Project title: Belowground carbon sequestration potential of apple trees

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Project leader: Prof Mark Tibbett and Prof Martin Lukac University of Reading and Dr Flora O'Brien and Dr Mark Else NIAB EMR

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Key staff: Catherine Chapman

Location of project: NIAB EMR and the University of Reading

Industry Representative: National Association of Cider Makers and Worldwide Fruits LTD

Date project commenced: October 2019

DISCLAIMER

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Catherine Chapman

PhD student

University of Reading and NIAB EMR

Signature Catherine Chapman Date 5th November 2021

[Name]

[Position]

[Organisation]

Signature .... Date ....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

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GROWER SUMMARY

Headline

Can apple orchards help to mitigate rising atmospheric Carbon dioxide levels?

I am investigating the attributes that may affect belowground carbon sequestration of apple trees. This includes differences between commercially used rootstock, scion-rootstock interactions, increased temperature on scion-rootstock interaction, tree age and carbon levels in the soil between alleyway and tree stands, and what happens at the end of orchard life to the soil carbon post grubbing up.

Background

Climate Change is affecting global weather patterns, resulting in predicted temperature increases and droughts and flooding episodes, this will put greater pressure on food production. Greenhouse gases (GHGs) in the atmosphere are still rising; in May 2021 the level of carbon dioxide (CO_2) in the atmosphere had risen to 419.05ppm, representing an increase of over 26% since June 1969 when it was 326.76ppm (noaa.gov). The UK signed up to the Kyoto protocol (UNFCCC, 2014) where they agreed to reduce global carbon (C) emissions, this was followed in 2015 by the Paris agreement (*UNFCCC*, 2015) in which 196 countries, committed to keep the global temperature rise below 2^{o}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 put in a legal frame work 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 CO_2. The Industrial Strategy Challenge Fund representing a major part of the strategy to achieve this.

Perennial crops such as apple trees could help mitigate rising atmospheric CO_2 levels through sequestering C belowground via the roots into the soil. Soil is the second largest active C cycling pool after the oceans (Fry et al., 2018), and it is believed that soil can store more carbon as it is not at full capacity (Stewart et al., 2007). Ledo et al (2020) found that approximately 30% of land is covered with perennial crops such as apple orchards, they suggest that perennial crops over their life time become carbon zero if not C negative as they continually absorb and store carbon as the soil is not being disturbed and releasing CO_2 back into the atmosphere.

Current rootstock breeding has promoted carbon uptake by the fruit, thereby limiting the amounts sent to the roots and out into the soil. Currently, it is not known if any particular apple rootstock has a greater ability to store C below ground, and this is what this study aims to determine. The amount of C that a tree can sequester varies dependent upon tree types (such as fruiting trees, other non-fruiting deciduous and evergreen trees) and the partitioning of carbon in different compartments, such as above ground in stems, branches, and fruit, or belowground in roots or released via exudation into the rhizosphere. This can also be affected by microbial (bacterial and fungal) communities in the rhizosphere, 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 all have an impact on the rate of photosynthesis, growth, storage, and production as well as on the soil microbial processes and GHG emissions. In a climate experiment that is currently being carried out at the National Fruit Collection at Brogdale in Kent, UK (*Climate change could alter the face of apple growing in Britain*, 03/10/2018), 20 different dessert apple varieties grafted onto M9 337 rootstock, are being grown under six environmental conditions to see what effect increased temperature and changes in rainfall have on the growth, flowering, fruiting and fruit storage of the apples. Currently, they have no plans to look at belowground carbon sequestration.

Carbon that has been sequestered in the soil can be released back in to the atmosphere as CO_2 via soil respiration which can increase as a result of disturbance e.g. tillage (Schlesinger & Andrews, 2000). Dessert orchards have a commercial life span of 15-20 years, whereas cider orchards are between 50-80 years, after which they are grubbed up, which causes soil disturbance, and the aboveground growth is generally burnt.

This project aims to see what affects the ability of an apple to sequester carbon belowground, such as root system architecture via different rootstocks sizes, scion-rootstock interaction, temperature and watering and other factors.

