *	4.10
under 6	3.8 *	2.6	859.36 *	607.30	0.40 *	0.28	6.57 *	4.43
over 8	2.4	2.3	651.92	622.48	0.25	0.25	4.14	3.92

Soil nutrients: The soil phosphorus and potassium concentrations under the trees under 6 and across all 23 orchards were significantly higher than those found under the alleyways. Magnesium concentrations in the alleyways were higher than under the tree rows across the 23 orchards, under 6 and over 8-year-old orchards, but these were not significantly different (**Fig 5.6 A, B, and C**).





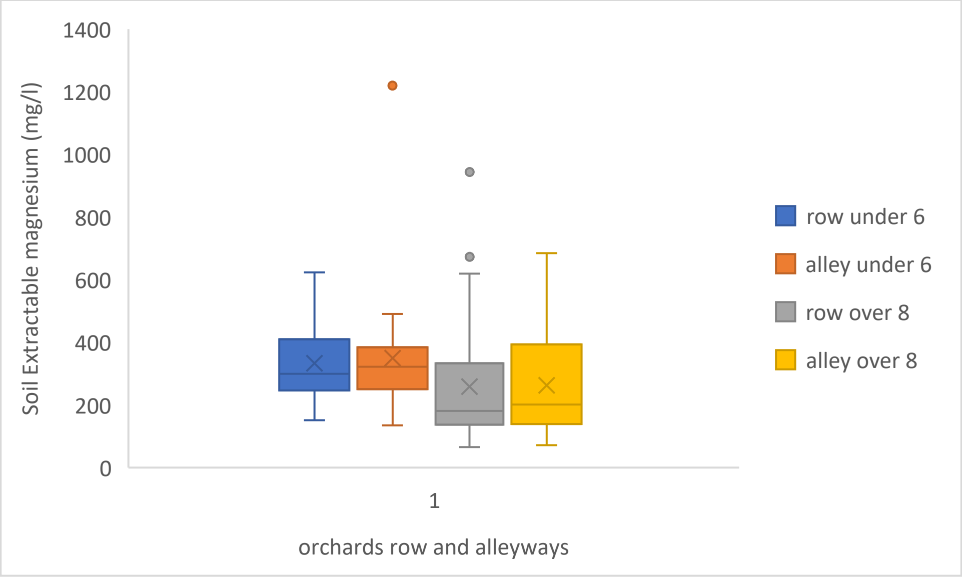


Fig 5.6 A, B, and C. Extractable soil nutrients (**A** Phosphorous, **B**, Potassium, and **C**, Magnesium) for orchards six year of age and under, and orchards eight years of age and over, showing differences between tree rows and grass alleyways. Overall, 23 orchards tree rows were significantly higher than the grass alleyways for both Phosphorus and Potassium. Stars indicate significant differences (P = <0.05) in the 6 years and younger orchards. Small circles indicate the outlier within orchard.

5.4.3. Nutrients and soil C sequestrations.

The extractable nutrients investigated (magnesium, potassium, and phosphorous) showed that potassium was the only nutrient to have any significant relationship with soil TC in the alleyways. The relationships between potassium and TC were positively correlated (**Fig 5.7**). In the orchards under 6, R^2 0.672 (P = <0.00001), and in the orchards over 8 the relationship had a R^2 value of 0.646 (P = <0.00001).

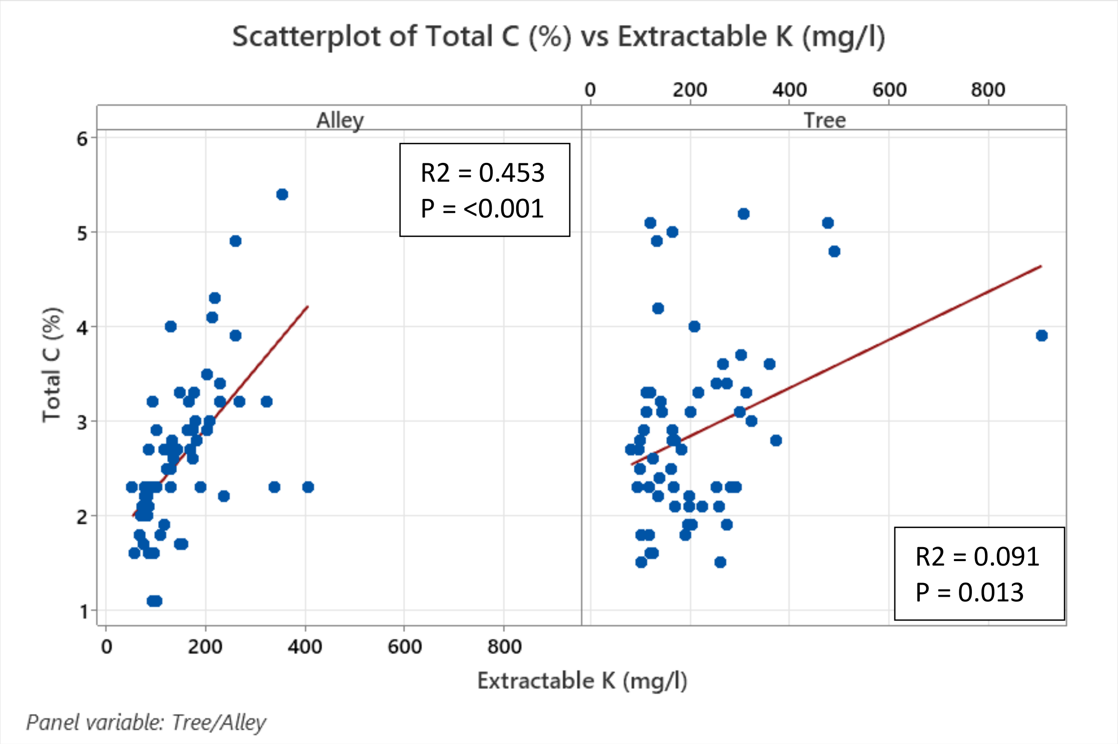


Fig 5.7. Scatterplots showing the Spearman pairwise correlation between soil total carbon (%) and soil extractable potassium (mg/l) across all orchards for the alleyways and tree rows. Alleyways had a R^2 = 0.453, P = <0.001 and tree rows had a R^2 = 0.091 and P = 0.013.

5.4.4. Farm, rootstock, and scions.

The three farms showed differences in correlations with orchard age and soil TC (**Appendix 3 A, B, and C).** Stone House Farm showed that only TC was significantly positively related to age, but other C fractions had no significant relationship to either age or soil TC. Bulk density, and pH were also positively related to age and soil TC, but differences between soil nutrients and age and soil TC were observed between the different areas of the orchard (under rows and alleyways). Both Thatcher's and Orchard Park Farm orchards showed that TC and TN had a significant negative relationship with increasing orchard age.

Of the 23 orchards 5 different rootstocks were used for the trees, two rootstocks were discounted as they only had one orchard being grown on them (A2 and M116), and the remaining 3 rootstocks (M25, M111 and MM106) were assessed for correlations between age and soil TC (**Appendix 4 A**). There were differences in the relationship between age and to soil TC between the different rootstocks. M111 did not have any significant relationship between C or N and the age of the orchards. Regarding the relationship with soil TC, all three rootstocks had the three fractions of C and N positively related. However, there were differences between the number of other relationships for M25, M111, and MM106. However, the soil bulk density was found to have a significant negative relationship to soil TC under each of the three rootstocks.

The three grafted scions (Dabinett, Tremlett bitter and Somerset Red streak) impacted the number of correlations between age and soil TC (**Appendix 4 B**). The three scions' soil TC and C fractions, TN and bulk density were significantly related to age but differed on the numbers of other relationships with the age of orchards. When looking at the relationships to the soil TC, all C fractions and TN across the three scions were significantly related. The other relations included soil nutrients, clay content and the bulk density.

5.5. Discussion.

Soil C sequestration has been suggested as one possible means of reducing atmospheric CO_2 levels in the fight against climate change and protecting the world's food security (Robertson, Paul and Harwood, 2000; George, 2010; Gregg. Ruth and Morecroft, 2021). Many studies have provided or have used computer modelling to project the potential estimates of C sequestrations for forests and orchards, but few on single trees (Phani Kumar *et al.*, 2010; Lefebvre *et al.*, 2021; Zellweger *et al.*, 2022). This study into what was happening to soil C in ageing apple orchards goes against the projections made by many studies, including Lefebvre *et al*. (2021) and Zellweger *et al*. (2022). These studies suggest C in orchards is constantly increasing (these studies include soil, tree biomass both above and belowground, which this current study does not). Still, in contrast to previous research this study showed C is lost over time.

5.5.1. Relationship between orchard age and soil C sequestrations.

The orchards were divided into two age ranges: those six and under, and those 8 years and older. This division was due to the youngest orchards having had soil amendments added (either mushroom-based or farm compost (100 product)) around planting to aid establishment and improve soil texture. This addition of soil amendments is reflected in the results of the experiments for soil C contents, but this addition of C into the soil is shown to be lost by the time the orchards have reached 6 years of age. This is consistent with Pacchiarelli et al. (2022) and Guo and Gifford's (2002) investigations. The results of soil C loss over increasing ages of orchards contradict the result of Deurer et al. (2009) who showed that adding soil amendments over 12 years increased the concentrations of SOC found in the soil. As the soil amendments decomposed, the C contained within could be leached from the amendments and soil layers as rainwater percolates through the upper layers. The C losses could also be due to the C being used by soil microbes and recycling CO_2 back into the atmosphere through respiration (Sathya, Vijayabharathi and Gopalakrishnan, 2016), mineralisation of the soil (Nicolardot, Recous and Mary, 2001) as the roots penetrate deeper into the soil aerating and allowing oxidation of the SOC. The C inputs in the older orchards would also be limited to windfall fruit left following harvesting, leaf litter and any prunings that are left on the soil surface both in the rows and alleyways, and the amounts of root exudation that occurred at the depth of sampling or transferred down the soil's profile.

The increase in the orchards soil bulk density, in the first 6 years could be due to the breakdown and incorporation of the soil amendments from planting, filling up the pore spaces created at the time of planting within the tree rows. Also, as the soil settles as the tree establishes and farm machinery runs over the soil in the alleyways, this compacts the soil (Farrakh Nawaz *et al.*, 2013) and increases the density of the soil. The decrease in the bulk density from eight years could come from root systems penetration and soil biota opening the soil structure allowing more oxygen, which would then release C. The amounts of leaf litter and organic material entering the soil would also increase as the trees develop larger canopies, helping to reduce the bulk density of the soil across the orchards. Soil compaction has been shown to impact C mineralisation, increase N_2O release from the soil, limit soil porosity, limit aeration and can affect root growth and crop yield (De Neve *et al.*, 2000; Farrakh Nawaz *et al.*, 2013). This is contradicted by work by Deurer et al. (2012), which suggested that soil C concentrations increase under soil compaction.

5.5.2. Relationship between rows and alleyways for soil C concentrations.

The higher levels of TC, TOC, active C, TN, and the phosphorous and potassium nutrients in the tree rows is likely due to the initial soil amendments at planting time, especially in the youngest orchards. There would also be the incorporation of root exudation in the soil, transporting the photosynthetic products from the tree into the soil across the whole age range of orchards under investigation. The organic C could be due to higher soil microbes, which have a symbiotic relationship with the tree, feeding on the root exudates and providing the tree with nutrients in return. The increased active C would be from the increase in the organic C as the microbes would consume and break down the C and release it back into the atmosphere as CO_2. The tree rows were kept clear of grass coverage, but the alleyways were grass covered due to herbicide spraying which limits plant growth and therefore limits the sequestration of C via photosynthesis. These results are inconsistent with those of Xiang et al. (2022). The results from the meta-analysis revealed that orchards with grass coverage increased the amount of SOC stocks compared to clean tillage within the first 30 cm of the soil. They concluded that the age of grass in the orchard and the percentage of clay content within the soil were the main causes of the difference observed in the soil organic C stocks in grass-covered orchards.

