Project title:	Understanding Resilience of Soil Beneficials to Combat Apple Replant Disease
Project number:	SF/TF 170: CTP_FCR_2018_5
Project leader:	Louisa Robinson-Boyer, NIAB EMR., Naresh Magan, Cranfield University., Xiangming Xu, NIAB EMR.
Report:	Annual report, October 2020
Previous report:	Annual report, October 2019
Key staff:	Chris Cook
Location of project:	NIAB EMR, Kent and Cranfield University, Bedfordshire
Industry Representative:	N/A
Date project commenced:	October 2018

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

Chris Cook

PhD Student

NIAB EMR

Signature ..... Date .....

Louisa Robinson-Boyer

Researcher in Pest & Pathogen Ecology

NIAB EMR

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

Effect of climate change stresses (CO_2 and temperature increase) on bulk soil microbiome populations over a 5 week period.

Background

There is very little information on how abiotic factors may impact the prevalence of apple replant disease (ARD). The IPCC report suggest an increase in temperature of between 2-4^{o}C by 2050. Atmospheric CO_2 exposure is expected to increase by at least two times of the current levels (400 vs 800-1000 ppm CO_2) (Intergovernmental Panel on Climate Change, 2014).

Elevated temperature has a profound impact on microbial activity and this reaches an optimum at around 30^{o}C and then declines at >30-35^{o}C quite rapidly (Figure 4; BÁRCENAS-MORENO *et al.*, 2009), however soil temperatures, particularly deeper soils, will be unlikely to reach these values in temperate climates. Increased temperature will also lead to increased drought stress if increased water is unavailable. Root length colonisation (RLC) by AMF is increased in drought conditions, exhibiting a shift in the reliance on the mutualistic fungus in dry conditions. This reliance is due to hyphal spanning of air gaps between shrinking roots and soil, increasing water absorption of the target plant (Robinson-Boyer *et al.*, 2009) (Augé, 2004). Drought stress genes also are supressed when inoculated with Rhizobacteria microorganisms in Arabidopsis (Zolla *et al.*, 2013) highlighting the importance of the interaction between beneficial microorganisms and plants in an increasingly stressful climate.

In conditions of elevated atmospheric CO_2, soil organic carbon degradation increases showing how carbon sinks may become carbon sources, increasing global warming (Carney *et al.*, 2007). This change was attributed to increased relative abundance of fungal populations and increased activity of soil organic matter degrading enzymes. Increased CO_2 concentrations also increase soil bacterial diversity initially but the bacterial populations decrease exponentially as atmospheric CO_2 concentrations increase from < 5000 to > 10,000 ppm (Ma *et al.*, 2017).

In this experiment, we aim to understand whether and, if so, effect climate change stresses (namely increased temperature and CO_2 elevation) will have on the bulk soil microbiome of apple orchards. We will be running short term trials exposing bulk soils from both an organic and conventionally managed plot to extreme CO_2 and temperature increases. Next

generation sequencing techniques were used to see the impact on soil microbiome populations due to the climate stresses both individually and in consortium.

Summary

In this study, short term work over a 5 week period was conducted to assess the impact of extreme CO_2 and temperature increase on soil microbiome populations using sequencing technologies to compare diversity of fungal and bacterial populations. Bulk soil cores were exposed to increases CO_2 concentrations and temperature increase then populations compared between treatments. The results indicated CO_2 concentration increase did not significantly impact bacterial or fungal diversity in the soils. A temperature increase of 4ºC lowered fungal diversity but did not significantly affect bacterial diversity. Site management highlighted a 50% reduction in diversity on an organic orchard compared to a conventionally managed orchard. Further work on population and functionality differences in apple microbiome will be conducted to supplement this data. These experiments will be cross-referenced with long-term growth data to demonstrate a comprehensive assessment of the effectiveness and potentiality of standardising biological soil amendments to mitigate the effects of ARD in a wider project.