Summary

In my first year of work, I set up a short-term glasshouse experiment to investigate if there was a difference between three UK commercially used apple rootstocks (M9, M116, and MM106) in the amount of carbon they sequester both in the roots and the soil. The rootstocks were all grafted with Cox's Orange Pippin to avoid the influence of the scion-rootstock interaction and were grown in 1 meter x 30cm x 3cm rhizotron boxes in fumigated soil. Soil samples and images were collected at monthly intervals and 12 trees were harvested every six weeks, with the final 18 trees being harvested 19 weeks after planting.

Root samples were sent away and showed that nitrogen levels were the same across the three rootstocks, but carbon levels were higher in the larger rootstocks. Soil carbon levels significantly increased over the experiment in the two soil regions under investigation (Bulk and Root zone (1cm around the root)).

This year I have set up two experiments one based at NIAB EMR and the other at the National Fruit Collection at Brogdale Farm in Kent.

The first of these aims to determine whether scion-rootstock interactions affect the levels of C stored belowground. This experiment takes place in the poly-tunnels and glasshouses at NIAB EMR for up to 18 months. I am using 90 trees; 60 trees are being grown in pots (which are in the poly-tunnels) and 30 in rhizotrons which are in the glasshouse. It uses 3 commercial dessert apple varieties (Cox's Orange Pippen, Braeburn and Gala) and 2 cider apple varieties (Dabbinett and Michelin) all of which are grafted on to the same rootstock (M9).

The second experiment looks at the effect of increasing temperature on scion rootstock interaction on belowground C sequestration. This experiment will run for 2 years and uses the climate change experiment running at Brogdale for the past 5 years, funded by the National Fruit collections Trust. The trees are planted directly in the ground and covered by poly tunnels. One of the tunnels is open sided to maintain the outside temperature while being slightly protected from the weather conditions. In contrast, the other two are fully closed and vented when temperatures exceed +2^{o}C and+ 4^{o}C. They have about 20 different dessert apples all grafted on to M9 rootstocks, out of these I have chosen to use 8 varieties for my experiment (listed below) with 6 trees per tunnel per variety (153 samples in total including 3 alley ways).

Table 1: Show the varieties of apples that I am collecting soil samples from underneath.

Scion variety	**Scion variety**	**Scion variety**	**Scion variety**
Gala	Braeburn	Cox's Orange Pippen	Winter Pearmain
Discovery	Tropical Beauty	Brambly seedling	George Cave

I am taking soil samples under the trees and alley ways every three months to see how carbon sequestration alters seasonally within the root zone of the tree (30cm from the tree trunk), as this will show root exudate carbon in the root zone. These samples will be taken to the lab for a variety of tests to determine carbon levels, including total nitrogen/carbon/ inorganic and organic carbon levels, microbial biomass carbon.

I have a further two experiments planned to begin this winter and through next year. They are comparing soil C levels associated with different ages of apple trees and determining what happens to the stored C after the orchard has been grubbed up at the end of the orchard's commercial lifespan. These experiments will just be looking at the total carbon and nitrogen levels in the soil.

Financial Benefits.

There are currently no financial benefits from this project at this stage.

Action Points

There are currently no action points at this stage.

SCIENCE SECTION

Introduction

Soil is a highly complex and sensitive environment that needs to be carefully managed to provide food security for the future in the current changing climate. The soil is a vital and yet an under-used store for C that could help with mitigation against rising levels of atmospheric CO_2 and other GHGs. Soil is the largest terrestrial store of carbon (Fry et al., 2018). With atmospheric CO_2 still rising rapidly, it is important to find and improve ways to mitigate against changing climate and increased occurrences of flooding and droughts. Perennial crops like apple trees use atmospheric CO_2 during photosynthesis. The carbon that the tree gains during the photosynthetic process is converted into energy for growth (above and belowground), fruit production and excess released into the soil via exudation.

Apple rootstocks are the foundations of apple production, providing multiple functions for the tree, including, and most importantly, anchoring the tree into the soil. The root system takes up nutrients and water from the soil and transports them to the leaves and fruit via the vascular network. In reverse, the photosynthesis in the leaves supplies the roots with energy in the form of carbohydrate-containing photosynthates, some of which are released into the soil as root exudates. Root exudates have been traditionally classed into two groups. The first group, which is believed to make up most root exudates, is the low molecular weight compounds that include amino acids, organic acids, sugars, phenolics, and secondary metabolites. The second group is the high molecular weight compounds such as proteins and polysaccharide sugars (mucilage). Roots exudates are an important and significant route for plant-derived C to enter the soil.

