



Final Report

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Title: Belowground carbon sequestration potential of apple trees.

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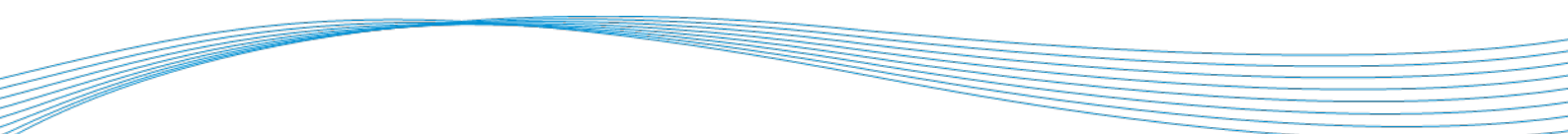
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1. Industry Summary

Atmospheric carbon dioxide (CO₂) levels are rapidly rising due to anthropogenic activities, and the effects of climate change are already being felt. The need to mitigate against the impacts of rising CO₂ to ensure food security for the future is therefore vital. Trees, including apple orchards, could provide a natural sink for CO₂, by storing carbon (C) both above ground and below ground in the soil. Soil is one of the largest terrestrial C pools– although the amount of carbon stored within the soils can vary between soil types and land use.

The aim of this project was to investigate what factors affected the belowground carbon sequestration ability of apple trees. Five factors were selected for investigation: - rootstock variety, scion variety, increasing atmospheric temperature, orchard age and end of orchard life practices (grubbing).

Rootstocks: A 4.5-month experiment was conducted using three rootstocks (M.9, M.116 and M.M.106) which are commonly used to grow apples commercially in the UK. The results showed that over this period there were no significant differences in soil C sequestration between rootstock varieties. Total soil C increased over time across all rootstocks with the highest concentrations found in the rootzone region (1cm around the roots).

Scions: Five scion varieties (Cox's Orange Pippin, Braeburn, Gala, Dabinett and Michelin) were studied over two growing seasons. The results showed that there were no consistent significant differences between the five scion varieties in the amount of C sequestered into the soil.

Atmospheric temperature increases: This 1-year experiment was conducted at the National Fruit Collection at Brogdale using a pre-existing long-term climate polytunnel. The results indicated that a 2°C increase in ambient atmospheric temperature significantly increased the concentration of soil C. However, increasing the atmospheric temperature to 4°C above those of current ambient temperatures appeared to decrease the soil C sequestration ability of trees.

Orchard age: A space-for-time-substitution study was carried out across three farms in Somerset, comparing soil sampled from the tree row to the alleyways from orchards between the ages of 2 and 46 years old. Results showed that the soil C decreased as the orchards aged, except for inorganic carbon which increased with orchard age. There were significant differences between the amount of stored soil C found under the tree stands compared to concentrations in the grass alleyways.

Post-grubbing soil carbon: A nine-month field study investigated how soil carbon concentrations responded following soil disturbance caused during the grubbing process. Soil total carbon decreased in the first two months following grubbing in the former tree stands and grass alleyways. The grass alleyways were the first to recover from the soil disturbance and gradually became significantly higher in soil total carbon % than either of the former tree stands.

In conclusion, of the various aspects studied, age, increasing atmospheric temperature, and the grubbing up process showed the most potential to impact soil carbon storage. Rootstock variety and the grafted scion cultivar could potentially have some impact, but this was not evident during this study. All these factors need to be considered when calculating carbon footprints of apple production and the carbon sequestration potential of apple orchards. Further research is required to gain a deeper understanding of the factors that influence the ability of apple trees to sequester carbon belowground and to bring all the research together to help shape the development of global climate mitigation policies and aid farmers to become net zero in their production.

2. Introduction

Climate Change is affecting global weather patterns and temperatures, with the occurrence of extreme heatwaves, severe droughts and flooding episodes predicted to increase, which will put greater pressure on food production. Greenhouse gases (GHGs) in the atmosphere are still rising; in May 2021 the level of carbon dioxide (CO₂) in the atmosphere had risen to 419.05ppm, representing an increase of over 26% since June 1969 (noaa.gov). The UK is a signatory of the Kyoto protocol (UNFCCC, 2014) and also to the following 2015 Paris agreement (UNFCCC, 2015) in which 196 countries committed to keep the global temperature rise below 2°C and to achieve net zero carbon emissions by the second half of this century. In 2019 the UK became the first major economy to set out in a legal framework a commitment to achieve net zero GHG emissions by 2050 (UK Gov). Consequently, the UK government has invested in technologies and projects aiming to mitigate and capture atmospheric CO₂, with the Industrial Strategy Challenge Fund representing a major part of their strategy to achieve this.

Perennial crops such as apple trees could help mitigate rising atmospheric CO₂ levels through sequestering C belowground via the roots into the soil. Soil is the second largest active C cycling pool after the oceans (Fry, De Long and Bardgett, 2018), and it is believed that most soils have the capacity to store more carbon than they currently do (Stewart *et al.*, 2007). Ledo *et al* (2020) found that approximately 30% of land globally is covered with perennial crops such as apple orchards,

and suggested that over their lifetime, perennial crops become carbon neutral, if not C negative as they continually absorb and store carbon.

Apple orchards have been reported to be able to continually sequester C over their life span (George, 2010; Gregg *et al.*, 2021), aiding climate mitigation and therefore helping to protect food security. Research has shown that both soil C and tree biomass C increase as orchards age, but other studies have shown that the ability of orchards to sequester C reaches a peak before it declines (Wu *et al.*, 2012a). The peak age of C storage has been suggested to differ between fruit crops but Wu *et al.* (2012) suggested that for apple this is at 18 years old.