Financial Benefits

It is too early to calculate the financial benefits of this work from this early preliminary data. This work feeds into the larger project concerned with reducing the negative impacts of apple replant disease (ARD) on young replanted trees using biological soil amendments. As ARD is a prevalent disease in both nurseries and in fruit production and ARD onset can be 1-2 years after planting, significant economic losses can occur for growers from both management and prevention of ARD. Fumigation is particularly an expensive pre-plant option, so a transition to using non-chemical soil amendments applied at planting would save growers both money and time managing ARD. This work aims to identify the impact on soil microbiome bacterial and fungal populations due to climate change stresses to inform the long-term work within the project on what impacts climate change stress may have on both biological soil amendments and ARD causal agents. This will benefit growers moving away from chemical amendments.

Action Points

There are no action points for growers as the project is still at an early stage of a 4 year project.

SCIENCE SECTION

Introduction

Apple Replant Disease (ARD), previously termed “replant problem” is a disease where previously high yielding perennial fruit orchards show unsatisfactory growth and yield in replanted trees (Mai & Abawi, 1981). ARD has become increasingly difficult to control as finding virgin land to establish new orchards becomes increasingly difficult. Apple (*Malus domestica)* can be severely affected by ARD both in newly planted orchards and particularly in nursery orchards where tree turnover and successive replanting of crops is far more frequent than fruit production orchards where older well-established trees may have a chance to recover from ARD. Modern systems of apple growing require much higher investment to induce higher yields and earlier fruit production (Hoestra, 1968), increasing the number of cases of ARD.

ARD causes a host of negative impacts on the replanted apple trees, such as stunted growth, discolouration of apple skin, reduced yield, reduced fruit size/weight, altered fruit aroma and tree death (Mazzola & Manici, 2012; Zhu *et al.*, 2014; LIU *et al.*, 2014). These changes through ARD symptoms may decrease profitability by 50% during the orchards life (van Schoor *et al.*, 2009). The symptoms of ARD can be easily missed as stunting is often subtle and early stage ARD can only be detected when fumigated and un-fumigated soils are compared (JACKSON, 1979; Jaffee, 1982a). Young apple trees, particularly in nurseries, are of particular concern as the symptoms of ARD can occur as early as 1 year after establishment in the orchard. If death of these young trees does not occur, then characteristic ARD symptoms emerge. Additional to the above ground effects described above, discoloured roots, root tip necrosis and reduction in root biomass are all evident below the surface (Mazzola & Manici, 2012).

There is debate as the cause of ARD being caused by biotic or abiotic factors. It is generally accepted that the cause is biotic due to basic soil properties remaining unaffected in ARD affected tree soils (Simon *et al.*, 2020). The most accepted hypothesis is that changes in the soil microbiome is the basis for the onset of ARD (Mazzola & Manici, 2012). The non-specific interaction of multiple pathogenic microorganisms with each other and the host may be responsible for the onset of ARD. Changes in key components, beneficial or otherwise, in the soil microbiome is also hypothesised due to the absence of speculated ARD pathogens in affected soils (Nicola *et al.*, 2018). It is thus likely that the hypothesis that soil microbiome composition is pivotal in either the overall health of the plant through rhizosphere microbe interaction with the roots or by the interaction of pathogenic microorganism complexes forming in ARD affected soils.

Various approaches have been made to identify the causal agents of ARD meaning a plethora of different pathogenic microorganisms have been associated with ARD. There is a general agreement that a number of oomycete and fungal genera contribute to the disease globally. These include the oomycetes *Pythium* and *Phytophthora* and the fungi *Cylindrocarpon*, *Rhizoctonia* and *Fusarium* (Mazzola & Manici, 2012). The nematode *Pratylenchus penetrans* has also been associated with ARD and acts to exacerbate the disease further, leading to reduction in vegetative growth of affected apple trees and seedlings (Jaffee, 1982b). It is important to be careful when associating pathogens with ARD as some reported causal agents including *Bacillus subtilis*, *Penicillium* spp., and *Mortierella* spp. are not usually associated with being root pathogens but increased populations in ARD affected soils lead to mis-labelling of them as ARD associated pathogens (Mazzola & Manici, 2012).

There is very little information on how abiotic factors may impact the prevalence of ARD. The IPCC report suggest an increase in temperature of between 2-4˚C by 2050. Atmospheric CO_2 exposure is expected to increase by at least two times of the current levels (400 vs 800-1000 ppm CO_2) (Intergovernmental Panel on Climate Change, 2014).