The aims and questions I would like to answer for my PhD project are:

Aim:

To investigate the attributes that increase or inhibit the potential of apple trees to sequester carbon belowground.

Research questions:

1. Are there significant differences between commercially used rootstock in the amount of C that is sequestered belowground?
2. Do the rhizosphere soil microbial communities vary significantly under different rootstocks?

3. Does the scion-rootstock interaction affect the amount of C being sequestered belowground?
4. How will increased temperatures affect apple trees ability to sequester C belowground?
5. How does grubbing up affect the level of C sequestered belowground during an apple tree's lifetime?
6. Does soil C content differ significantly with orchard age?

In my first experiment I focused on the first two questions to determine if any commercially used rootstock sequesters increased levels of belowground carbon than the others and whether there is a difference in the soil microbial communities.

The second experiment which is in progress is focused on investigating question 3 regarding the influence of the scion has on the M9 rootstock for carbon sequestration into the soil.

The third experiment is running alongside of experiment 2 and is looking at trying to answer question 4 but can also include question 3. This is also running over 2 years.

I am currently planning two further small-scale experiments to investigate the last two questions; of which I hope to start this winter.

Materials and methods

Experiment one: Rootstock effect on belowground Carbon sequestration**.**

This experiment was conducted at NIAB EMR, Kent in a glasshouse using the three rootstocks most commonly used commercially in the UK (M9, M116 and MM106). The rootstocks were all grafted with Cox's Orange Pippin to remove any effect of scion-rootstock interactions. These rootstocks were planted into rhizotrons (1 meter x 30cm x 3cm) to allow monitoring of the root growth over the experimental period of four and a half months. The rootstock varieties were randomised across the compartment to allow for heat, cooling, and sunlight. The rhizotrons were all watered daily via drip irrigation that also incorporated a general-purpose feed. The volume of water they were given varied through the growing season according to changes in water uptake, which were assessed by weighing the rhizotrons at 24 hours intervals. A total of 54 trees were used in this experiment (18 per rootstock) in addition to four soiled filled "blank" rhizotrons. At the start of the experiment, bulk soil samples were taken, and 12 trees (four of reach rootstock) were randomly selected to be used for base-line assessments. The remaining 42 trees were planted into the rhizotrons. The plants were monitored for bud/leaf break, flowering, and overall tree health (pests and pathogen symptoms).

Figure 1 Apple trees growing in rhizotrons in the glasshouse at NIAB EMR.

<u>Imaging and soil samples</u>

Root system images were taken monthly using a camera rig, and soil samples were collected at three harvest points. The soil collected at the three harvest points comprised bulk, root zone (1cm around the roots), and rhizosphere soil (soil attached to roots was brushed off post-harvest). The rhizosphere soil samples were stored in the freezer (-20°C) before being used for DNA extraction. The root imaging was also before planting, and at harvest once all the soil was removed. A ruler was included in each photo to allow for measurements to be made. These images are analysed using the SmartRoot plug-in in Image J to measure primary and secondary root length and to monitor changes in the root system architecture (RSA)

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Figure 2 Bare roots of grafted tree before planting into rhizotrons

Root systems were carefully excavated by hand to maintain as much of the root system intact as possible, and any roots that broke off were placed into labelled bags. These were washed in tap water and root samples were taken to assess arbuscular mycorrhizal fungi (AMF) colonisation later. The roots of trees harvested at the end of the experiment were collected and sent to Forest Research for total C and nitrogen (N) analysis. The remaining roots were placed in an oven and dried over 3 days at 60°C to determine the dried biomass to estimate of the carbon content based on the total C/N analysis results.

<u>Soil laboratory tests</u>

Soil DNA was extracted using the Qiagen DNeasy® Powersoil® kit following the manufacturer's protocol. Soil DNA extracts were also taken from the fumigated soil before planting to give a baseline microbial community for the soil. Baseline bulk soil DNA extractions (pre-planting) and the rhizosphere samples from the endpoint of the experiment of sufficient quality and quantity will be used for sequencing of the 16S and ITS rRNA genes to compare the bacterial and fungal communities associated with the different rootstocks.