Rootstock breeding has generally promoted carbon uptake by the fruit, thereby limiting the amount allocated to the roots and exuded into the soil. Currently it is not known if any particular apple rootstock has a greater ability to store C below ground and so this was one of the experimental aims of this project. The amount of C that a tree can sequester varies between tree types (e.g. fruiting trees, other non-fruiting deciduous and evergreen trees), which also differ in the allocation of carbon to different compartments, such as above ground in stems, branches, and fruit, or belowground in roots which release C into the soil via exudation. Soil C sequestration can also be affected by rhizosphere microbial (bacterial and fungal) communities, which can promote nutrient uptake from the soil by the roots, in return for root exudates which feed these soil communities (Kell, 2012). Other factors that can affect C sequestration include abiotic stresses such as droughts and flooding, which can impact the rate of photosynthesis, growth, C storage, production of fruit, soil microbial processes and soil GHG emissions of apple trees.

Carbon sequestered in the soil can be released back in to the atmosphere as CO₂ via soil respiration which can increase as a result of disturbance events such as tillage (Schlesinger and Andrews, 2000). Dessert apple orchards typically have a commercial life span of 15-20 years whereas cider orchards are between 50-80 years, after which they are grubbed up, causing substantial soil disturbance, and the aboveground growth is generally burned. This burning of aboveground growth releases CO₂ back into the atmosphere, increasing the orchards' carbon footprint.

The overarching aim of this project was to investigate different factors that could potentially increase or inhibit the ability of apple trees to sequester carbon belowground. Each of the five factors investigated had their own experimental aims.

3. Materials and methods

The project used a combination of glasshouse, polytunnels and field studies. Laboratory analyses were conducted at NIAB East Malling; Forest Research, UK; NRM (Reading, UK); LGC Genomics Ltd (Germany) and the University of Reading. Laboratory analysis of soil samples included the measurements of soil total C and N, nitrate, active C, microbial biomass C, pH and texture, in addition to measurements of the total C, N and protein content of root samples (experiments 1 and 2) and above and belowground biomass C (experiments 1 and 2). Additionally, experiment 4 (effect of orchard age) included measurements of soil organic and inorganic C, bulk density, and nutrients (phosphorous, potassium and magnesium).

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

At NIAB East Malling, soil moisture content was determined using the gravimetric method described by Forster (1995) and soil pH was measured 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) 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 ninhydrin-reactive N assay described by Amato and Ladd (1988) was used to determine both the microbial soil biomass nitrogen and carbon (MBN/MBC) content following the chloroform fumigation extraction method as described by Vance et al (1987). The soil nitrate concentration was analysed using the colorimetric method described by Cataldo et al (1975). Some modifications were made to the ninhydrin and nitrate assay methods, which are noted below. Aboveground and belowground biomass C was measured following the method and calculations described by De Oliverira et al (2019), Petersson et al (2012), and Manickam et al (2014).

Soil Nitrate – The concentrations of the standard solutions were increased from 10 up to 25 µg NO₃⁻-N mL⁻¹. Some samples needed diluting up to 70 % with deionized water for the absorbance reading to be in range.

Soil microbial biomass C –The maximum concentration of the standard for the calibration curve was decreased from 1000 $\mu\text{m NH}_2\text{-N L}^{-1}$ to 600 $\mu\text{m NH}_2\text{-N L}^{-1}$. The volume of extracted soil sample used in the assay was decreased from 1 mL to 0.6 mL, and the volume of citric acid buffer used was increased from 1 mL to 1.4 mL.

Soil DNA was extracted using the Qiagen DNeasy PowerSoil Kit and protocol. DNA extracts were sent to LGC Genomics GmbH, Berlin, Germany for amplicon sequencing of ITS and 16S ribosomal RNA (rRNA) to enable analysis of the fungal and bacterial communities in the commercial rootstock experiment.

For the experiment investigating the effect of orchard age on soil C, all analyses were conducted by NRM in Reading, UK using their “carbon checker plus” package. This includes analysis of soil total nitrogen, carbon, organic C and inorganic C concentrations, which were determined using the Dumas Combustion method as described by AOAC official analysis methods (1990), BS EN 15936:2012. The fraction of soil active carbon, was determined using the permanganate oxidisable carbon method as described by Culman et al (2012). The soil bulk density was measured using the method described by DL Rowell (Rowel D L, soil science -methods and applications. ISBN 0-582-08784-48). A second round of tests for the orchard age experiment were also carried out by NRM on 142 samples using their “Standard Soil Plus” package and soil textural classification. The package included soil pH using a suspension of soil and water with the pH probe. The soil texture classification was determined using laser diffraction and the UK soil classification. The final set of analyses determined the available phosphorus (P) using the Olsen’s extraction method and the levels of potassium (K) and magnesium (Mg) by the ammonium nitrate method.

The statistical analyses were conducted using Microsoft Excel 365. Statistical tests included, one-way ANOVAs and Pearsons analysis, and the XLSTAT add-in was used to create ternary plots. R Studio 4.1.1 was used to analyse the amplicon sequencing data. Minitab 21.2 was used to conduct regression analysis, data transformation using angular transformation (arcsine) and Box-Cox transformations with optimal lambda (λ) and Spearman pairwise analysis as the data was not normally distributed. Post hoc Tukey tests were conducted for the orchard age experiment. Statistical significance was indicated by p- values of <0.05 .