Elevated temperature has a profound impact on microbial activity and this reaches an optimum at around 30˚C and then declines at >30-35˚C quite rapidly (Figure 4; BÁRCENAS-MORENO *et al.*, 2009), however soil temperatures, particularly deeper soils, will be unlikely to reach these values in temperate climates. Increased temperature will also lead to increased drought stress if increased water is unavailable. Root length colonisation (RLC) by AMF is increased in drought conditions, exhibiting a shift in the reliance on the mutualistic fungus in dry conditions. This reliance is due to hyphal spanning of air gaps between shrinking roots and soil, increasing water absorption of the target plant (Robinson-Boyer *et al.*, 2009) (Augé, 2004). Drought stress genes also are supressed when inoculated with Rhizobacteria microorganisms in Arabidopsis (Zolla *et al.*, 2013) highlighting the importance of the interaction between beneficial microorganisms and plants in an increasingly stressful climate.

In conditions of elevated atmospheric CO_2, soil organic carbon degradation increases showing how carbon sinks may become carbon sources, increasing global warming (Carney *et al.*, 2007). This change was attributed to increased relative abundance of fungal populations and increased activity of soil organic matter degrading enzymes. Increased CO_2 concentrations also increase soil bacterial diversity initially but the bacterial populations decrease exponentially as atmospheric CO_2 concentrations increase from < 5000 to > 10,000 ppm (Ma *et al.*, 2017).

In this experiment, we aim to understand whether and, if so, effect climate change stresses (namely increased temperature and CO_2 elevation) will have on the bulk soil microbiome of

apple orchards. We will be running short term trials exposing bulk soils from both an organic and conventionally managed plot to extreme CO_2 and temperature increases. Next generation sequencing techniques were used to see the impact on soil microbiome populations due to the climate stresses both individually and in consortium.

The project hypothesis were:

- Increasing CO_2 concentration and temperature will increase diversity of bacteria and fungi in bulk soil.
- Population diversity will become more dissimilar when exposed to elevated CO_2 concentrations and temperature increase.
- Soil at a lower depth will respond differently to the climate conditions that shallower soils
- Soil management type (conventional vs organic) will impact the microbiome populations present in the soils.

Materials and Methods

Soil exposure to climate change stress in relation to CO2 concentration increase and temperature increase

There were two climate change stresses, temperature increase and CO_2 concentration increase tested across two different soil types, organically managed soil and conventionally managed soil. Each soil type was exposed to three different climate conditions: 10,000ppm CO_2 concentration and temperature increase +4˚C (29°C), 5,000ppm CO_2 concentration and temperature increase +4˚C (29°C) or a control (400ppm concentration and 25°C). As well as the three climatic conditions, two different depths of soil of each type were exposed to each condition. The two depths were: 10-17cm (10) and 18-25cm (20). Four replicates for each depth were exposed to each condition to study the effect of climate condition and depth on microbiome populations.

Soil core collection

Soil cores were collected from 2 different sites, a conventionally managed and organically managed tree station at NIAB EMR, East Malling, Kent, UK in March 2019. The tree stations were recently grubbed and soil cores were collected from the tree stations where the previous trees had been. A 15cm soil corer was used to take the samples. The top 10cm of soil was

discarded and soil cores A and B were then collected from the same core. 12 cores of each depth were collected on each site making 48 total cores across both sites. Each core was placed into a separate polythene bag and immediately returned to the lab for storage at 4˚C after collection.

Growth Incubator CO_2 calibration and set up

Soil cores were transported to Cranfield University, Cranfield, Bedfordshire, UK. Growth incubators were used to create climate change conditions and were initially calibrated to check concentrations of CO_2 in each chamber. Incubators were set to 29˚C (+4˚C on ambient) and 1% CO_2 (10,000ppm). A tray of water and was placed at the bottom of the incubator and refreshed once per week during the experiment to prevent CO_2 drying air in the incubator. Pipes were placed at the base of the incubator (figure 1) to prevent CO_2 accumulation and increased concentration of CO_2. Gas chromatography was used to test the peak area (pA) of CO_2 in each of the incubators converted to target ppm values. In chamber 1, 0.3% CO_2 equalled 5,000ppm and in chamber 2, 0.5% CO_2 equalled 10,000ppm.