The most likely reason for the alleyways having a higher soil bulk density than the tree rows is the fact that the alleyways would be compacted as the farm machinery moves through the orchards (Deurer *et al.*, 2012; Farrakh Nawaz *et al.*, 2013). The youngest orchards tree rows would have been disturbed and structure separated at the time of planting and the addition of soil amendments would have loosened the density of the soil texture. The soil amendments would have been the cause of the increased organic matter within the soil, which could allow extra oxygen to penetrate the soil, but this would then oxidise any available C and release it back into the atmosphere. Every year, the rows would receive new organic matter in the soil, altering the bulk density via vegetative inputs from leaves, twigs, and waste fruit decomposition (Adhikari and Bhattacharyya, 2015).

5.5.3. Relationship of soil nutrients and soil C concentrations.

Soil nutrients are important in crop production and overall soil health, which could aid soil C sequestration (Gransee and Führs, 2013; Macdonald *et al.*, 2018; Qaswar *et al.*, 2019; Wu *et al.*, 2022). Potassium's only significant and positive relationship to soil TC could be due to the soil amendments added in the early years increasing the soil's organic matter. The higher concentrations of soil organic matter can increase the negative charge of the soil, which binds potassium to the clay content within the soil (Adhikari and Bhattacharyya, 2015). This experiment showed a positive relationship between potassium and clay. Still, they were not significant, which backs up the research by Wu et al (2022) on the relationship between soil nutrients and SOC in paddy fields. The changes observed in the nutrients in this study could be due to changes in the organic matter under the trees as the orchards aged.

In this study, soil magnesium (Mg) became negatively related to soil TC. Research has shown that the trees needs soil magnesium to ensure plant health, especially in producing chlorophyll (Gransee and Führs, 2013) in the leaves to conduct photosynthesis. Therefore, it is most likely that the tree is removing Mg from the soil to enable the trees to produce chlorophyll, convert the absorbed C, and transfer this to the root exudates, increasing the soil C content and aiding CO_2 mitigation.

5.5.4. Relationships of farms, rootstocks, and scions to soil C concentrations.

Stone House farm showed a difference in how TC was related to orchard age; this could just be down to the number of orchards sampled at this farm (2 orchards), limited ages as well as the difference between the two orchard locations (one by a river the other on a ridge). The three scions incorporated in this study showed no significant impact on soil C sequestrations. These results were similar to those observed in the investigation on scion-mediated effects on belowground C sequestrations (**Chapter 3**) on M9 rootstocks. Any differences in relationships under the different rootstocks, could be due to the number of orchards grown on the rootstock altering the patterns of the data as well as the age range of these orchards. In the previous study (**Chapter 2**) on the effects of rootstocks, there were no significant differences in their C sequestration ability. However, the rootstock study did show that C changes throughout the growing seasons, so the soil collection and analysis timings could impact soil C results.

5.5.5. The overall study.

This study has shown that the assumption that soil C under ageing orchards would increase and therefore aid in mitigating climate change through the absorption of atmospheric CO_2, is not shown here. This contradicts several studies and reports showing that C sequestrations in orchards and other land uses will increase over time (Guo and Gifford, 2002; George, 2010; Wu *et al.*, 2012; Zhang *et al.*, 2021; Pacchiarelli *et al.*, 2022). A recent study by Duddigan et al. (2024) in heathlands also challenged the established belief that C sequestration will occur in soils after successful restoration. In an earlier study by Guo et al (2008) it was determined that soil C was lost over 16 years of the pine plantation when compared to the native pasture, but that the overall ecosystem of the pine plantations made significant gains in the amount of C sequestered. The current study also showed that soil TC did not peak at any age before declining, which contradicts the study by Wu et al. (2012) and Yang et al. (2021), but showed a constant decline in soil TC, TN, active and organic C concentration and that the rate of decline was much more rapid in the younger aged orchards before slowing down in the older orchards, in both the tree rows and alleyways.

5.6. Conclusion and future work.

This investigation on how orchard age affects the belowground C sequestered in the soil has shown that for the 23 orchards of different ages, most fractions of soil C decreased as the age of the orchards increased. The exceptions to the overall decreases in soil C fractions was inorganic C, which increased. The concentrations of C and N found in the soil under the trees and in the corresponding alleyways showed that trees maintain significantly higher concentrations than those found in the alleyways. These findings show that soils under apple trees may not help to mitigate rising atmospheric CO_2 levels. The C in the soil is being turned over through microbial activities, releasing the C back into the atmosphere. The soil nutrients' relationship with the soils TC was shown to be significant and positive for potassium in both the youngest and the older orchards, only within the alleyways. This study backs up the increasing body of work showing that soil C sequestration may not have the climate-mitigating potential as first believed.

There is still a need for further work on changing C under ageing orchards as the full age range was not gathered, as cider orchards can last for another 20 - 30 years before they reach the end of their economic lifespan. This would also need to be compared to see if the same results would be found on different soil types, as all these were grown on clay-rich soils and determine if this would be the same for dessert apple orchards.

5.6.1 Limitations of study

There were several limitations to this investigation. Firstly, there was no data from a full range of orchard ages, as there were some big gaps between some orchards, with most of the orchards concentrated under 10 years of age, where most had two orchards of the same age. Secondly, this study was not a true chronosequence (space-in-time substitution), as it was reliant on different study sites to collect the data, from which the assumptions have been made of the effect of age on soil C. Thirdly, the time of year may have impacted the soil C as the trees were entering winter dormancy and pruning was beginning. Fourthly, the soil collection was spread over two weeks and at different times of day; some were frosty mornings, and others were early afternoons in the sun. Fifthly, this study only collected one layer of soil between 20 and 30 cm, and not the full profile of the soil, as C concentrations are likely to change over soil depth. Orchard soil depths are likely to vary as well as the under lying geology which could be playing a part in soil C. Finally, the soil textures were all similar, silty clay, so future studies would need to compare different soil types to determine whether the results gathered in this study show a consistent trend or are area specific.

5.7. References.

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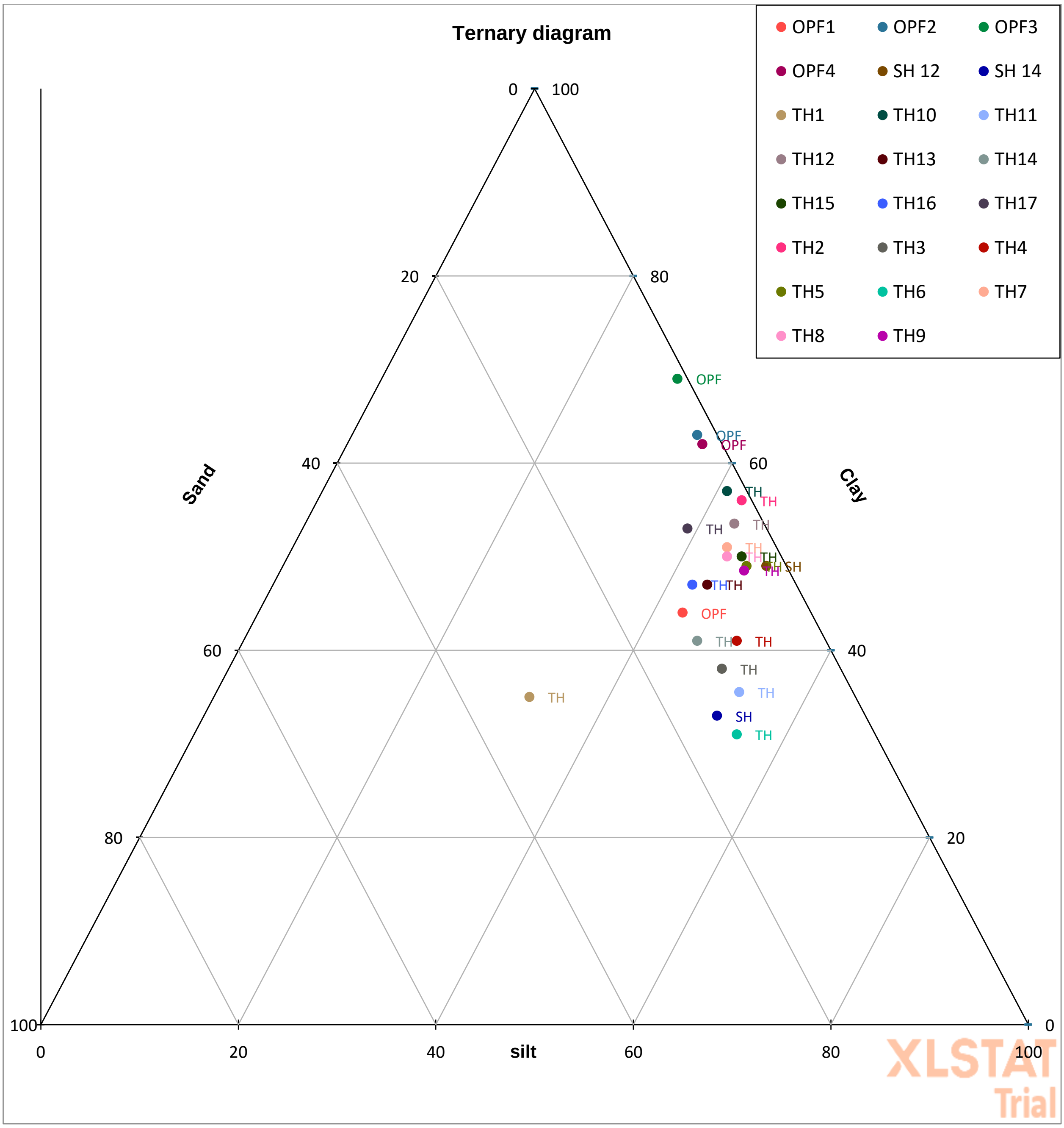
Appendix 1: Ternary plot of soil texture.



Appendix 2: Mean orchard values for all 23 orchards of the soils total organic carbon, active carbon, total nitrogen and organic matter. Significant differences indicated by asterisk (*) for tree rows compared to alleyways.