3.1. The impact of commercial apple rootstocks on belowground C sequestration (2020).

3.1.1. Aim:

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, rootzone (1 cm around the roots) and rhizosphere soil), and
- 3) whether the diversity and composition of soil microbial communities (both fungal and bacterial) differed significantly between rootstock varieties and soil regions.

Method: This glasshouse experiment was conducted at NIAB East Malling, UK. Three rootstock varieties were used in this study (M9, M116 and MM106, all supplied by F.P Matthews) selected for their different growth patterns ranging from dwarfing to semi-vigorous and are used in different sectors of apple industry. Eighteen rootstocks were used per variety (54 in total), all of which were grafted with Cox's Orange Pippin to remove any effect of the scion cultivar on the rootstocks. The grafted trees were planted into 1m tall Perspex rhizotrons (**Fig 1 A and B**) and placed in a glasshouse compartment in a randomized block design, with daily fertigation, which increased over the course of the growing season. Four grafted trees of each rootstock variety were not planted to provide a baseline for biomass carbon content. The planted trees were divided into three harvest groups, with four of each rootstock being destructively harvested at 6- and 13-weeks post-planting, and the remaining six of each rootstock were destructively harvested at 19 weeks after planting. The destructive harvest consisted of separating the aboveground material, cutting the trunk at the top edge of the rhizotron, meaning it contained part of the rootstock below the graft union. This was done to maintain consistency between harvests, since the graft unions were at different heights for each tree. Belowground growth was placed into labelled bags ready for further processing in the lab. Two homogenised soil samples were taken at the time of destructive harvest from across the rhizotron – bulk and rootzone (1cm around the roots), placed in labelled bags and kept in the fridge for analysis. A third soil sample was collected directly from the roots (rhizosphere soil) which was used for DNA extraction and amplicon sequencing analysis of fungi and bacteria.

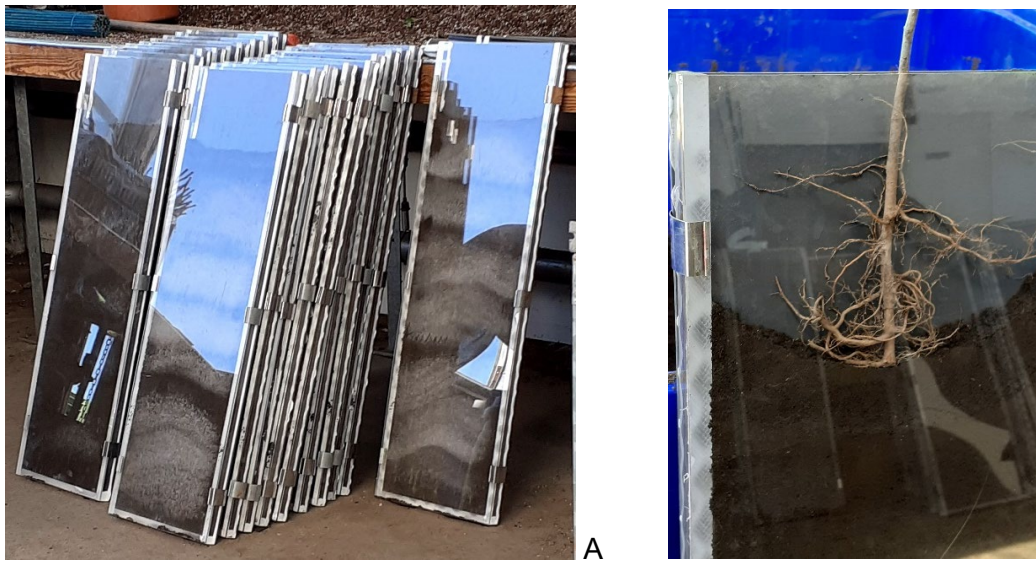


Fig 1 A and B. Rhizotrons (100cm x 30cm x 5cm) used in the experiment, and a tree ready to be planted. (Photos by Catherine Chapman)

3.2. Scion-mediated effects on belowground C sequestration (2021-2022)

3.2.1. Aim:

To determine whether different scion cultivars and their interactions with M9 rootstocks affect amount of C sequestered into the roots and the surrounding soil regions (root zone and bulk soils).

Method: This glasshouse and polytunnel experiment was conducted at NIAB East Malling, UK. The scion investigation used 90 M9 rootstocks (F. P. Matthews) which were divided into five groups of 18 and grafted with different scion cultivars (three dessert varieties: Cox's Orange Pippin, Gala and Braeburn and two cider varieties: Dabinett and Michelin). Following grafting, the 18 trees of each scion were randomly divided into three groups of 6 trees and assigned a destructive harvest point and planted in March 2021. The first 30 trees (6 of each scion) were planted into rhizotrons and placed into a glasshouse compartment for 6 months (March – September 2021) in a randomised block design. The remaining 60 trees (12 of each scion) were planted into 3 L pots and placed into plastic crates using a randomised block design in a polytunnel

until they were harvested at 12 and 18 months (March and September 2022). The destructive harvest process used the same protocol as the rootstock experiment (3.1).

3.3. Impact of increasing atmospheric temperature on belowground C sequestration (2021)

3.3.1. Aim:

To investigate whether increasing atmospheric temperatures influenced the amounts of belowground C sequestration of different scions across three different temperature ranges.