To calculate the gravimetric soil moisture content, a sample of bulk and root zone soils was dried for 48 hours at 105°C.

Soil samples from both bulk and root zone were subdivided into two, with one half fumigated in chloroform for soil microbial biomass C determination. The Fumigated and unfumigated soil samples were then extracted in a solution of potassium sulphate (0.5M K_2SO_4). The soil extracts were then used for colorimetric-based determination of plant-available ammonia and nitrates via spectrophotometry. Permanganate oxidisable C content of both fumigated and non-fumigated soil will be performed. Roots and soil samples from the final harvest were sent to Forest research to be analysed for total C/N levels. The soil samples also had the levels of total organic and inorganic carbon analysed. All these results will help provide an indication of the effect of the different rootstocks on the C content and C/N ratio of the soil during a single growing season.

Experiment 2: Scion-rootstock interaction on belowground carbon sequestration.

This experiment into the effect of scion-rootstock interaction is being carried out at NIAB EMR, Kent over a 2-year period (started March 2021) in a mixture of glasshouses using both rhizotrons to monitor root growth differences and poly tunnels with 3 litre pots.


Figures 3 and 4: Experimental set-up in the glasshouse and poly tunnel with the trees randomised across the boxes and frames.

Three desert scions – Cox's Orange Pippen following on from the experiment conducted last year, Gala and Braeburn, and two commonly grown cider apples Michelin and Dabbinett are being used in this experiment. All the scions were grafted on to M9 rootstock, which was selected as it is the most widely used commercially rootstock for the dessert apple industry. Although it is not generally used with cider apples, it was important to incorporate these apples as they are grown in the UK in the highest numbers compared to the rest of the world. Six replicates of the 5 scions will be destructively harvested at 3 points over 18 months. Trees are being crop walked weekly to monitor tree health (e.g., pest or diseases incidences) and phenological stages. Fifteen of the trees that are being grown pots are being monitored for their soil moisture content using Decagon 10HS soil sensors and Decagon Em50G remote loggers.

In this experiment, 30 trees will be harvested every 6 months to see how C may be increasing in both the bulk and root zone soils and tree roots. The rhizotron grown trees have now been harvested, and roots have been collected for total C analysis.

<u>Soil laboratory test and root analysis</u>

The root system photographs will be analysed using Image J and the Smart Root plug-in to determine the overall root length and to see if there was a scion effect on the amount of root growth during this season.

5grams of roots from half the trees harvested this year were collected to test for total C/N in the roots. This will be repeated for the next two harvest points (March and September 2022) to see if and how the C changes within the roots.

The soil tests will be the same as for experiment one at each harvest point. This will include total N/C/inorganic and organic C levels, soil microbial biomass C, pH levels, and soil nitrate and ammonium levels. A baseline for each of these will also be assessed from before the trees were planted in March 2021.

Experiment 3: Effect of temperature on belowground C sequestration

This is being conducted at the National Fruit Collect, Brogdale, Kent, using the climate change experiment being run by the University of Reading. Their experiment looks at the effect of increased temperature, and an alteration in the levels of rainfall has on 20 different grafted scions on M9 rootstocks. This experiment looks at the effect of increased temperatures has on the different scion's ability to sequester C into the root zone soil. The temperatures under investigation are ambient (in an open-sided poly-tunnel) and increase of plus 2°C and 4°C that are in completely closed tunnels.

I have selected 8 varieties (Cox's Orange Pippen, Gala, Braeburn, Discovery, Tropical Beauty, George Cave, Brambly seedling, and Winter Pearmain) to study over the two years. Cox's Orange Pippen is being used as a standard to compare against. Soil samples are being collected with a hand corer within the 30cm of the tree trunk to stay within the influence of the root system at depths of 20 and 30cm to reduce the likelihood of roots from grass and weeds influencing soil carbon levels. However, some grass species roots will get down to this depth.

Soil sampling occurs every three months to determine whether seasonal soil C levels occur over the two years of sampling. After taking the soil core, the excess soil is replaced, and a marker is placed to avoid disturbing the same area at a later sampling date.