	Mean total organic carbon		Mean active carbon		Mean total nitrogen		Mean organic matter	
age	*Tree*	*Alley*	*Tree*	*Alley*	*Tree*	*Alley*	*Tree*	*Alley*
2	4.4 ± 0.7 *	2.8 ± 0.2	1105.25 ± 155.47	773.25 ± 69.51	0. 44 ± 0.05 *	0.31 ± 0.02	7.53 ± 1.13 *	4.79 ± 0.34
2	6.4 ± 0.5 *	4.8 ± 0.4	1052.50 ± 49.01 *	854.63 ± 50.10	0.64 ± 0.04 *	0.51 ± 0.04	11.08 ± 0.88 *	8.18 ± 0.69
3	2.9 ± 0.1 *	2.4 ± 0.1	946.38 ± 40.52 *	662.00 ± 57.86	0.34 ± 0.01 *	0.26 ± 0.02	4.95 ± 0.17 *	4.10 ± 0.22
3	4.9 ± 0.1 *	3.1 ± 0.1	981.25 ± 19.92 *	675.75 ± 18.21	0.32 ± 0.01 *	0.49 ± 0.01	8.41 ± 0.25 *	5.34 ± 0.13
4	2.8 ± 0.1 *	1.5 ± 0.1	758.25 ± 22.65 *	425.50 ± 48.73	0.31 ± 0.01 *	0.18 ± 0.01	4.81 ± 0.11 *	2.54 ± 0.19
4	4.8 ± 0.2 *	2.8 ± 0.2	938.75 ± 33.80 *	520.75 ± 33.73	0.51 ± 0.01 *	0.30 ± 0.03	8.26 ± 0.28 *	4.46 ± 0.23
5	2.4 ± 0.1 *	1.8 ± 0.1	582.13 ±28.35 *	421.00 ± 25.15	0.24 ± 0.01 *	0.19 ± 0.01	4.21 ± 0.13 *	3.01 ± 0.23
6	2.0 ± 0.1	1.8 ± 0.1	618.38 ± 26.90 *	525.50 ± 29.51	0.22 ± 0.01	0.19 ± 0.01	3.43 ± 0.22	3.04 ± 0.15
8	2.6 ± 0.2	2.2 ± 0.2	719.50 ± 53.25	575.50 ± 48.24	0.29 ± 0.02	0.24 ±0.02	4.41 ± 0.39	3.78 ± 0.34
8	2.7 ± 0.2	2.8 ± 0.1	841.00 ± 54.63	474.25 ± 23.35	0.27 ± 0.02	0.29 ± 0.02	4.60 ± 0.30	4.89 ± 0.25
9	2.1 ± 0.1	2.2 ± 0.1	598.50 ± 42.13	561.38 ± 28.37	0.23 ± 0.01	0.25 ± 0.01	3.70 ± 0.25	3.79 ± 0.16
9	2.0 ± 0.1 *	1.7 ± 0.1	628.63 ± 22.89 *	474.25 ± 23.35	0.20 ± 0.01	0.18 ± 0.01	3.40 ± 0.14 *	2.75 ± 0.11
10	1.9 ± 0.1	2.2 ± 0.1	664.43 ± 59.70	695.00 ± 48.39	0.23 ± 0.02	0.26 ± 0.01	3.20 ± 0.25	3.73 ± 0.21
11	1.7 ± 0.1	1.9 ± 0.1	520.75 ± 40.00	579.00 ± 25.42	0.18 ± 0.01	0.20 ± 0.01	2.94 ± 0.23	3.26 ± 0.13
20	2.6 ± 0.1	2.6 ± 0.1	659.75 ± 27.79	672.00 ± 23.13	0.29 ± 0.11	0.28 ± 0.11	4.51 ± 0.15	4.39 ± 0.12
23	2.8 ± 0.1	2.6 ± 0.1	566.50 ± 20.40	528.13 ± 20.23	0.32 ± 0.01	0.31 ± 0.01	4.80 ± 0.09	4.48 ± 0.18
23	2.6 ± 0.1	2.5 ± 0.2	803.63 ± 137.68	947.13 ± 65.57	0.29 ± 0.02	0.30 ± 0.02	4.47 ± 0.23	4.30 ± 0.26
24	2.4 ± 0.1	2.5 ± 0.2	623.88 ± 21.61	623.25 ± 26.99	0.31 ± 0.02	0.29 ± 0.01	4.35 ± 0.29	4.08 ± 0.18
25	2.5 ± 0.3	2.6 ± 0.2	640.50 ± 110.11	704.63 ± 111.37	0.27 ± 0.03	0.29 ± 0.03	4.23 ± 0.47	4.41 ± 0.45
29	4.0 ± 1.8	2.5 ± 0.6	631.00 ± 78.00	523.50 ± 123.45	0. 26 ± 0.03	0.24 ± 0.03	6.44 ± 2.56	4.38 ± 1.22
31	2.5 ±	2.6 ± 0.1	777.25 ±	629.63 ±	0.28 ±	0.28 ±	4.34 ±	4.41 ±

	0.2		55.84 *	26.91	0.2	0.01	0.27	0.17
44	1.8 ± 0.1 *	2.1 ± 0.1	500.25 ± 37.75	554.63 ± 42.02	0.19 ± 0.01	0.22 ± 0.01	3.19 ± 0.15	3.64 ± 0.16
46	2.1 ± 0.2 *	1.4 ± 0.1	681.63 ± 31.61*	471.13 ± 23.74	0.23 ± 0.01 *	0.17 ± 0.01	3.74 ± 0.25 *	2.43 ± 0.17

Appendix 3: Farm correlations

A) Overall:

Farm	Thatcher's			Orchard Park Farm			Stone House Farm		
Correlations	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**
Age/TC (%)	-0.224	0.050	0.000	-0.420	0.176	0.001	0.778	0.605	0.000
Age/TN (%)	-0.205	0.042	0.001	-0.408	0.166	0.001	0.187	0.035	0.305
Age/ SOM (%)	-0.199	0.040	0.001	-0.388	0.151	0.002	-0.122	0.015	0.505
Age/SOC (mg/Kg)	-0.202	0.041	0.001	-0.387	0.15	0.002	-0.144	0.021	0.435
Age/Active C (mg/kg)	-0.202	0.041	0.001	-0.362	0.131	0.003	-0.254	0.065	0.161
Age/ Phosphorous	0.007	0.000	0.946	-0.272	0.074	0.198	0.242	0.059	0.449
Age/ Potassium	-0.19	0.036	0.056	-0.549	0.301	0.005	0.579	0.335	0.048
Age/ Magnesium	-0.232	0.054	0.019	-0.417	0.174	0.043	-0.822	0.676	0.001
Age/ Bulk density	0.003	0.000	0.960	0.168	0.028	0.185	0.507	0.257	0.003
Age/ soil pH	-0.239	0.057	0.015	0.103	0.011	0.632	0.886	0.785	0.000
Age/ Clay (%)	-0.061	0.004	0.543	0.034	0.001	0.874	0.874	0.764	0.000
TC/ TN	0.897	0.805	0.000	0.979	0.958	0.000	0.342	0.117	0.056
TC/SOM	0.867	0.752	0.000	0.969	0.939	0.000	0.072	0.005	0.695
TC/SOC (mg/Kg)	0.867	0.752	0.000	0.970	0.941	0.000	0.075	0.006	0.685
TC/Active (mg/Kg)	0.760	0.578	0.000	0.807	0.651	0.000	0.003	0.000	0.988
TC/phosphorous	0.087	0.008	0.389	0.372	0.138	0.074	0.340	0.116	0.280
TC/ potassium	0.357	0.127	0.000	0.513	0.263	0.010	0.513	0.263	0.088
TC/magnesium	0.274	0.075	0.006	-0.189	0.036	0.377	-0.600	0.360	0.039
TC/bulk density	0.504	0.254	0.000	-0.783	0.613	0.000	0.492	0.242	0.004
TC/soil pH	0.176	0.031	0.080	-0.095	0.009	0.658	0.846	0.716	0.001
TC/Clay	0.316	0.100	0.001	-0.001	<0.000	0.995	0.747	0.558	0.005

B) Trees

Farm	Thatcher's			Orchard Park Farm			Stone House Farm		
Tree Correlations	correlation	r squared	P value	correlation	r squared	P value	correlation	r squared	P value
Age/TC (%)	-0.336	0.113	0.000	-0.438	0.192	0.012	0.789	0.623	0.000
Age/TN (%)	-0.337	0.114	0.000	-0.369	0.136	0.038	0.286	0.082	0.284
Age/ SOM (%)	-0.331	0.110	0.000	-0.388	0.151	0.028	0.041	0.002	0.880
Age/SOC (mg/Kg)	-0.335	0.112	0.000	-0.383	0.147	0.030	0.041	0.002	0.880
Age/Active C (mg/kg)	-0.275	0.076	0.001	-0.349	0.122	0.050	-0.258	0.067	0.335
Age/ Phosphorous	0.044	0.002	0.757	-0.546	0.298	0.066	0.891	0.794	0.017
Age/ Potassium	-0.158	0.025	0.268	-0.580	0.336	0.048	0.878	0.771	0.021
Age/ Magnesium	-0.346	0.120	0.013	-0.444	0.197	0.148	-0.891	0.794	0.017
Age/ Bulk density	0.097	0.009	0.261	0.038	0.001	0.835	0.137	0.019	0.613
Age/ soil pH	-0.254	0.065	0.072	-0.190	0.036	0.555	0.933	0.870	0.007
Age/ Clay (%)	-0.191	0.036	0.180	0.188	0.035	0.558	0.878	0.771	0.021
TC/ TN	0.907	0.823	0.000	0.966	0.933	0.000	0.357	0.127	0.175
TC/SOM	0.886	0.785	0.000	0.978	0.957	0.000	0.242	0.059	0.366
TC/SOC (mg/Kg)	0.889	0.790	0.000	0.979	0.958	0.000	0.273	0.075	0.306
TC/Active (mg/Kg)	0.749	0.561	0.000	0.480	0.230	0.005	-0.043	0.002	0.875
TC/phosphorous	-0.134	0.018	0.353	-0.207	0.043	0.519	0.882	0.778	0.020
TC/ potassium	0.094	0.009	0.515	0.175	0.031	0.586	0.812	0.659	0.050
TC/magnesium	0.407	0.166	0.003	-0.424	0.180	0.170	-0.794	0.630	0.059
TC/bulk density	-0.459	0.211	0.000	-0.678	0.460	0.000	0.293	0.086	0.272
TC/soil pH	0.136	0.018	0.345	-0.480	0.230	0.115	0.832	0.692	0.040
TC/Clay	0.400	0.160	0.004	0.431	0.186	0.162	0.754	0.569	0.084

C) Alleyway:

Farm	Thatcher's			Orchard Park Farm			Stone House Farm		
Alleyway Correlations	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**
Age/TC (%)	-0.336	0.113	0.000	-0.611	0.373	0.000	0.776	0.602	0.000
Age/TN (%)	-0.337	0.114	0.000	-0.583	0.340	0.000	0.083	0.007	0.760
Age/ SOM (%)	-0.331	0.110	0.000	-0.572	0.327	0.001	-0.300	0.090	0.258
Age/SOC (mg/Kg)	-0.335	0.112	0.000	-0.563	0.317	0.001	-0.345	0.119	0.191
Age/Active C (mg/kg)	-0.275	0.076	0.001	-0.732	0.536	0.000	-0.217	0.047	0.420
Age/ Phosphorous	0.044	0.002	0.757	-0.341	0.116	0.277	0.098	0.010	0.854
Age/ Potassium	-0.158	0.025	0.268	-0.530	0.281	0.076	0.878	0.771	0.021
Age/ Magnesium	-0.346	0.120	0.013	-0.376	0.141	0.229	-0.878	0.771	0.021
Age/ Bulk density	0.097	0.009	0.261	0.302	0.091	0.092	0.803	0.645	0.000
Age/ soil pH	-0.254	0.065	0.072	0.309	0.096	0.329	0.878	0.771	0.021
Age/ Clay (%)	-0.191	0.036	0.180	-0.034	0.001	0.916	0.891	0.794	0.017
TC/ TN	0.907	0.823	0.000	0.967	0.935	0.000	0.241	0.058	0.369
TC/SOM	0.886	0.785	0.000	0.940	0.884	0.000	-0.132	0.017	0.627
TC/SOC (mg/Kg)	0.889	0.790	0.000	0.936	0.876	0.000	-0.173	0.030	0.523
TC/Active (mg/Kg)	0.749	0.561	0.000	0.829	0.687	0.000	0.109	0.012	0.687
TC/phosphorous	-0.134	0.018	0.353	0.242	0.059	0.448	0.486	0.236	0.329
TC/ potassium	0.094	0.009	0.515	0.466	0.217	0.127	0.886	0.785	0.019
TC/magnesium	0.407	0.166	0.003	0.200	0.040	0.533	-0.600	0.360	0.208
TC/bulk density	-0.459	0.211	0.000	-0.626	0.392	0.000	0.712	0.507	0.002
TC/soil pH	0.136	0.018	0.345	-0.176	0.031	0.583	0.886	0.785	0.019
TC/Clay	0.400	0.160	0.004	-0.129	0.017	0.691	0.841	0.707	0.036

Appendix 4 Rootstock and scion correlations.