Method: This was a field-based polytunnel experiment conducted at the National Fruit Collection at Brogdale Farm in Kent, using their climate change polytunnels consisting of three sets of three interconnected tunnels each being maintained at different temperatures: Ambient, +2°C and +4°C (**Fig 2 A and B**). Each tunnel contained 27 different scion cultivars grafted on M9 rootstocks, which were planted in 2013. Soil sampling was carried out once every three months between March and December 2021. Eight scion varieties (Cox's Orange Pippin, Braeburn, Gala, Tropical Beauty, Bramley seedling, Winter Pearmain, Discovery, and George Cave) were selected for this experiment with six trees of each variety being sampled. The soil samples were collected over a period of four days by hand, at a depth of 20-30cm and within a 30 cm proximity of the tree trunk, at each sampling time point, to ensure samples were collected within the rooting area. Three soil samples from the alleyways were also collected from each of the three temperature tunnels. Soil samples were placed into labelled plastic bags and kept in cool boxes before returning to the lab where they were stored in the fridge.



Fig 2 A and B. Exterior (A) and interior (B) of the interconnected polytunnels used at the National Fruit Collection, Brogdale Farm, Kent, UK, for the climate change experiment conducted by FAST and the University of Reading. Images were taken by Catherine Chapman on 10th March 2021.

3.4. Soil carbon content across different ages of cider orchards (2021)

3.4.1. Aims of the 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.

Method: This was a field-based experiment carried out over a two-week sampling period in late November and early December 2021, across 27 different cider apple orchards from three farms in Somerset. The 27 orchards sampled ranged in age from 2 – 46 years, with the trees being grown on different intermediate vigour rootstocks (M111, M116, MM106, M25 and A2) and contained a single variety (either Dabinett, Somerset red streak or Tremlett Bitter). Soil sampling was performed across each orchard in the standard “W” pattern, to include orchard edges, middle and ends of the rows to ensure an even spread across each orchard as they varied in size and shape (**Fig 3**). Soil samples were taken in the tree rows 30 cm from the trunk (“rootzone”) and in the middle of the corresponding alleyway (eight samples in the rows and eight in the corresponding alleyway).



Fig 3. One of the sampled cider orchards showing two rows of trees with herbicide strip, the grass alleyway, and the downhill slope of the orchard towards a stream. Image taken by Catherine Chapman on 23rd November 2021.

3.5. The effect of orchard grubbing on stored soil carbon (2022)

3.5.1. Aim:

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.

Method: This was a field-based study using a former experimental orchard which investigated tree spacing, comprising two apple varieties (Gala and Ruben). This orchard had been grown for only nine years before being grubbed, which is atypical for a commercial dessert orchard which are usually grubbed between 15 and 20 years. Twenty-seven sampling locations were randomly selected (using an adapted W pattern) across the orchard to account for different varietal rows, orchard edges and alleyways. Locations of each sampling site were recorded using the “what3words” app, and these were plotted on the orchard plan. There were 9 soil sampling time points, one prior to grubbing and the other 8 were post-grubbing over a six-month period (**Table 1**). Soil samples were collected by hand at a depth of 20-30cm and homogenized in a labelled plastic bag that was stored in the fridge until processing for analysis. This experiment only investigated the total soil C and N content of the soil.

Table 1. Timing and dates of soil sampling from the grubbed orchard

Soil collection points	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-month post grubbing	16.05.2022
3-month post grubbing	13.06.2022
4-month post grubbing	11.07.2022
5-month post grubbing	08.08.2022
6-month post grubbing	05.09.2022

4. Results

4.1. Commercial rootstock comparison

The mean total C (TC (%)), total organic C (TOC) total inorganic C (TIC) and organic matter for the bulk or rootzone soil samples at the three destructive harvests are shown in **Table 2**. The soil organic matter under the M.M.106 rootstock increased significantly in the rootzone soil between 13- and 19-weeks ($P = 0.04$). There was a significant increase in soil organic matter concentration under M.M.106 between the bulk soil prior to planting and the rootzone soil at 19 weeks ($P = 0.01$). M.M.106 had significantly higher concentrations of soil organic matter than those found under M.9 rootstock at nineteen weeks ($P = 0.04$). At the second destructive harvest of the M.M.106 trees at thirteen weeks soil TIC had significantly decreased in comparison to soil collected prior to planting ($P = 0.03$). Under the M.M.106 trees, soil TOC was significantly higher at nineteen weeks than samples collected prior to planting ($P = 0.01$). The total C in the soil under the three rootstocks did not significantly change over nineteen weeks of tree growth.

Table 2. Mean total soil C (%) and its fractions for three collection points for all rootstocks. Asterisks (*) indicate significant changes over time and blue triangles indicate significant differences between rootstocks at individual harvests.

Harvest, soil region and rootstock	Total carbon %	Total organic carbon %	Total inorganic carbon %	Organic matter %
Pre-planting - bulk soil	5.378*	4.386*	0.992*	7.56*
13 weeks post planting rootzone soil				
M.9	5.410	4.547	0.863	7.84
M.116	5.507	4.617	0.890	7.96
M.M.106	5.361	4.513	0.848*	7.78*
19 weeks post planting rootzone soil				
M.9	5.606	4.676	0.930	7.78 ▲
M.116	5.761	4.878	0.882	7.86
M.M.106	5.684	4.774*	0.910	8.26* ▲

At 13 weeks post planting soil active carbon was higher in rootzone soil samples than those found in the bulk soil prior to planting under all rootstock varieties. M.9 and M.M.106 rootstocks both exhibited significant differences in POXC concentrations between the two soil regions ($P = 0.02$ and $P = 0.01$ respectively), with rootzone being higher than the bulk soil. At 19 weeks post planting, there were no significant differences in the soil active carbon content between the three rootstocks. The concentrations of soil active carbon in the samples taken at the 19-week harvest had significantly decreased from those of the soil sampled prior to planting.