Figure 5: Climate change experiment poly tunnels at Brogdale (first tunnel is the +2°C above ambient, next is the +4°C above ambient and the final is the ambient tunnel)

Soil laboratory tests

Once it has been transferred to the laboratory, the soil is checked for any stones which are then removed. The soil samples are then weighed, and the gravimetric soil moisture content is measured. After the first soil collection, the soils water holding capacity (WHC) was determined as well as the soil pH across the three tunnels in the allies and under the tree. The Laboratory tests will include soil total C/N/ inorganic and organic C, microbial biomass C, nitrate, and ammonia levels.

Experiment 4: End of orchard life carbon

This will be up to a year's study based at NIAB EMR, Kent looking at what effect grubbing up an orchard and the soil disturbance has on the sequestered soil carbon levels. The orchard being sampled was growing two varieties of apple (Ruben and Gala) on M9 rootstocks. The orchard site will have regular hand-cored soil samples of 18 former tree stands and 18 alleyways to cover both varieties, and a set of samples will be collected about a week before grubbing occurs to provide a base line for the soil C levels.

Soil Laboratory tests

The laboratory tests will include the soil texture, soil pH, total C/N/organic and inorganic C levels.

Experiment 5: How does the age of the orchard influences soil carbon

This is a one-off sampling across a range of different aged orchards to determine the percentage difference of bulk soil C between alleyways and under the trees. This is to determine how much soil C levels increase over the whole of cider orchard's commercial life as they are longer in the ground than the dessert apple orchards and are also grown on larger growing rootstocks. This project will be carried out in 2 or 3 the cider apple farms of Somerset this winter.

Soil Laboratory tests

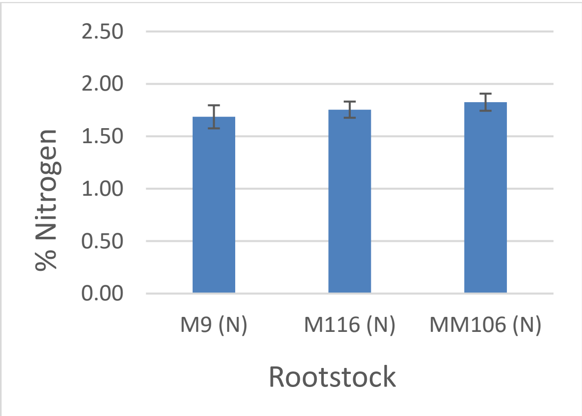
The laboratory tests will include the soil texture, soil pH, total C/N/organic and inorganic C levels.

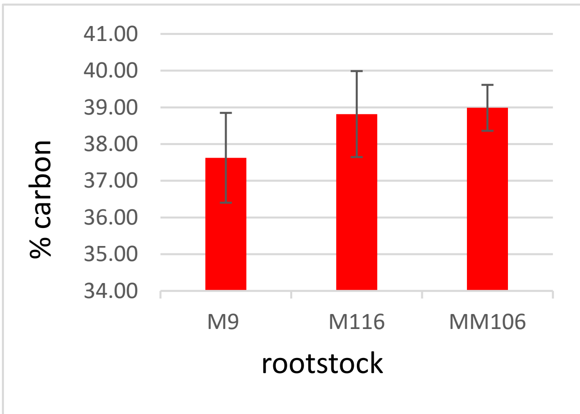
Results

Currently, I am still processing root images for the first experiment, but from some of the preliminary data, there was no real effect on the rootstock on leaf bud break above the graft union across the three rootstocks, but there were two graft unions that failed to grow.

The soil's pH did change both in the bulk soil and from the root-zone (1cm around the roots) very slightly but nothing significant from the initial soil collected from before the trees were planted from 6.8$CaCl_2$ in March 2020 to 6.9$CaCl_2$ for the root-zone and 7.1$CaCl_2$ in September 2020 in the bulk soil.