A) Rootstock

Farm	M25			M111			MM106		
Rootstock correlations	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**
Age/TC (%)	0.307	0.094	0.034	-0.006	<0.001	0.939	-0.483	0.233	0.000
Age/TN (%)	-0.299	0.089	0.039	0.008	<0.001	0.914	-0.522	0.272	0.000
Age/ SOM (%)	-0.481	0.231	0.001	-0.047	0.002	0.542	-0.459	0.211	0.000
Age/SOC (mg/Kg)	-0.487	0.237	0.000	-0.049	0.002	0.524	-0.459	0.211	0.000
Age/Active C (mg/kg)	-0.485	0.235	0.000	0.092	0.008	0.227	-0.238	0.057	0.012
Age/ Phosphorous	-0.125	0.016	0.621	0.165	0.027	0.186	-0.008	0.000	0.958
Age/ Potassium	-0.227	0.052	0.365	-0.024	0.001	0.848	-0.269	0.072	0.085
Age/ Magnesium	-0.818	0.669	0.000	-0.332	0.110	0.006	-0.565	0.319	0.000
Age/ Bulk density	0.010	0.000	0.948	-0.168	0.028	0.027	0.120	0.014	0.206
Age/ soil pH	0.827	0.684	0.000	0.001	0.000	0.994	-0.108	0.012	0.494
Age/ Clay (%)	-0.149	0.022	0.556	0.385	0.148	0.001	-0.305	0.093	0.050
TC/ TN	0.525	0.276	0.000	0.833	0.694	0.000	0.972	0.945	0.000
TC/SOM	0.339	0.115	0.018	0.787	0.619	0.000	0.984	0.968	0.000
TC/SOC (mg/Kg)	0.343	0.118	0.017	0.789	0.623	0.000	0.984	0.968	0.000
TC/Active (mg/Kg)	0.325	0.106	0.024	0.798	0.637	0.000	0.716	0.513	0.000
TC/phosphorous	0.099	0.010	0.697	0.248	0.062	0.049	-0.176	0.031	0.266
TC/ potassium	0.384	0.147	0.115	0.472	0.223	0.000	0.188	0.035	0.232
TC/magnesium	-0.620	0.384	0.006	-0.068	0.005	0.593	0.639	0.408	0.000
TC/bulk density	-0.308	0.095	0.033	-0.368	0.135	0.000	-0.713	0.508	0.000
TC/soil pH	0.456	0.208	0.057	0.345	0.119	0.005	-0.059	0.003	0.712
TC/Clay	-0.064	0.004	0.802	0.168	0.028	0.178	0.465	0.216	0.002

B) Scions

Farm	Dabinett			Tremlett Bitter			Somerset Red streak		
Scions' correlations	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**	**correlation**	**r squared**	**P value**
Age/TC (%)	-0.336	0.113	0.000	-0.438	0.192	0.000	-0.683	0.466	0.000
Age/TN (%)	-0.366	0.134	0.000	-0.443	0.196	0.000	-0.697	0.486	0.000
Age/ SOM (%)	-0.421	0.177	0.000	-0.456	0.208	0.000	-0.626	0.392	0.000
Age/SOC (mg/Kg)	-0.425	0.181	0.000	-0.457	0.209	0.000	-0.624	0.389	0.000
Age/Active C (mg/kg)	-0.406	0.165	0.000	-0.588	0.346	0.000	-0.540	0.292	0.000
Age/ Phosphorous	0.173	0.030	0.116	-0.423	0.179	0.040	-0.193	0.037	0.306
Age/ Potassium	-0.387	0.150	0.000	-0.371	0.138	0.074	-0.466	0.217	0.009
Age/ Magnesium	-0.487	0.237	0.000	-0.097	0.009	0.652	-0.319	0.102	0.086
Age/ Bulk density	0.189	0.036	0.005	-0.347	0.120	0.006	0.478	0.228	0.000
Age/ soil pH	0.149	0.022	0.175	-0.254	0.065	0.231	-0.554	0.307	0.001
Age/ Clay (%)	-0.489	0.239	0.000	-0.065	0.004	0.764	-0.236	0.056	0.209
TC/ TN	0.917	0.841	0.000	0.725	0.526	0.000	0.972	0.945	0.000
TC/SOM	0.864	0.746	0.000	0.698	0.487	0.000	0.956	0.914	0.000
TC/SOC (mg/Kg)	0.865	0.748	0.000	0.692	0.479	0.000	0.955	0.912	0.000
TC/Active (mg/Kg)	0.772	0.596	0.000	0.748	0.560	0.000	0.826	0.682	0.000
TC/phosphorous	0.030	0.001	0.789	0.260	0.068	0.242	0.013	0.000	0.947
TC/ potassium	0.569	0.324	0.000	0.235	0.055	0.292	0.416	0.173	0.022
TC/magnesium	0.163	0.027	0.140	-0.517	0.267	0.014	0.447	0.200	0.013
TC/bulk density	-0.573	0.328	0.000	0.161	0.026	0.214	-0.775	0.601	0.000
TC/soil pH	0.179	0.032	0.103	0.494	0.244	0.019	0.182	0.033	0.335
TC/Clay	0.512	0.262	0.000	0.090	0.008	0.676	0.128	0.016	0.499

Chapter 6. The fate of stored soil carbon following grubbing of an apple orchard.

6.1. Abstract

The economic life span of an apple orchard varies between dessert, cider, and culinary apples, after which they are mechanically removed through the process of grubbing which causes soil disturbance and release of C from the soil. This preliminary study investigated the effect that grubbing had on the levels of soil TC and TN of a nine-year-old orchard for the six months post the grubbing of an orchard. The results showed that soil carbon under the former tree-stands significantly declined between March and April (1 day to 1 month after grubbing), before starting to recover. Soil TC and TN in the alleyways were always significantly higher than the soil where the trees had been. Overall soil TN significantly increased across the whole site across the time of the study. The decline in TC is likely to be from the disturbance of the soil enabling oxygen to enter the soil, oxidising the C, and releasing CO_2 back into the atmosphere. In conclusion significant amounts of TC are lost from the soil in the first two months of grubbing, but this C is recoverable over time, and worth further study.

6.2. Introduction

Commercial orchards typically have a life span of between 15 and 25 years for dessert apples, or up to 50-80 years for culinary and cider apples, depending on fruit yield and quality, and the health of the orchard. Once an orchard reaches the end of its commercial life, the trees are removed via grubbing - the process of mechanically uprooting the trees and their subsequent removal (**Fig 6.1**).

Fig 6.1. Grubbing of a nine-year-old apple orchard on the 14th of March 2022, which had a mixture of Rubens and Gala trees grown on M9 rootstocks at NIAB East Malling. Image taken by Catherine Chapman on 15th March 2022.

Fig 6.2 A and B. Different amounts of roots left on grubbed trees (rootstock M9) that have been pushed or pulled over by mechanical means. Images taken by Catherine Chapman on 15th March 2022.

Mechanical grubbing leaves a proportion of the root system within the soil, as the trees are pushed over rather than dug out. The number of remaining roots is likely to vary between trees and rootstocks used, as more vigorous root systems extend deeper into the soil than those of M9 used in this experiment (**Fig 6.2 A and B, Chapter 2**). Carbon contained in the remaining roots is either stored in the soil or released into the atmosphere through enzymatic activity, depending on various factors such as root position (soil layer), soil temperature, soil moisture, and the degree of soil disturbance which allows oxygen to enter the soil which may lead to oxidation of SOM (Gill and Burke, 2002). The roots which remain can be left undisturbed depending upon the use of the land following orchard removal. Some orchards will be brought straight back into cultivation, either as a new orchard (but in a different position) or for other crops, so the roots could be chopped up through ploughing in preparation for new plantings (**Fig 6.3**), this could potentially enhance any soil C losses that occur from grubbing. Alternatively, the orchard sites may be left to go fallow for a couple of years before being brought back into use, which could allow for any potential loss of soil C to be replaced by new plant growth. Orchards are generally not replanted straight away with another apple crop in the same stand positions on the same rootstock due to the risk of soil borne diseases (syndromes), such as apple replant disease, that can stunt growth and production which affect the newly-planted trees (Leisso, Rudell and Mazzola, 2017; Sheng *et al.*, 2020).

The aboveground biomass and roots that are removed during tree grubbing are processed using various methods. Burning is the most common method and usually takes place at the edge of the orchard, releasing CO_2 into the atmosphere. Other methods less commonly used due to the economic cost to the grower include the chipping of healthy wood, used as feedstock for biodigesters to generate biogas, or converting the biomass into biochar and returning it into the soil as a means of longer-term C storage. However, these processes still release CO_2, albeit at a reduced and slower rate, than burning at the edge of the orchard (Anthony, 2013; Zhao, Ta and Wang, 2017; Sheng and Zhu, 2018; Duan *et al.*, 2021).

Fig 6.3. A grubbed apple orchards that has been prepared through ploughing for new planting with the chopped roots of the old trees visible at the surface. Image taken on 16th February 2023 by Catherine Chapman.

When soil is disturbed, stored C can be released into the atmosphere through oxidation of stored C, erosion, and enhanced microbial activity on organic matter decomposition (Reicosky and Archer, 2007; Ostle *et al.*, 2009; Zummo and Friedland, 2011). The amount of C that remains in the soil after orchard removal can be affected by the land use that follows. If the soil is left fallow, it is more susceptible to weathering and erosion, which can lead to C loss. Planting a cover crop or a new orchard can help protect the soil against these damaging effects. However, ploughing the soil to plant cover crops or new orchards can cause further disturbance, resulting in damage to the soil organic matter allowing for oxidization of C and increased microbial actively leading to the release of C into the atmosphere (Tiefenbacher *et al.*, 2021). The soil type, moisture and pH can all have an impact on the soils ability to sequester carbon both in the short and long term as discussed in Chapter 1.

Orchard management practices can have significant impacts on the concentrations of C that enter and accumulate in the soil over the lifetime of the orchard, including those from seasonal pruning, leaf litter and windfall apples. In some cases, such as in a diseased orchard, tree prunings, leaf litter, and windfall apples are removed from the orchard system; this practice reduces the organic matter (C) that enters the soil, thus limiting the availability of nutrients for the trees and C supply for soil biota. The limited availability of soil nutrients can lead to an increase in the breakdown of organic matter by microbes and enzymes to release nutrients for plants and soil microbial consumption, but this can also result in a large proportion of C being released back into the atmosphere, estimated at 75-100 Peta grams (Pg $=1x10^{15}$ grams) C yr^{-1} CO_2 released from forest soils (Grayston, Vaughan and Jones, 1997; Zhou *et al.*, 2017; Lee *et al.*, 2023). The use of heavy machinery can damage soil structure and porosity, although Deurer et al (2012) reported that soil C concentrations can increase with compaction. These authors concluded that increases in C from compaction were likely to be due to the lack of dissolvable C being washed away via water filtration and a slight reduction of microbial activity.

There is currently little research on the fate of soil C under apple orchards after grubbing and the associated soil disturbance. This is important to consider when assessing the overall C sequestration ability of an orchard as a land use, since potential losses after grubbing could reduce the positive C store that has been built up in the soil during their productive lifespan, through C being released back into the atmosphere following soil disturbance. Thus, the aim of this preliminary study was to investigate the fate of stored soil C under apple orchards over six months following destructive tree removal and soil disturbance. The results of this study will help to determine if further investigations on a larger scale are needed and if any observed trends are consistent or site-specific.