4.2. Scion-mediated effect on M.9 rootstock

Overall, there were limited significant differences between the five scions under investigation, but these were not consistent across all soil parameters measured, harvest points, or soil regions. The soil total C (%) (**Fig 4**) in the rootzone soil at the first harvest point in September 2021 was significantly higher under Gala in comparison to Cox's Orange Pippin. At the March 2022 harvest, Dabinett, Michelin and Cox's Orange Pippin had significantly higher concentrations of soil TC in both soil regions than Gala and Braeburn. By the final harvest in September 2022, all but four pairwise scion comparisons were significantly different from each other in the amounts of soil TC, these were COP and Dabinett, Dabinett and Michelin, Braeburn and Gala, and COP and Michelin.

Tree biomass C was assessed at each destructive harvest and the root total C, N and protein were analysed for the September 2021 and September 2022 destructive harvests. No significant differences were found in the root C, N, or protein content between any of the five scions. By September 2022, three out of the five scions had significantly lower for root TN (%) and protein (%) content relative to the September 2021 samples. These three scions were 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 $P = 0.001$).

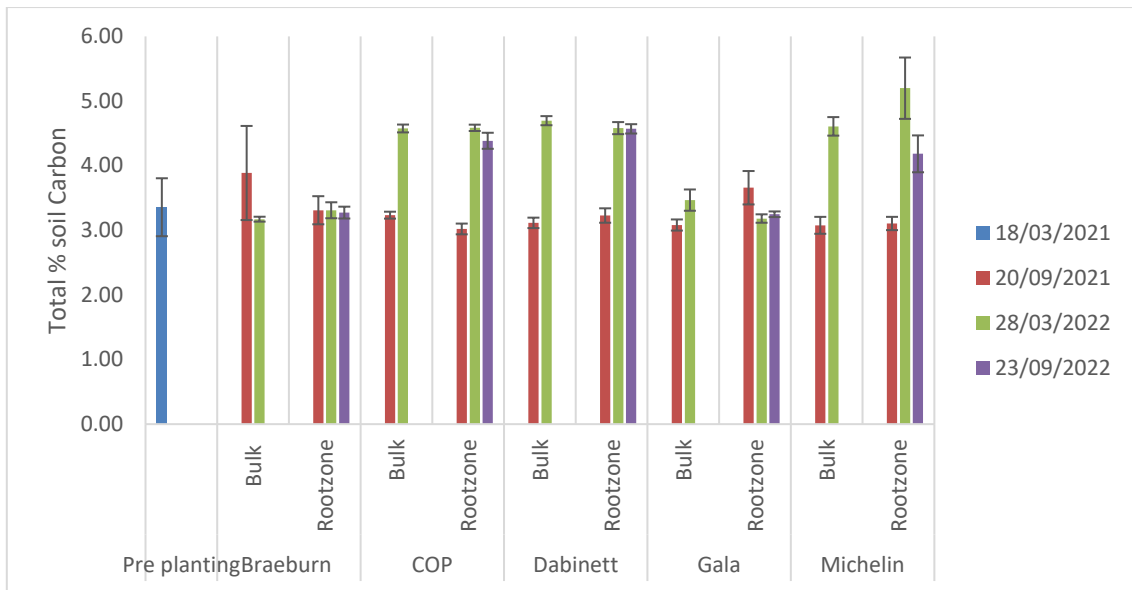


Fig 4. The mean total % soil C for the five scions and two soil regions at each harvest point, with standard error bars (n = 6 with exceptions of Braeburn, Gala in March 2022 and COP September 2022 where n = 5).

4.3. Effect of increasing atmospheric temperature.

Soil TC (%) significantly increased between September to December ($P < 0.01$) in the ambient tunnel. When comparing the three different temperature treatments, soil samples from the +2°C tunnel had a significantly higher content of TC than the ambient and +4°C tunnels at each soil collection time point. In December, the ambient tunnel was also significantly higher in TC content than the +4°C tunnel.

In September 2021, the soil microbial biomass C concentrations differed significantly across all tunnels, the +4°C tunnel had the highest and the ambient tunnel the lowest concentrations. Soil total N concentrations were significantly higher throughout the experiment in the +2°C tunnel compared to the other two temperature tunnels.

Table 3. Pairwise comparison of the overall P values of soil total carbon content of samples taken from tunnels at the four collection time points in 2021. Significant differences are indicated with an asterisk (*).

Soil collection point	Ambient v +2°C tunnels	+2°C v +4°C tunnels	Ambient v +4°C tunnels
March	P <0.001 *	P <0.01 *	P = 0.4
June	P <0.001 *	P = 0.001 *	P = 0.5
September	P <0.0001 *	P = 0.001 *	P = 0.2
December	P = 0.01 *	P <0.00001 *	P = 0.01 *

4.4. Orchard age

The relationship between soil C, N, bulk density, and nutrients to age, showed that soil TC, organic C, active C, and TN all significantly declined with orchard age, across the 23 orchards sampled. The most significant declines in soil concentrations of C and N were found in the youngest orchards (orchards 6 years old and under). However, soil bulk density and organic matter were significantly positively correlated to the age of the orchard.

Soil C and N concentrations were higher in the tree rows than those found in the alleyways but were only significant in the youngest orchards (under 6 years of age). In contrast, soil bulk density in the younger orchards (under 6 years of age) was significantly higher in the alleyways than the tree rows.