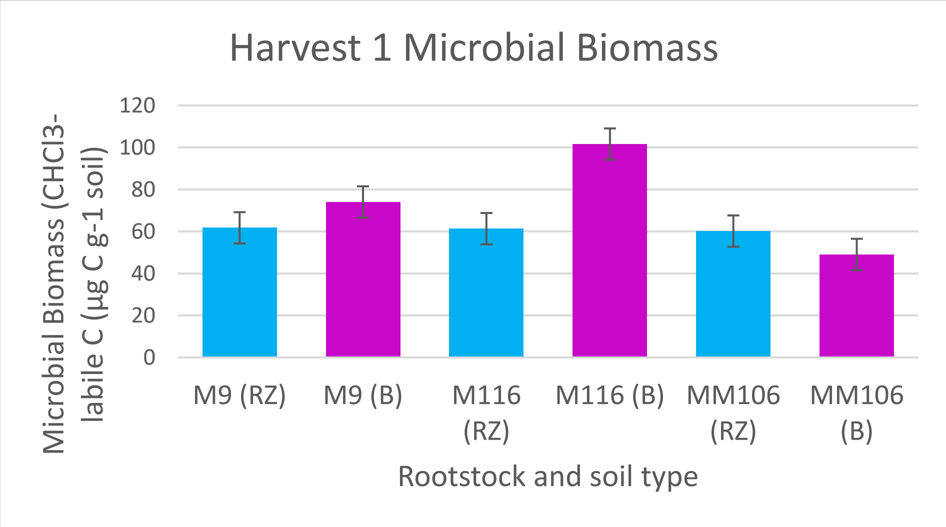
Root samples from the final harvest in September were sent away for analysis at Forest Research for the total C/N levels. Data shows that there is no significant difference between the rootstocks for N levels as shown in the graph below. Carbon, on the other hand shows that the larger two rootstocks (M116 and MM106) had increased levels of C but whether this is significant about further statistical work is needed.

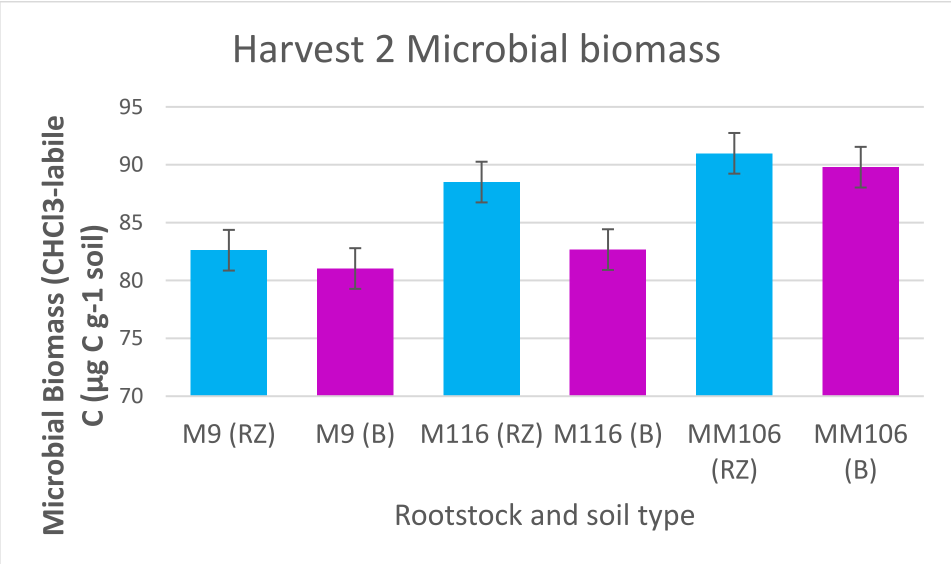




Figures 6 and 7: Root nitrogen and carbon levels for the roots collected at the end of the growing season.

Data from the soil microbial biomass assessments for the first two harvest time points shows fluctuations between soil regions and rootstocks and harvests. MM106 both soil regions increased in the levels of soil microbial biomass found. In the M9 rootstock again both soil regions increase but not as dramatically as the largest rootstock MM106. The root-zone levels increase with the M116 rootstock, but the bulk soil decreases in the amount of microbial biomass found.





Figures 8 and 9: Microbial biomass carbon for the first and second destructive harvests.

Experiments 2 and 3 are currently being processed and further data still to be gathered now, no results are ready to present.

Discussion and Conclusions

For a short 4 and half months of growing there doesn't seem to be a significant difference in the levels of carbon that the 3 commercial rootstocks sequester. This may change as the trees grow older due to the differences in rootstock size, but this would need a long-term study which is not in the remit of this PhD.

Experiment 2 and 3 are ongoing with sampling, laboratory analysis and data gathering so no conclusions are available.

Knowledge and Technology Transfer

- Presented a poster of my project at the AHDB student conference
- Presentation to the GCRI trust

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