6.3. Materials and methodologies

The experimental work was conducted at NIAB East Malling in Kent, using a nine-year-old apple orchard (51.28717 N, 0.44754 E) that was grubbed on the 14th of March 2022. The soil texture was a sandy silt loam (confirmed by laser diffraction), with a slightly acidic pH (pH_{CaCl2} 5.99). There were two apple cultivars Gala and Ruben, each grafted on to M9 rootstocks. The orchard was planted in April 2013, as an experiment examining the effect of planting density (**Fig 6.4**), where the trees had been planted either at 0.5 or 1 metre apart. This is not the typical time frame for grubbing dessert apple orchards, but this apple orchard was no longer needed for experiments. Despite its relatively short lifespan, it was thought that this orchard could still give an indication of what might happen to the stored soil C concentrations following grubbing. In the two years prior to grubbing (2020/21), two herbicide sprays were applied, targeting the tree rows for weed control. In 2020 a combination of Pendimethalin, and Isoxaben was used, and in 2021 Glyphosate was applied to the tree rows. Eight trickle fertigation events were applied to the soil (last application August 2021), using different combinations of nutrients at each spray including Kemira Urea (nitrogen), Mono potassium and phosphate, Magnesium sulphate and nitric acid (nitrogen).

The locations of soil sampling sites were determined as randomly as possible (adapted W pattern), considering the arrangement of the two cultivars across the orchard (**Fig 6.4**), to try to account for variability across the orchard. The locations of each collection points were recorded using the “what3words” app, and these were plotted on the orchard plan. In total, 27 soil samples were collected across the orchard at each of the nine sampling dates (**Table 6.1**). Eighteen samples were taken from below the tree stand (rows) and nine within the corresponding alleyways for comparison, from January to September 2022. The soil samples were collected using a hand-held soil corer between 20 cm and 30 cm in depth, which covered the areas of high root concentration. Following grubbing (**Fig 6.5**) the aboveground material was removed and burnt. The site was left fallow (except for being mowed on 7th July 2022) allowing for tree regrowth from the remaining roots (**Fig 6.6**), and grass and weeds to grow in the former tree stands.

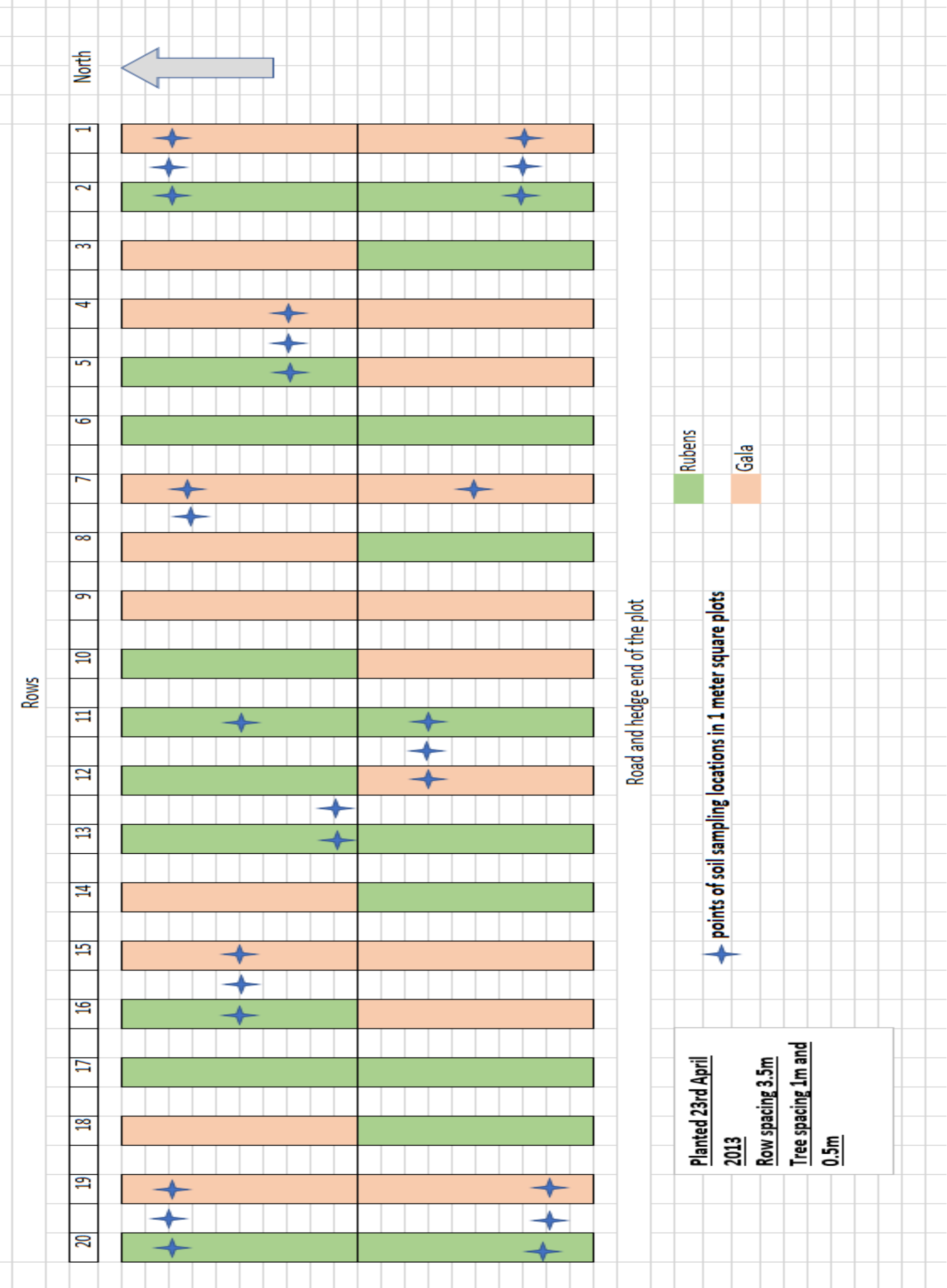


Fig 6.4. Layout of the grubbed orchard, indicating the positions of the different scion varieties under investigation and with sampling locations indicated by crosses.

Fig 6.5. The grubbed apple orchard under investigation for soil carbon changes at NIAB East Malling. The photo was taken the morning after grubbing had occurred prior to the removal of biomass for burning. Image taken by Catherine Chapman on the 15th of March 2022.

Table 6.1. Timing of soil collection from the grubbed orchard.

Timings for soil collection	Date of sampling
Before orchard grubbing	11.01.2022
Day after grubbing	15.03.2022
1 week after grubbing	22.03.2022
1 month post grubbing	19.04.2022
2-months post grubbing	16.05.2022
3-months post grubbing	13.06.2022
4-months post grubbing	11.07.2022
5-months post grubbing	08.08.2022
6-months post grubbing	05.09.2022

Fig 6.6. Regrowth of apple trees from the broken roots left in the ground following grubbing of the apple orchard. Image taken by Catherine Chapman on 30th May 2022.

6.3.1. Soil analysis

Different soil components were analysed at NIAB East Malling, the University of Reading, and by NRM. The soil analysis conducted at NIAB East Malling, included soil pH measured using the $CaCl_2$ method (Schofield and Taylor, 1955), and soil moisture content which was measured using the gravimetric method described by Forster (1995). Daily rainfall data was collected from the onsite weather station. The rainfall data was collected from 1st January 2022 to the final soil sampling on 5th September 2022. Soil texture was determined by NRM in Reading UK, using laser diffraction, while soil TN and TC percentages were determined at the University of Reading, UK, using a LECO CHN628 analyser.

6.3.2. Statistical analysis

Statistical analysis was conducted via Microsoft Excel 365 using one-way ANOVAs to identify statistically significant differences in soil moisture content and pH values between rows and alleyways and soil collection points. Pearson's correlation coefficient was used to determine the strength of relationships between soil moisture content, TC, and TN at each of the soil collection points. The statistical analysis of the soil TC and TN over the course of the preliminary study, between alleyways and the former trees stands (both Rubens and Gala) was conducted in R studio (version 4.1.1) using a continuous one-way ANOVA and post hoc tests, to determine significant changes over time. Significant differences between samples at each soil sampling point in the former tree stands and alley ways are indicated by $P = <0.05$.

6.4. Results

In January 2022, before grubbing occurred on 14th March 2022, the orchard soil had an average soil pH $_{(CaCl2)}$ 5.99, with some variations across the site (variance of 0.21). The soil in the Gala apple rows differed significantly in pH to that in the alleyways (P = 0.001) in January but not between Gala and Ruben or Ruben and the alleyways (**Fig 6.7**). In September 2022 there was a decrease across the site in soil pH $_{(CaCl2)}$ (5.75 variance of 0.17) and there were no significant differences between sampling sites or apple variety.

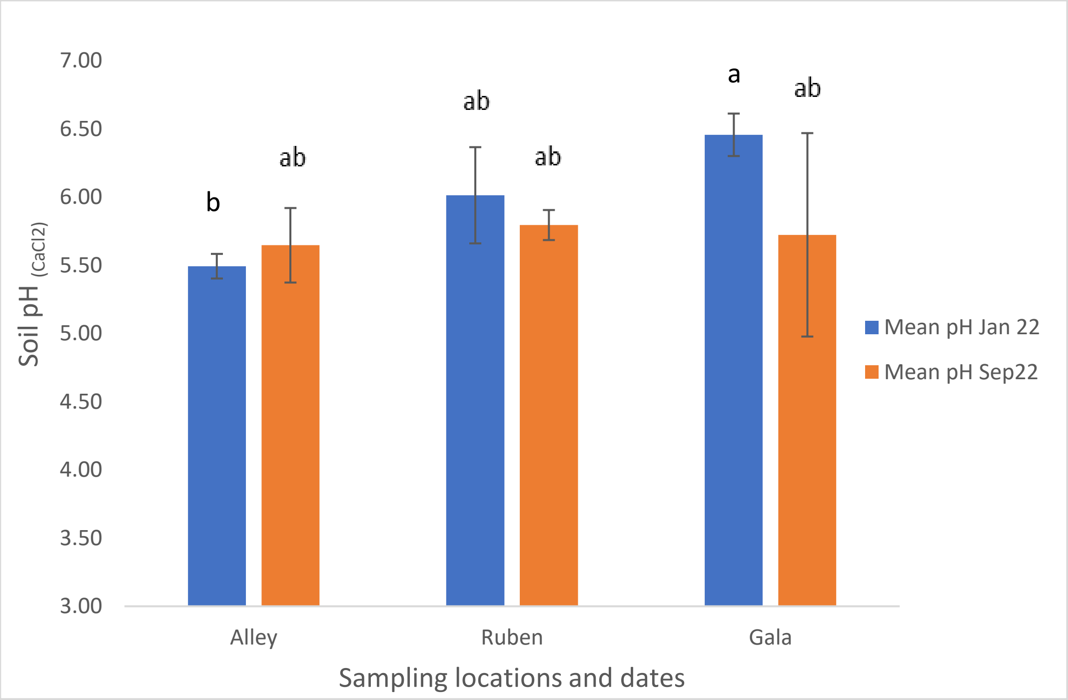


Fig 6.7. Mean soil pH (n=3) with standard deviation, before grubbing and at the experiment end (a and b, indicate significant differences; ab no significant differences).

From the initial soil samples taken in January to the final collection in September 2022, soil moisture content (**Fig 6.8**) decreased significantly (Gala, Ruben, and alleyway P = <0.00001). There was a significant increase in moisture content in September due to rainfall in the preceding days (Gala and alleyways P = <0.00001, and Rubens P = 0.0001). This decline in soil moisture content was due to the high temperatures (highest temperature recorded at NIAB East Malling of 38.4^{o}C mid July 2022) and limited amounts of rainfall for several months (a drought was declared in this location Summer 2022), with only 218 mm of rainfall from the 1st of January to 5th September 2022 falling at East Malling. Over the same period the year before 479.6 mm of rain fell at this location.

The day after grubbing, soil moisture content in both former tree stands was significantly higher than in the alleyways (Gala: P = 0.03 and Ruben: P = 0.01). The former Gala stands were significantly higher in soil moisture than the alleyways in April, May, and June (P = 0.04, P = 0.03 and P =0.02 respectively). In August, both former tree stands had significantly higher levels of soil moisture contents than those in the alleyways (Gala: P = 0.04 and Ruben: P = 0.02).