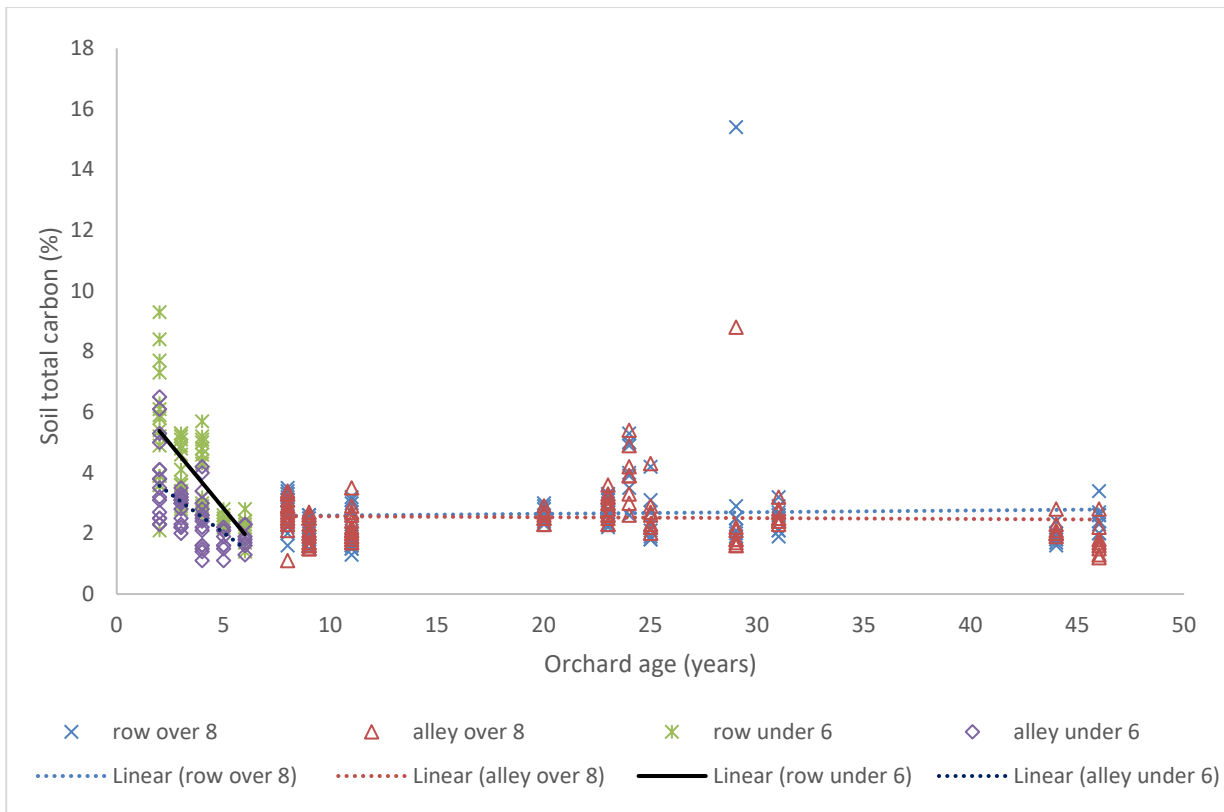


Fig 5. Soil total percentage carbon across all orchards, divided into two age groups (≤ 6 years old and ≥ 8 years old) for tree rows and alleyways.

Soil potassium was the only nutrient to have a significant positive correlation with the concentration of soil total C. The correlation between orchard age and soil total C differed between the three farm sites showed. At one farm, total C was positively correlated with age, whereas at the other two sites total C was significantly negatively correlated with age. Soil total C under the M.25 rootstocks (3 orchards) was significantly positively related to age, whereas the active and organic C were significantly negatively related to age. Soil total C, organic and active C were all significantly negatively related to increasing tree age under the M.M.106 rootstock variety (8 orchards). Under the three scion varieties, all soil C fraction concentrations and total N were all significantly negatively related to orchard age irrespective of farm site.

4.5. Fate of soil carbon after grubbing.

This preliminary study showed that soil total C (%) in the former trees stands declined significantly between 22nd March and 19th April 2022 (five weeks post-grubbing) (Ruben: $P = 0.04$ and Gala: $P = 0.01$). The soil TC 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 percentage C in the alleyways was significantly higher than in the former tree stands for both cultivars at the final soil collection, six months after grubbing ($P = 0.0001$).

The total soil nitrogen (N) increased significantly across the former orchard site throughout the sampling period from pre-grubbing to the day after grubbing (alleyway $P = 0.003$, Gala stands $P = 0.03$ and the Ruben stands $P = 0.01$). Soil total N in the alleyways was significantly higher than the soil under the former tree stands from May onwards ($P < 0.02$).

