Project title: Belowground carbon sequestration potential of apple trees

Project number: CTP_FCR_2019_5

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

Worldwide fruits LTD

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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

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GROWER SUMMARY

Headline

A short-term (4 and a half months) trial looking at the difference in carbon sequestration of commercially used rootstocks in the UK.

Background

As climate change affects weather patterns globally, with predicted rises in temperature, and an increase in the occurrence of severe droughts and flooding episodes this is expected to have a major impact on global food production. Greenhouse gases (GHGs) in the atmosphere are still rising, in June 2019 the level of carbon dioxide (CO_2) in the atmosphere had risen to 413.92ppm, representing an increase of about 26% since June 1969 when it was 326.76ppm. 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) which 196 countries, agreeing to keep the global temperature rise below 2^{o}C and commit to further discussions on ways 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 emission by 2050 (*GOV.UK*, 2019). Consequently, the UK government has put money aside to help mitigate and remove CO_2 from the atmosphere, with 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 carbon belowground via the roots into the soil. Soil is the second largest active carbon cycling pool after the oceans (Fry, De Long and Bardgett, 2018), and it is believed that soil is currently able to store more carbon as it is not at full capacity (Stewart *et al.*, 2007). Current rootstock breeding has promoted carbon uptake by the fruit, thereby limiting the amounts being sent to the roots and out in to 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 where in the tree carbon is stored, such as above ground in stems, branches, and fruit, or belowground in roots or released in to the rhizosphere. This can also be affected by microbial (bacterial and fungal) communities in the rhizosphere, which can help 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.

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), 15 different dessert apple varieties grafted onto M9 337 rootstock, under six environmental conditions to see what affect increased temperature and changes in watering have on the growth, flowering, fruiting and fruit storage of the apples. Currently they have no plans to look at belowground carbon sequestration. 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. This PhD intends to investigate the factors which effect the ability of an apple to sequester carbon belowground, such as root system architecture via different rootstocks sizes, scion- rootstock interaction, temperature and irrigation and other factors.

Summary

In the first year of this PhD a small short-term glasshouse experiment was set up 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 high 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 at the end of September. Soil and root samples are still being processed in order to determine any differences in carbon levels using a variety of laboratory techniques

Financial Benefits

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

Action Points

There are currently no action points at this early stage in the project.

SCIENCE SECTION

Introduction

Apple rootstocks are the foundations of apple production, providing multiple functions for the tree including, 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 the majority of roots 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 carbon to be lost from plants and to enter the soil.

Soil is a complex but fragile environment that needs to be carefully managed in order to provide food security for the future in the current changing climate. The soil is a vital and yet an under-used store for Carbon that could help with mitigation in the fight against the rising levels of atmospheric CO_2 and other greenhouse gases.

Many different techniques are used to study rootstocks. These have included observational techniques such as excavating the full root system, trenching, and installing glass panels (root windows, or rhizotrons) or tubes at the root-soil interface to see how the roots grows *in-situ* within orchards. Specialist underground facilities have been built that can be used to study root systems without disturbance to the root system as the trees are planted within the facility such as rhizotrons/lab (tunnels). One such ‘Rhizo-lab’ can be seen at The National Root Laboratory at NIAB EMR in East Malling, Kent. Rhizotrons, or rhizoboxes, can also be purpose built boxes made from Perspex or glass that are usually very thin, approximately 3cm wide which the tree is planted into. This forces the roots to grow next to the clear panel in order to be visible for study and photographed. These methods can have their issues. Roots can be damaged by digging trenches and clear panels/tubes into the soil after the tree has been planted, while rhizotron boxes limit the root spread so altering the growth of the natural architecture of the root system. Studies have also looked at DNA of the rootstocks to understand dwarfing, pest and disease resistance and their ability to influence scions. Few of these have looked at them to determine their ability to sequester carbon in to the soil and longer term storage, and this is the starting point of my PhD project.