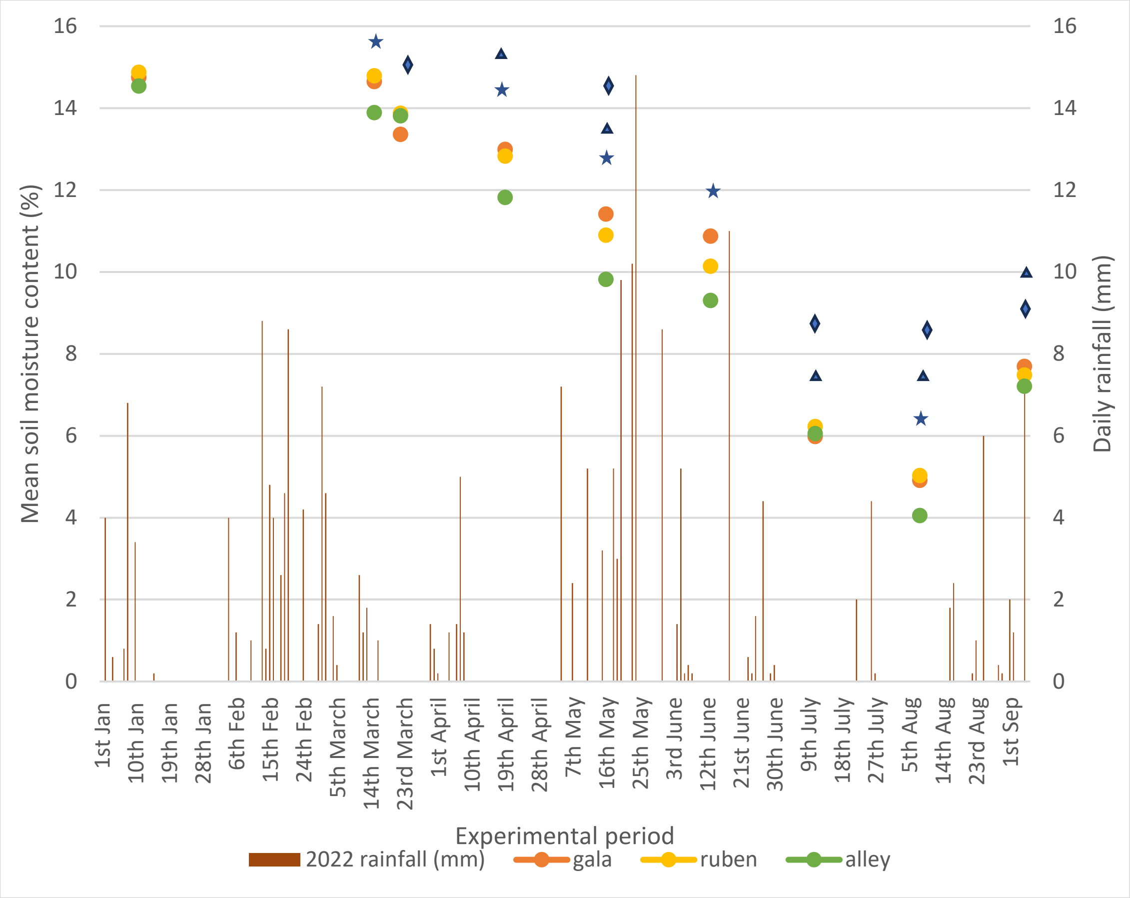


Fig 6.8. Changes in mean soil moisture content (using the gravimetric method) and rainfall totals to the date of sampling, over the experimental period from pre grubbing in January to the final soil collection at six months post grubbing in September 2022 per collection point. Grubbing occurred 14th March 2022 (green line). Statistically significant differences ($P = <0.05$) are indicated as follows; asterisk - difference between soil under former tree stands and alleyways, the blue triangles (alleyways) and diamonds (former tree stands) indicate changes between soil collection points.

Soil TC and TN percentage across the orchard site fluctuated during the sampling period (**Fig 6.9 and 6.10**). The locations where trees had stood had greater fluctuation in C and N concentrations than the grass alleyways. After grubbing, the site was left unmanaged, allowing for grass, weeds, and tree regrowth to occur in the alleyways and rows (**Fig 6.6 and Fig 6.11**).

The total % C differed significantly ($P = <0.0001$) across the tree stands and alleyways over the entire sampling period (**Fig 6.9**), but this was not consistent across each sampling point. The soil TC in the former trees stands declined significantly (Ruben: $P = 0.04$ and Gala: $P = 0.01$) between 22nd March and 19th April 2022 (five weeks post grubbing). From April onwards, the soil C content in the alleyways increased significantly ($P = <0.02$) compared to values under the former tree stands (**Fig 6.9**). The soil under the former Gala tree stands started to increase a month earlier than the former Ruben trees (April compared to May respectively). The soil total % C in the alleyways was significantly higher than in the former tree stands at the final soil collection made six months after grubbing (Ruben v Alley and Gala v Alley $P = 0.0001$).

The total % soil N (**Fig 6.10**) increased significantly across the site throughout the experimental period ($P = <0.0001$) unlike total C (**Fig 6.9**). Soil total N increased significantly at all sampling points across the orchard (alleyway $P = 0.003$, Gala stands $P = 0.03$ and the Ruben stands $P = 0.01$) from sampling before grubbing occurred to the day after grubbing. Soil total N in the alleyways increased from a week to five months post grubbing, these changes were not significant, but when compared to the soil N under the former tree stands from May onwards the alleyways had significantly higher concentrations of soil TN ($P = <0.02$).

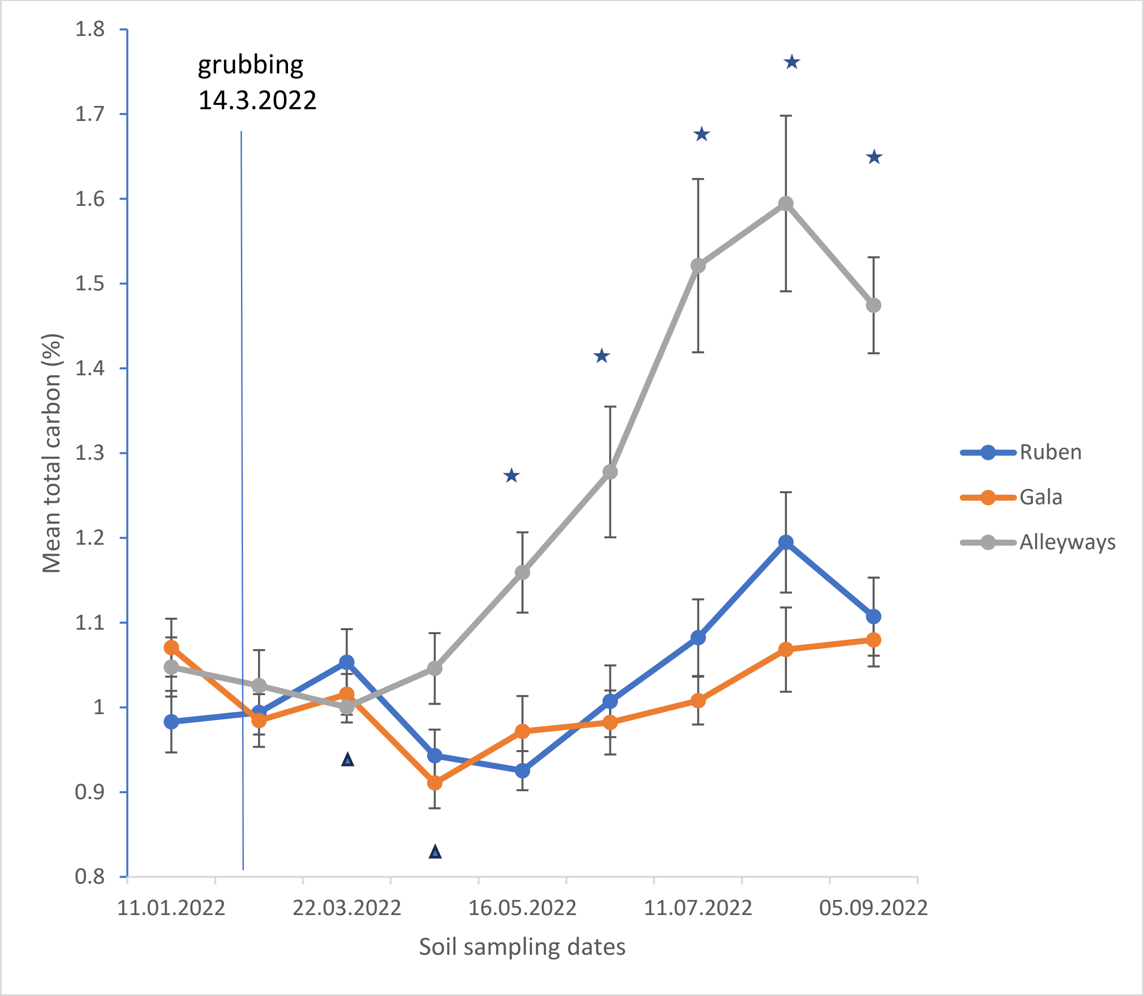


Fig 6.9. Mean soil total % carbon and standard errors across the orchard for the alleyways, Gala and Ruben former tree stands from two months before grubbing (14th March 2022 (green line)) to six months post grubbing (September 2022). Significant differences (P = <0.05) indicated by the asterisk (*) are between the alleyways and the former tree stands which occurred from May onwards, and the blue triangle indicated the significant decrease under the former tree stands between March and April. There were no significant differences in soil total C between the Gala and Ruben tree stands.