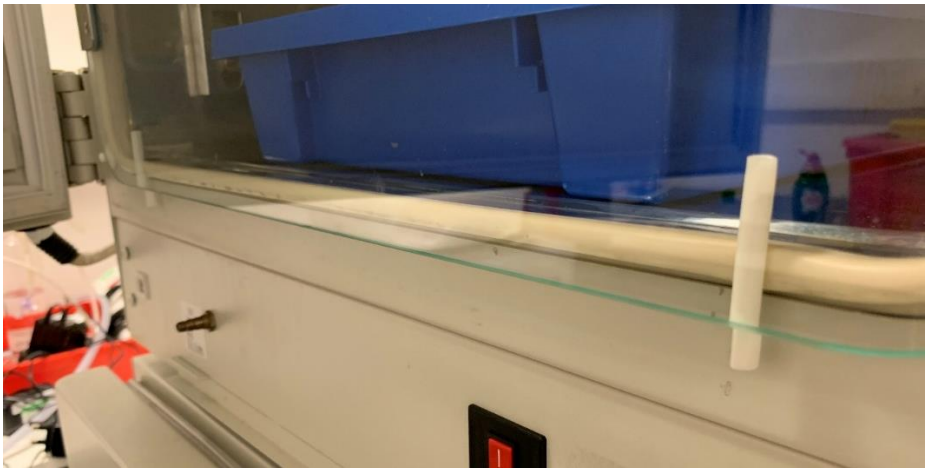

Figure 1: Pipes placed in bottom of the incubator to prevent CO_2 accumulation in the incubators.

Soil cores were placed in 8cm tall glass jars and sealed with a porous lid to allow for gas exchange within the jars. The jars were randomly distributed in a 4x4 lattice design within the incubator to prevent positional bias in the experiment (figure 2). The samples were left in the incubators for a 5 week period prior to sampling.

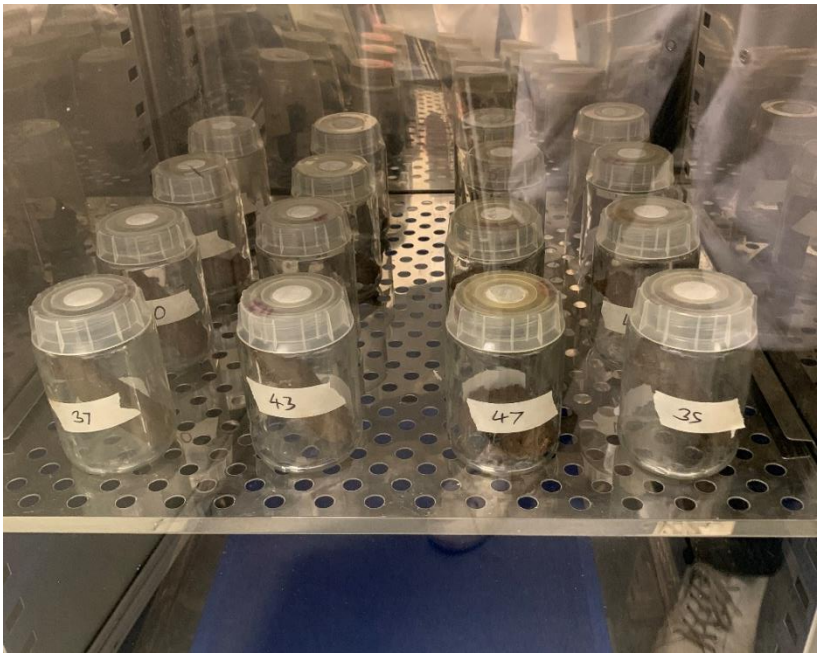

Figure 2: 4x4 lattice design of samples in growth incubator

The control samples were placed in a 25°C growth room with ambient CO_2 concentration (400ppm). The samples were placed in glass jars with porous lids inside a box with loose lid allowing for gaseous exchange. Beakers of water were placed within the box to prevent drying of the samples. The samples were similarly placed in a 4x4 lattice design and left in the growth room for 5 weeks.