The aims and objectives of my PhD project are:

- To determine if any apple rootstock has a greater ability to sequester more carbon below ground than others
- To compare dessert and cider apple varieties on commercial rootstocks (M9, M116, MM106), to see what different roots stock architecture has on carbon storage ability
- To see how scions influence rootstocks architecture and carbon storage ability
- To see what effect extremes of weather could have on rootstock architecture and the ability of the tree to sequester carbon

In this first experiment I will be focusing on the first aim of trying to determine if any commercially used rootstock sequesters increased levels of belowground carbon than the others.

Materials and methods

This experiment was conducted at NIAB EMR in Kent in a glasshouse using the three rootstocks most commonly used commercially in the UK (M9, M116 and MM106). This experiment was a month and a half shorter than originally planned due to Covid- 19 restrictions. The rootstocks were all grafted with Cox's Orange Pippin to remove any effect of scion-rootstock interactions. These rootstocks were planted into rhizotrons 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 variations in environmental conditions. 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 over 24 hours. A total of 54 trees were used in this experiment (18 per rootstock) in addition four soil filled "blank" rhizotrons. At the start 12 trees (four of reach rootstock) were randomly selected from the 54 trees to be used for base line assessments. Before planting soil was collected to provide carbon and DNA base lines.

Figure 1 shows the apple trees growing in rhizotrons in the glasshouse.

Imaging and soil samples

Root system images and soil samples were collected at monthly intervals from the point of planting and at each harvest point. The soil samples that were taken each month comprised bulk soil that was collected from various spots up the length of the rhizotron. The soil collected at the three harvest points comprised bulk, root zone (1cm around the roots) and rhizosphere soil (soil attached to roots that 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 performed monthly using a camera rig, 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 will be analysed with Image J to measure primary and secondary root length and to monitor changes in the root system architecture (RSA).

Figure 2, show the bare root before planting

Figure 3, example of root growth within the rhizotron at the final harvest

Figure 4, imaging frame with rhizotron.

Root systems were carefully excavated by hand to maintain as much of the root system on one piece and any that broke off were placed into labelled bags. These were washed and root samples were taken for later assessment of both arbuscular mycorrhizal fungi and staining of cellular structures. The roots of trees harvested at the end of the experiment were collected and sent to Forest Research for total carbon and nitrogen analysis. The remaining roots were placed in an oven and dried over 3 days at 60 °C to determine the dried biomass to provide an estimate of the carbon content.

Soil laboratory tests

Soil DNA extractions will be conducted to help determine what soil born bacteria, fungi and other microbes were present. This will give an indication of carbon-based life in the soil. Soil DNA and Nano-drop testing has been carried out on the fumigated soil before planting to give a baseline for the soil. The rhizosphere and root zone soils collected at tree harvests will be extracted and then the nano-drop will give an idea of the amount of DNA that is within the soil (this can provide an indicator of the soil microbial biomass), only samples of sufficient quality and quantity will be used for sequencing of the 16s and ITs.

A sample of bulk and root zone were dried for 48 hours at 105 °C to calculate the soil moisture content, I have only done these two soil samples as the amount of rhizosphere that I could collect is very small.

Soil samples will be split into two, those that will be fumigated, and those which will not be fumigated and then extracted in a solution of potassium sulphate. The reason for testing both fumigated and non-fumigated soils allows for the determination of microbial activity in the levels of carbon that the soil contains. From this point samples are going to be tested to determine the levels of ammonia and nitrates via colour spectroscopy. I will also be using testing for oxidisable carbon content of both fumigated and non-fumigated soil. All of these results will help provide information about the carbon content and the carbon/nitrate ratio of the soil through this short growing season.

Results

Work on-going. Currently no results to present as still processing soil, root samples and root images.

Discussion and Conclusions

Work on going. Conclusion and discussion will be possible once the results are available.

Knowledge and Technology Transfer

- Presented my project at the AHDB student conference
- Presented a poster at the NIAB EMR/AHDB tree fruit day
- Presented a power point of my project to the Tree Fruit Panel
- Produced an industrial update of my project for the NACM (September 2020)

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