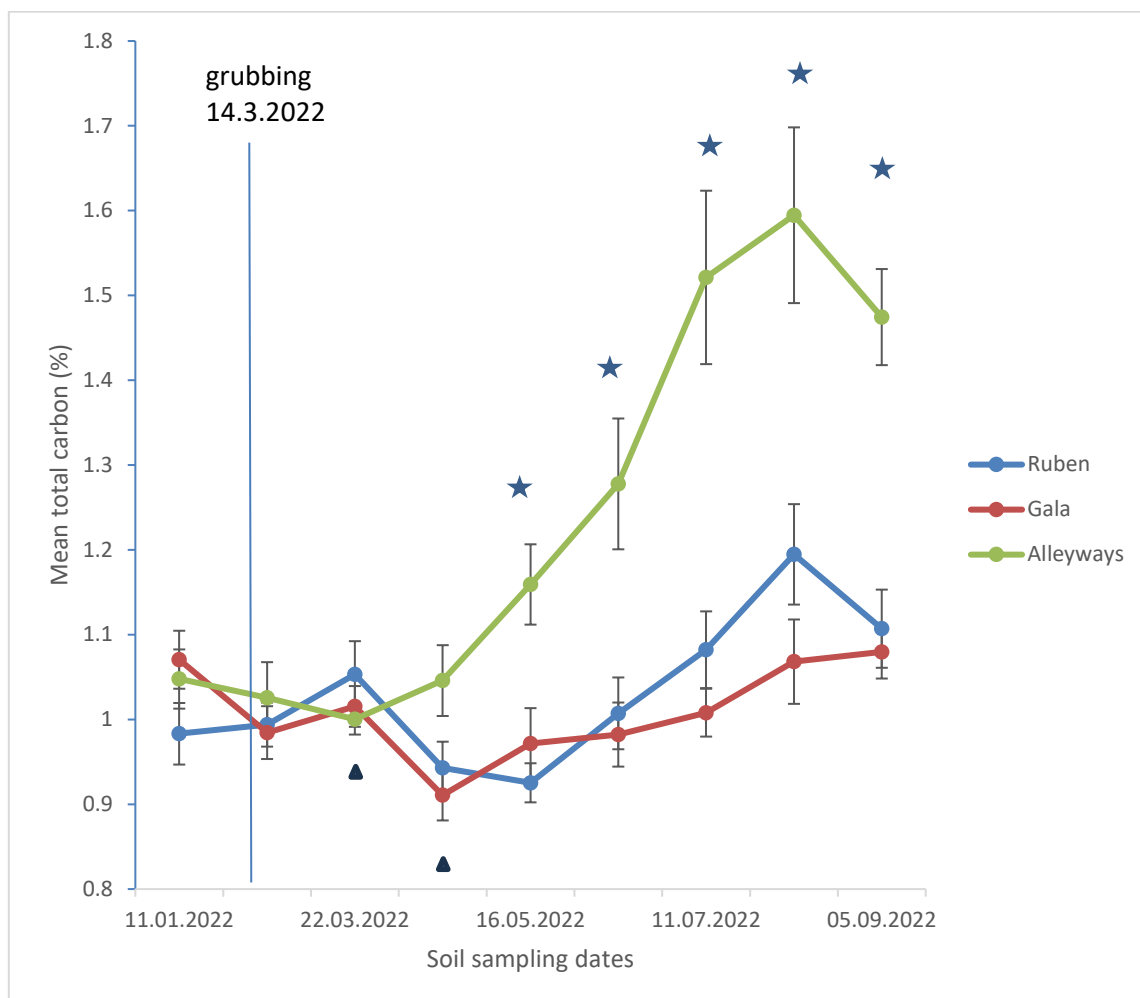


Fig 6. Mean soil total carbon (%) of the samples collected from the orchard pre- and post-grubbing in the alleyways, and the former Gala and Ruben tree stands from two months before grubbing (14th March 2022 (blue line)) to six months post grubbing (September 2022). Error bars indicate

standard errors and significant differences ($P < 0.05$) are indicated by asterisks (*) for comparisons between the alleyways and the former tree stands, and blue triangles indicate significant differences between sampling dates. There were no significant differences in soil total C between the Gala and Ruben tree stands.

5. Discussion, conclusion, and future work.

5.1. Discussion.

Soil C sequestration has been suggested as a potential method to mitigate rising atmospheric CO₂ concentrations since most soils have yet to reach their full potential of C storage, although the amount of C that can be stored in most soils does have 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 selected factors, (rootstock varieties, scion varieties, increasing atmospheric temperatures, orchard age, and grubbing), did show differing impacts on the potential amounts of C that apple trees can sequester. Rootstock and scion varieties showed little to no influence on the ability of apple trees to sequester soil C. In contrast, increasing atmospheric temperature by 2°C had 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, orchard age and grubbing both were associated with declining soil C concentrations.

Four out of five of these experiments were conducted over a period of time ranging from only four months to two years. Due to the time limitations, these studies can only indicate what might be happening, but cannot offer a comprehensive understanding of each factor's effects, highlighting the need for further studies. Several of the experiments (rootstocks, scions and increasing atmospheric temperature) indicated a strong seasonal fluctuation in soil C and N concentrations, highlighting the need to determine the best time of year to gain the most information regarding annual C changes and that sampling is carried out at the same time each year. 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 decline in soil microbial biomass C concentration at the September harvests in three experiments (rootstocks, scions and increasing atmospheric temperature) compared with harvests earlier in the year. The other two experiments could not support this trend due to differences in the timing of soil collections and the subsequent laboratory analyses.

All five of the experiments showed significant differences in the concentrations of soil C and N between the different soil regions, and 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). The results showed that the C in the rootzone under the trees had higher concentrations than the bulk soil of the rhizotrons or the alleyways in the field-based studies. This would be expected as tree roots release root exudates, dead roots and cells, and the soil surrounding the roots usually has higher abundance of soil microorganisms (Holz *et al.*, 2018; Canarini *et al.*, 2019). In two of the experiments (rootstock and scion comparisons) the soil samples were homogenised at the time of collection, before being analysed for soil TC and other C fractions, as well as soil N within the rhizotrons bulk and rootzone soil. This therefore did not account for potential variation across the soil C profile of the rhizotron. It has been suggested that C will be transferred within the soil 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).

Significant differences in both above and belowground biomass C of the trees were observed between varieties across the study periods in both the rootstock and scion experiments. This is consistent with previous studies on biomass C content of both fruiting and non-fruiting trees and would be expected as trees age and grow (Pettersson *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 trees, such as the fertigation of the rhizotrons (which was not investigated), and the time of year that the biomass is collected. In the scion experiment, trees were grown in pots with limited nutrient availability, which could have restricted tree growth. These pot-grown trees did not receive any fertigation and became pot bound by the end of the experiment.