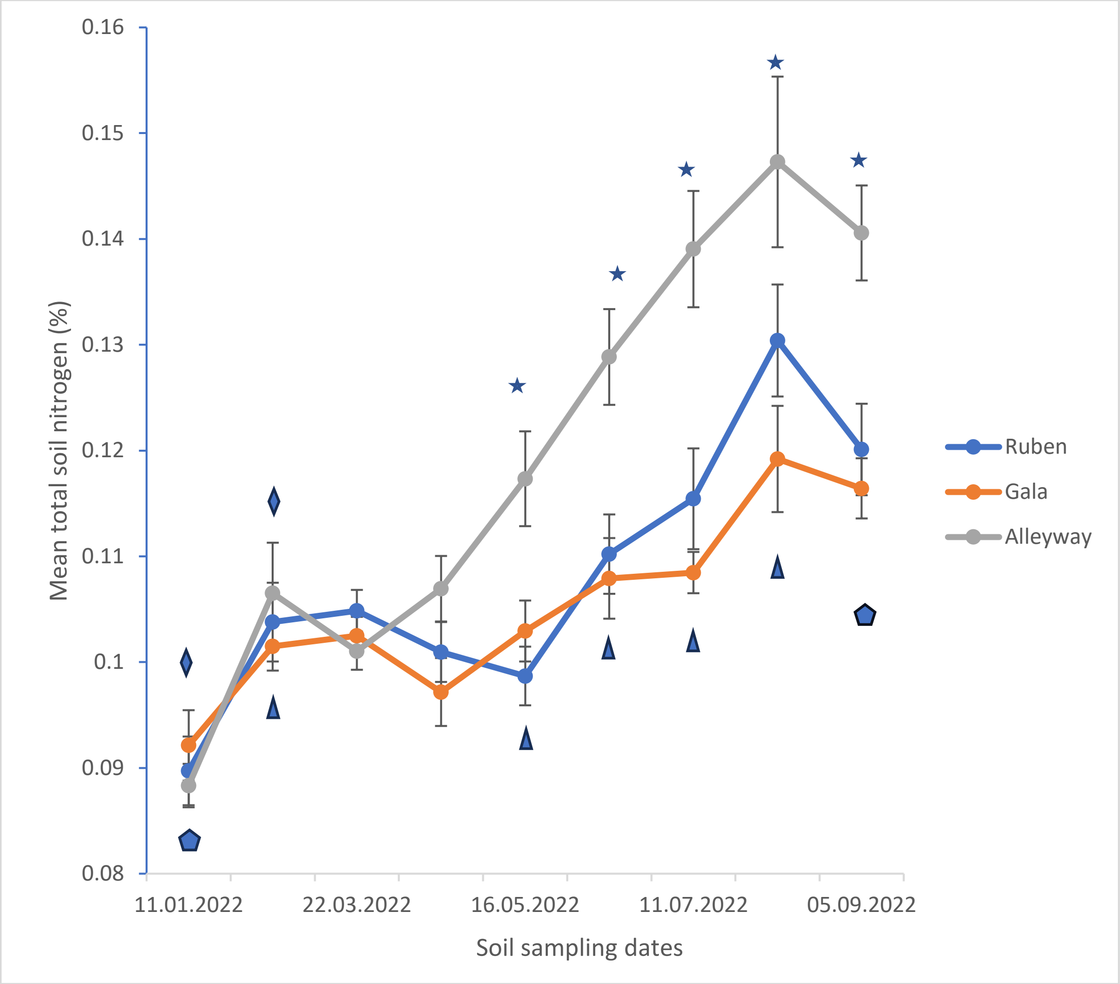


Fig 6.10. Mean soil total nitrogen % across the orchard for the alleyways, Gala and Ruben former tree stands from two months before grubbing (14th March 2022 (green line)) to six months post grubbing (5th September 2022) with standard errors marked. Statistically significant differences (P = <0.05) are indicated as follows; Asterisks differences between soil N in the alleyways and former tree stands, Diamonds (Rubens) and triangles (alleyways and Gala) indicate changes between soil collection points (time), and the hexagon signifies the significant increase in soil N across the length of the study.

The results of the Pearsons correlations coefficient analysis (**Table 6.2**) showed that over the period of soil sampling the total C and N had a significant positive relationship, except for the first sampling in January (P = 0.14). Soil moisture was not significantly correlated (either positively or negatively) with either total C or N throughout this preliminary study, and the soils moisture content showed the most variability at different collection points to TN and TC.

Table 6.2. Pearson's correlation coefficients across the site at each sampling point. Difference levels of significance indicated with an asterisk, P= < 0.01 *, P < 0.001 ** and P < 0.0001 ***.

before grubbing	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	0.44	1	
T%C	0.20	0.53	1
Day after	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	0.34	1	
T%C	0.33	0.85 *	1
1 week post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	0.42	1	
T%C	0.43	0.85 *	1
1 month post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	-0.15	1	
T%C	-0.18	0.91 **	1
2 months post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	-0.49	1	
T%C	-0.44	0.95 ***	1
3 months post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	-0.42	1	
T%C	-0.54	0.95***	1
4 months post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	0.04	1	
T%C	-0.03	0.96 ***	1
5 months post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	-0.41	1	

T%C	-0.47	0.95 ***	1
6 months post	*soil water content %*	*T%N*	*T%C*
soil water content %	1		
T%N	-0.13	1	
T%C	-0.13	0.96***	1

6.5. Discussion

This 9-month preliminary study was undertaken to investigate the fate of stored soil C in the six months following the grubbing of an apple orchard. This site was atypical in age and spacing of the trees compared to a commercial orchard, which could therefore influence the amount of stored soil C and may not represent stored soil C of a typical commercial orchard. Additionally, this site had been fertigated in the two years prior to grubbing, which likely had an impact on the levels of N and C in the soil at the time of initial soil sampling in the form of residual N from these applications which can enhance C sequestration in the soil, as discussed in Chapter 1 and by Reay et al (2008). However, following grubbing, N and C in the disturbed surface layers of soil would then have been exposed to oxidisation and mineralisation leading to partial release back into the atmosphere (Page, 2011; Zummo and Friedland, 2011) and an increased availability for plant uptake.

Although a significant difference in soil pH was observed in January 2022 between alleyway and Gala rows (**Fig 6.7**) no significant differences were found in soil moisture (**Fig 6.8**), total C (**Fig 6.9**) and N (**Fig 6.10**) among the three sampled areas. A possible explanation for the significant difference in the soil pH under Gala compared to the alleyways is an increase of root exudates at the depth of sampling and could be linked to the density of tree planting, but this was not true for the former Ruben stands. This is in contrast to work carried out on apple replant disease by Leisso et al (2017), which showed that apple rhizodeposits lowered soil pH under apple trees. The difference between the two apple varieties in their soil pH levels could be down to the types of root exudates, tree sizes and root systems, but this was not assessed as part of this study. Soils pH under the former Ruben stands did have the largest variability between the samples across the site (**Fig 6.7**) which could also be due to the root exudates, density of tree planting, underlying geology, as well as soil moisture. Soil pH has been cited as an important factor in the C storage ability of soil: modelling suggested that pH values between 4.2 and 6.5 led to a higher soil organic C density (Liao, Wu and Zhu, 2016; Zhou *et al.*, 2019). Soil organic C density is the amount of C that has been stored at certain depths of the soil profile (Liao, Wu and Zhu, 2016; Zhuo *et al.*, 2022). Although there was a change in the soils pH (not significant) across the site by six months post grubbing (**Fig 6.11**), decreases in the former tree stands could be a result of limited root exudates at the depth of soil sampling, limited microbial activity and lack of rainfall even though grass and weeds had taken over the former rows (Walker *et al.*, 2003; Zhang, Wu and Liu, 2019; Vives-Peris *et al.*, 2020). In contrast to the former tree stands, the alleyways had a constant supply of root exudation within the upper layers of the soil which was due to only having a small amount of disturbance of the grass and upper surface of the soil during grubbing.

Fig 6.11 A and B. Tree regrowth from the broken root systems as well as weed and grass coverage in the former tree stands which had previously been kept clear of weeds and grass by herbicide sprays. Images taken by Catherine Chapman 10th June 2022.

Soil moisture in this preliminary investigation had a significant decline from April to August 2022, due to the lack of rainfall that year, but over the same period soil TC across the whole site increased, with the most significant increases occurring in the alleyways. These results are in contrast to previous studies which have shown that a lack of soil moisture can reduce the ability of the soil to sequester C in several ways, including limiting plant growth, lowering evapotranspiration, prompting stomatal inhibition of photosynthesis, lessening excretion of exudates into the soil and effecting soil microbial respiration rates through limiting their activity levels (Six *et al.*, 2006; Bardgett, Freeman and Ostle, 2008; Yang and Zhou, 2013; Jia *et al.*, 2016; Humphrey *et al.*, 2021). The study by Yang and Zhou (2013) concluded that C sequestration was sensitive to alterations in seasonal rainfall, more than the annual totals and soil moisture content. Yang and Zhou (2013) suggested that seasonal rainfall was more important than soil moisture content because it can affect C within an ecosystem, through changing photosynthetic rates which alters plants growth and productivity, and the amount of sequestration that can occur. The results from this preliminary study showed that soil moisture across the former orchard site declined significantly over the study period, some of this was due to normal seasonal variations but in 2022 there was half the amount of rainfall compared to the same period the previous year and a drought had been declared (Kendon *et al.*, 2023).

The initial significant decrease of total soil C content in the former tree stands (both Gala and Rubens), agreed with previous findings on soil C dynamics following soil disturbance (Zummo and Friedland, 2011; Narayan Yadav *et al.*, 2021). This decrease in soil C in the first two months following grubbing could be due to soil aggregates being broken apart allowing oxygen to enter the soil to greater depths which cause C oxidisation to occur and releasing CO_2 back into the atmosphere (Narayan Yadav *et al.*, 2021). The increase in soil C following the initial decline could be due to the management of the site, or natural seasonal variations of day light and photosynthetic rates of the grass and weeds. The soil on this site was left undisturbed, allowing for grass, weeds and even tree regrowth in the previously planted tree rows (**Fig 6.11**

a and B), which could have replaced some of the lost C following grubbing (**Fig 6.9**). The increase in soil C could also be due to the breakdown of the root system releasing stored C from the roots into the soil through microbial activity on the organic tissue (Zhou *et al.*, 2017).

This study showed N (**Fig 6.10**) was not limited by the soil moisture content especially in the alleyway that showed significant increase over the preliminary study period. There was an initial decrease after grubbing in both alleyways and the former tree stands, although this was at two different points (day after grubbing occurred and between one week to two months post grubbing). This decline in soil N could be due to the mineralisation as oxygen was able to penetrate the soil (Kristensen, McCarty and Meisinger, 2000) and subsequent release back into the atmosphere, especially for the soil in the grubbed rows. The increase in soil N across the study period could be due to the movement of N in the soil from one area to another, trees no longer competing with the grass for N, root exudates from the grass and weeds, soil temperature, microbial activity and increased plant growth (Kemmitt *et al.*, 2005; Farrell *et al.*, 2011).

The interaction between the soils total C and N has been well documented (Ge *et al.*, 2013; Yao *et al.*, 2022; Amorim *et al.*, 2023), and can be seen in this study (**Table 6.2**) as the soils concentration of TN increased, the soil C concentration also increased, especially in the alleyways. This interaction of C and N is often quoted as a ratio, which indicates not only the health of the soil (including soil microbial activity) but its effect on SOM decomposition rates which can either increase or decrease CO_2 emissions from the soil (Post *et al.*, 1985; Lu *et al.*, 2021). The increase in soil N through root exudates and seasonal turnover over the course this preliminary study could account for some of the increased soil C, in all areas under investigation and the return of the former tree stands soil C concentrations to those collected before grubbing.

6.6. Conclusion and future work

This preliminary investigation on the fate of stored soil C following grubbing showed that, significant amounts of soil C was lost from the former tree rows in the first two months. Thereafter, soils TC slowly recovered to levels similar to those observed before grubbing occurred. The alleyways recovered their small loss of C and N first as these areas suffered less soil disturbance than the rows. The changes in the soil total percentage C and, N, and pH and moisture are likely to be accounted for by internal and external soil factors. These factors would include local weather patterns (the lack of rainfall that year and high summer temperatures) affecting the soil moisture content and temperature, natural seasonal variations of C and N cycling within the soil, amounts and depth of root exudations from the grass and weeds into the soil and microbial activity within the soil releasing CO_2 back into the atmosphere (which was not measured as part of this study).

Further work on belowground C sequestration and changes in soil C following orchard grubbing are needed to explain how effectively apple orchards could help mitigate against increased atmospheric CO_2 levels and contribute to carbon offsetting programmes to achieve net zero farming targets. The focus should be on different soil types, depths, pre and post grubbing practices, and whether the age of the orchard at grubbing has any effect on soil C concentrations.

6.6.1 Limitations of this study

This study has several shortcomings. Firstly, the depth at which the soil was sampled only showed what the soil C was in one point in the soil profile but does not show the entire profile of the C storage in the soil. This depth was selected as it was within the main rootzone of the tree. Secondly, this investigation was limited to one orchard, so no comparisons could be made to another grubbed, and non-grubbed orchards, to determine seasonal verses grubbing effect on the changing soil C. Thirdly, the limited number of soil assessments conducted were subject to time and budget limitations. Fourthly, this study does not give a true representation of a commercial orchard, where trees are older and may have sequestered more C into the soil. Also, the spacings between trees were not uniform due to the experiment that was conducted previously.

6.7. References

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Chapter 7. Discussion, conclusion, limitations, and future work.