The experiment investigating the effects of increased atmospheric temperatures indicated that an increase of 2°C could have a positive impact on the amount of C sequestered into the soil by all varieties of dessert apples included in this experiment, 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 reports that increased temperatures are detrimental to overall soil health and food production (Valverdi, Cheng and Kalcsits, 2019; Hartley *et al.*, 2021; Tiefenbacher *et al.*, 2021). Increasing atmospheric temperature would limit photosynthesis rates, thereby limiting the availability of C for tree growth, fruit production, and exudation into the soil (Moore *et al.*, 2021). The reduction in photosynthesis and exudation would potentially also limit the availability of soil trees to absorb nutrients for plant uptake due to a possible reduction in the population of soil microbes, which could lead to the plant to becoming weakened and may limit its productivity (Valverdi, Cheng and Kalcsits, 2019).

The results from the study of the effect of age on the soil carbon content were unexpected. They showed that the soil C and N content decreased with orchard age and that the most significant loss of C occurred in the first six years as the initial soil amendments were utilised. 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.*, 2012b; Yang *et al.*, 2021; Betemariyam and Kefalew, 2022).

The final experiment which investigated effects of grubbing on the stored soil C showed that after an initial C loss in the first two months following grubbing, soil C appeared to recover as vegetation colonised in the former tree stands. The initial loss of soil C was attributed to the soil disturbance caused by 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). However there is a potential opportunity to recoup some of the lost C by converting the grubbed biomass into biochar and its subsequent use as a soil C store/amendment (Jones *et al.*, 2011; Anthony, 2013; Tan *et al.*, 2020; Zhao *et al.*, 2020; Duan *et al.*, 2021). The soil C in the alleyways initially had a limited decline post-grubbing, but it subsequently increased during the period between one and five month following grubbing, 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).

This study is contributing new knowledge to deepen our understanding of what may be influencing the ability of apple trees to sequester C belowground, and whether apple orchards can be classed as C sinks, C neutral or a C source. Apple trees and other top fruit orchard crops are valuable economic crops and cover large areas of land across the globe with fruit tree production covering a total area of 1,295,407 ha in 28 EU countries in 2014 (Eurostat,2014). Previous research has shown that apple trees are good at sequestering C both belowground in the soil as well as in the

tree biomass (above and below-ground) (Scandellari *et al.*, 2016; Zahoor *et al.*, 2021). However, there may be a peak age at which maximum C sequestration level occurs before it starts declining.

With the Kyoto and Paris agreements both recommending that global C stores need to be protected and enhanced in order to mitigate against rising CO₂ and to keep global temperatures from rising to 2°C above that of pre industrial times, which is critical to ensure global food security (French, 1998; United Nations, 2018). Reports by Natural England (George, 2010; Gregg *et al.*, 2021), suggested that the planting of more trees (which could include apple trees), and conversion of land back into natural habitats will all be a positive way to mitigate climate change. Some 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 (Wang *et al.*, 2017; Koutika, 2022; Tian *et al.*, 2023; Duddigan *et al.*, 2024).

5.2. Conclusion.

In conclusion, this study has shown that different rootstocks and scions have little to no effect on the amount of C that an apple tree sequesters belowground. Both increasing orchard age and the grubbing process have a negative influence on soil C concentrations. However, in the grubbed orchard that was left fallow and allowed to return to being fully covered in grass the soil C concentration showed a potential recovery of lost C over time. A 2°C increase in atmospheric temperature had a positive impact on soil C concentrations, however at 4°C above ambient atmospheric temperature this became negative, showing soil C was being lost and had similar concentrations to those in ambient temperature tunnels. This suggests that belowground C sequestration abilities of apple trees appears to contradict the current thinking that they can aid in climate mitigation (increasing atmospheric CO₂ concentrations).

It is therefore crucial to consider these factors, along with others (e.g. soil type), for a comprehensive understanding when determining the belowground C sequestration ability of apple trees from pre-planting to the end of orchard 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 practices 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.

5.3. Future work.

The completion of this research has shown that there is still a need for further research to understand what is influencing 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 experiments on the rootstock and scion varietal effects in larger scale and longer-term studies would enhance the current findings, shedding light on the impact on the soil C as trees age.

Furthermore, research into the impact of varying atmospheric CO₂ levels and changing weather patterns, including the increased occurrence of winter flooding and summer droughts, is necessary. Given the reported global temperature increase in 2023 compared to previous years, understanding how these climatic changes affect photosynthesis and carbon sequestration is vital for developing more resilient agricultural practices (Met Office, 2024; NASA, 2024; NOAA, 2024). Understanding the impact of increasing atmospheric temperatures on the sequestration ability of trees, as explored in **Chapter 4**, is crucial. However, it is equally important to investigate how these global atmospheric temperature changes affect soil temperature and influence soil C stability and sequestration ability, and the soil microbial communities. This analysis would provide a comprehensive view of the intricate relationship between climate change, 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. Improving our understanding of C storage across different horizons could provide insights into the types of storage pools affected by sequestration.

The ability of apple trees to sequester atmospheric CO₂ should be investigated as a whole and not as separate storage compartments (e.g. soil, roots, or aboveground biomass) over their entire life span. Future studies need to determine soil C including the soil microbes and estimate tree biomass C before planting, as well as the C that is available in any applied soil amendments such as biochar, and what happens to the stored C in the aboveground biomass at grubbing, such as the loss of C through burning and the ash that is left.

Further information can be found in the PhD thesis.

6. References

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