7.1 Discussion.

Soil C sequestration has been suggested as a potential method to mitigate rising atmospheric CO_2 concentrations, and soil has not yet reached its full potential, but the amount of C that can be stored in most soils has an upper limit (Lal, 2004; Davidson and Janssens, 2006; Fry, De Long and Bardgett, 2018; Amelung *et al.*, 2020). Apple trees, being perennial, could provide a long-term method of sequestering C in soils where there is a deficit, typically due to past soil management. This project aimed to determine what may influence the potential of apple trees to sequester C belowground by investigating five factors that had limited previous research. The five factors, rootstocks (**Chapter 2**), scions (**Chapter 3**), increasing atmospheric temperatures (**Chapter 4**), age of orchards (**Chapter 5**), and grubbing (**Chapter 6**), did show differing impacts on the potential amounts of C that apple trees can sequester. Rootstock and scions showed little to no influence on the apple tree's ability to sequester soil C. In contrast, increasing atmospheric temperature showed that an increase of 2°C showed a positive effect on soil C but a further rise to 4°C above ambient temperatures had a negative impact on soil C sequestration. Finally, the increasing age of orchards and grubbing both negatively influenced soil C concentrations.

All these experiments were conducted over a period ranging from two weeks to two years. Due to the limitation, these studies indicate what might be happening, but they do not offer a comprehensive understanding of each factor's effects, highlighting the need for further studies. Several investigations (**Chapters 2 - 4**) confirmed strong seasonal fluctuation in soil C and N concentrations, highlighting the need to find the most informative time of year to understand the studied process. These findings support other studies showing seasonality in soil C concentrations (Franzluebbers, Hons and Zuberer, 1996; Bardgett *et al.*, 1997; Yang *et* *al.*, 2020; Singh and Kumar, 2021). The seasonal variation of soil C concentrations was demonstrated by the declining soil microbial biomass C concentration at the September harvests in three investigations (**Chapters 2 - 4**). The other two investigations could not support this trend due to differences in the timing of soil collections and the subsequent laboratory analyses.

All five of the investigations showed significant differences in the concentrations of soil C and N between the different soil regions, areas of orchards where soil had been collected (whether in the rootzone of the tree, bulk soil in the pots or rhizotrons or in the alleyways in the field-based experiments). Most studies showed that the C in the rootzone had higher concentrations than the bulk/alleyways. This would be expected as this is the location of the root exudates from the tree, dead roots and cells, and the higher concentration of soil microorganisms (Holz *et al.*, 2018; Canarini *et al.*, 2019). In two of the studies (Rootstock and scions, **Chapter 2 and 3**) the soil profile was homogenised to give an indication of the soil TC and other C fractions, as well as soil N within the rhizotrons bulk and rootzone soil. This was not a true representation of the soil C profile. It has been suggested that C will be transferred within the soil and C pools from those that are actively accessible to those of more stable and long-term storage (Jackson *et al.*, 2017; Sokol and Bradford, 2019; Witzgall *et al.*, 2021; Sokol *et al.*, 2022). The soil profile and depth were not considered within any of the other three studies, nor was the underlying geology which could have influenced the inorganic soil carbon levels (Ferdush and Paul, 2021; Sharififar *et al.*, 2023).

Significant differences in biomass C of the trees were observed across the investigation periods of the rootstock and scion experiments (**Chapters 2 and 3**). This is consistent with previous studies on biomass C content of the tree and would be expected as trees age and develop (Petersson *et al.*, 2012; Gao *et al.*, 2014; Sahoo, Nath and Lalnunpuii, 2021; Zahoor *et al.*, 2021). Several factors had possibly influenced the different growth rates of the tree's roots, shoots, and leaves, these could include the fertigation of the rhizotrons (which was not investigated), and the biomass collection points. In the scion experiment (**Chapter 3**), trees were grown in pots with a limited nutrient availability, inhibiting the trees' growth. These trees that had been grown in pots had no fertigation and had become pot bound by the end of the experiment.

The investigations into increasing atmospheric temperatures (**Chapter 4)** did clearly and significantly show that an increase in atmospheric temperature of 2°C, in the UK, could have a positive impact on the amount of C sequestered into the soil by all varieties of dessert apples under investigation, which agrees with the research carried out by Guttières et al. (2021). However, a further increase in atmospheric temperatures (up to 4°C above ambient) resulted in a significant decline in the ability of the soils to sequester C belowground, which is in line with previous research, which has shown that increased temperatures are detrimental to the soil's overall health and food production (Valverdi, Cheng and Kalcsits, 2019; Hartley *et al.*, 2021; Tiefenbacher *et al.*, 2021). Increasing atmospheric temperature would limit the trees' photosynthesis, limiting the availability of C for plant growth, fruit production, and exudation into the soil (Moore *et al.*, 2021). The reduction in photosynthesis and exudation would limit the ability of the trees to absorb nutrients from the soil due to a possible reduction in the population of soil microbes breaking down the nutrients, which would lead the plant to become weakened and limit productivity (Valverdi, Cheng and Kalcsits, 2019).

The results of **Chapter 5** investigating the effect of age on the soil C content were unexpected. They showed that as the age of the orchard increased, the soil C and N content decreased with age and that the most significant loss of C occurred in the first six years as the initial input of soil amendments was used. This contradicts several studies that have predicted that orchard trees would continually increase soil C (George, 2010; Lefebvre *et al.*, 2021; Zhang *et al.*, 2021). However, other studies have shown that C sequestration levels decline once the orchard reaches a certain age, however, this differs between fruit crops (Wu *et al.*, 2012; Yang *et al.*, 2021; Betemariyam and Kefalew, 2022).

The final investigation on the effects of grubbing on the stored soil C (**Chapter 6**) showed that after an initial C loss in the first two months following grubbing, soil C could recover as other vegetation grew in the former tree stands. The initial loss of soil C was due to soil disturbances occurring during the grubbing process, and this is supported by researchers who have investigated soil disturbances in other land uses (Ostle *et al.*, 2009; Zummo and Friedland, 2011). The soil C in the alleyways initially had a limited decline, but after a month following grubbing, soil C significantly increased over the remaining five months, which supports the finding that grass alleyways in orchards are beneficial to soil C sequestration (Xiang *et al.*, 2022), and soil compaction in the alleyways may be protecting the C due to limited pore space for air to enter (Deurer *et al.*, 2012). The grubbed biomass is waste material and the conversion of this material into biochar rather than just being burnt at the orchards edges, and subsequent use as a soil C store/amendment is still an active area of research (Jones *et al.*, 2011; Anthony, 2013; Tan *et al.*, 2020; Zhao *et al.*, 2020; Duan *et al.*, 2021).

Advancements in remote soil sensor technology that detect soil C and N, could potentially reduce the need to disturb the soil layers that release stored soil C, as shown in several studies, including in this project (**Chapter 6**). However, soil disturbance cannot be prevented when the orchard is grubbed. Remote sensing would also reduce the time required to collect and analyse the samples and data. Different sorts of sensors are becoming more widely available for farmers to help determine their soil C content. These include satellite imaging and estimation of soil C content such as those of Landsat (Gong *et al.*, 2013), hyperspectral or near infrared-shortwave infrared imaging, that use the light spectrum to detect soil C (Angelopoulou *et al.*, 2019) or even the multispectral analysis by remote means (Luo, 2019). The results from these technologies need to be compared to those found by the traditional methods done within the laboratory to ensure the soil C contents are as accurate as possible and that it is not detecting the CO_2 within the air above the soil or C in the biomass of plants.

This study is helping to build up a deeper understanding of what may be influencing apple trees' ability to sequester C belowground, and whether apple orchards can be classed as C sinks, C neutral or a C source. Apple trees (orchards) and other top fruit orchard crops are valuable economic crops and cover large areas of land across the globe. Previous research has shown that apple trees are good at sequestering C whether this is in the soil or in the trees' biomass (above and belowground) (Scandellari *et al.*, 2016; Zahoor *et al.*, 2021). However, they may be a peak age at which sequestration occurs before declining.

With the recommendations in the Kyoto and Paris agreements recommending that global C stores need to be protected and enhanced, to mitigate the rising CO_2 and temperatures to maintain global food security (French, 1998; United Nations, 2018). Reports by Natural England (George, 2010; Gregg *et al.*, 2021), as well as UK government subsidies such as those discussed in Chapter 1, suggested that the planting of more trees (which could include apple trees), conversion of land back into natural habitats such as heathlands and the protection of areas such as peat, will all be a positive way to mitigate climatic changes that are happening. Evidence is now being presented that contradicts this, or that the expected concentrations of C sequestration into the soil by land conversion is not being achieved (Guo *et al.*, 2008; Wang *et al.*, 2017; Koutika, 2022; Tian *et al.*, 2023; Duddigan *et al.*, 2024).

7.2 Conclusion.

In conclusion, this study has shown that rootstocks and different scions have little to no effect on the amount of C that an apple tree sequesters belowground. Both increasing ages of orchards and the grubbing process have a negative influence on soil C concentrations. However, after a couple of months following grubbing, in an orchard that was left fallow and return to being fully covered in grass the soil C concentration can potentially recover the lost C over time. Increasing atmospheric temperature had a positive impact at an increase of 2°C on soil C. However, this changed at 4°C above that of the ambient atmospheric temperature and became negative showing that soil C was being lost and was found to be of similar concentrations of those in ambient temperature tunnels. This suggests that belowground C sequestration abilities of apple trees may not be what is expected and contradicts the current belief that they can aid in climate mitigation (increasing atmospheric CO_2 concentrations).

It is therefore crucial to consider these factors, along with others, for a comprehensive understanding when determining an apple tree’s potential for belowground C sequestration ability from pre-planting to the end of the orchard’s life. The above and belowground C sequestration ability of an apple tree also needs to be considered when determining if they are a C sink, store, or source, and the farming practises throughout their productive life. These considerations are important for producers when calculating their carbon footprints of apple production, aligning with the UK’s target to achieve net zero carbon by 2050.

7.3 Project limitations.

This project had several limiting factors. Firstly, none of the studies were repeated so the results gained show possible trends, rather than showing consistent results. Secondly, all field-based studies did not show the C of the full soil profile, or considered the underlying geology that may influence soil C. Thirdly, the soil types sampled were all clay based, so do not show what may happen to soil C in different soil types, such as sandier based. Finally, time and funding have limited the amount of laboratory analyses carried out and the length of several studies.

7.4 Future work.

The completion of this research has shown that there is still a need for further investigations to understand what influences the potential for apple trees to sequester C belowground. It is imperative to find strategies to combat climate change and means to adapt to future changes. Repeating the investigation on the rootstock and scion effects in larger and longer-term studies would enhance the current findings, shedding light on the impact on the soil C as trees age in controlled and field studies.

Furthermore, investigating the impact of varying atmospheric CO_2 levels and changing weather patterns, including the increased occurrence of winter flooding and summer drought, is necessary. Given the reported global temperature increase in 2023, understanding how these climatic changes affect photosynthesis and C sequestration is vital for developing more resilient agricultural practices (Met Office, 2024; NASA, 2024; NOAA, 2024). Understanding the impact of increasing atmospheric temperatures on trees' sequestration ability, as explored in **Chapter 4,** is crucial. However, it is equally important to investigate how these global atmospheric temperature changes alter soil temperature and influence on soil C stability and sequestration ability and the soil microbial communities. This two-fold analysis would provide a comprehensive view of the intricate relationship between climate changes, tree behaviour, and soil dynamics in the context of the C sequestration potential of apple orchards.

Exploring other aspects, such as the influence of differing soil types and the underlying geology of orchards on soil C sequestration, is vital. Unlike many studies that focus on fixed points within the soil profile, a more extensive examination of the soil profile of an orchard would be recommended. Understanding of C storage across different horizons can provide insights into the types of storage pools affected by sequestration.

Apple orchards (trees), ability to sequester atmospheric CO_2 should be investigated as a whole and not as two or three separate storage sites (soil, below and above ground biomass stores) over the entire life span. Future studies need to determine the soil C and estimate biomass C before planting, as well as the C that is available in any soil amendments that may be or have been applied such as biochar, to what happens to the biomass at grubbing, such as the loss of C through burning and the ash that is left.

7.4 References

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