Soil sample collection for next generation sequencing

Soil cores were removed from each pot and soil scraped from the outer edge of the of the soil core using a spatula, ensuring to collect soil from the entire length of the core and a 2ml Eppendorf tube filled with the soil. The spatula was washed in 70% ethanol and dried between each sample. The soil samples were then stored at 4°C until use for next generation sequencing.

Sample preparation for Illumina MiSeq loading

The DNeasy PowerSoil Kit (Qiagen, Hielden, Germany) was used to isolate genomic DNA from the soil samples following manufacturer's protocol in conjunction with SPEX CertiPrep™ Pulverizer and Cell Lyser 2010 Geno/Grinder. ITS and 16S regions were amplified using the Bakt_341F/Bakt_805R and EkITS1F/Ek28R (≡ 3126T). Amplified PCR product was library prepared for Illumina system using manufacturer's protocol and ITS and 16S samples were pooled together in a 1.5ml Eppendorf tube and stored at -20°C until sample loading onto Illumina MiSeq system.

Library denaturing and Illumina MiSeq sample loading

Before loading onto the MiSeq, reference library pooled DNA was denatured and diluted to 10pM along with the PhiX control. The MiSeq was then loaded with flow cell, cartridge and reagents as per manufacturer's protocol. Returned raw sequences were used for bioinformatics analysis.

Bioinformatics analysis of sequence reads

Sequences were processed following a previously reported pipeline (Deakin *et al.*, 2018). Briefly, operational taxonomic units (OTU) were generated and all sequences were aligned with the OTU representative sequences at 97% similarity to generate an OTU frequency table using the USEARCH 11.0 pipeline (Edgar, 2013). 16S and ITS OTU tables were created and analysed separately. Reference taxonomy databases RDP training set v16 (16S) and UNITE v7.1 (ITS) were used to create sintax taxonomy table for the OTU representative sequences.

Statistical analysis

All statistical analysis was performed in R Studio (R Version 3.5.1). All statistical analysis was conducted from the 97% similarity OTU tables generated from the unfiltered reads. The *metacoder* R package (Foster *et al.*, 2018) was used to analyse data and visualised with the *ggplot2* R package. Alpha diversity (α) was calculated using the Simpson's reciprocal index where:

$$\alpha = D/1$$

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

n = the total number of organisms of a particular species
N = the total number of organisms of all species

Results

CO_2 and temperature effect on alpha diversity.

Increasing concentration of CO_2 had differing effect on bacterial and fungal populations. Mean bacterial alpha diversity was highest in the 400ppm treatment (α = ~125) and mean diversity reduced in the both the 5,000ppm and 10,000ppm treatments with alpha scores of α = ~75 and α = ~80 respectively (Figure 3a). Fungal diversity was generally lower than the bacterial populations and the 5,000ppm treatment showed the highest mean alpha diversity (α = ~8) followed by the 400ppm treatment (α = ~7) and the 10,000ppm treatment (α = ~4) respectively (Figure 3b). None of the differences observed were significantly different for either bacterial or fungal alpha diversity using p significant threshold of 0.05. Increasing temperature effect on both bacterial and fungal populations showed a reduction in alpha diversity for both sets of organisms (Figure 4) and a statistically significant reduction in fungal populations when temperature was increased by 4°C (Figure 4b).

Alpha diversity due to site management and depth of sample soil.

The most influential factor affecting alpha diversity for both bacterial and fungal populations was the management of the site the soil was taken from (organic vs conventional). Figure 5a shows a reduction in bacterial diversity from mean alpha diversity score of α = ~140 on the conventional plot to a mean score of α = ~75 on the organic plot. Fungal alpha diversity is also reduced in the organic plot with α = ~4 in the organic soils and α = ~8 in the conventional samples (Figure 5b). Increasing depth of soil from 10-17cm to 18-25cm reduced the alpha diversity in only the fungal populations significantly reducing diversity from mean alpha diversity α = ~8 in the 10-17cm samples and α = ~5 in the 18-25cm samples (Figure 6a). There was no effect of deeper depths of soils on the bacterial alpha diversity (Figure 6b).

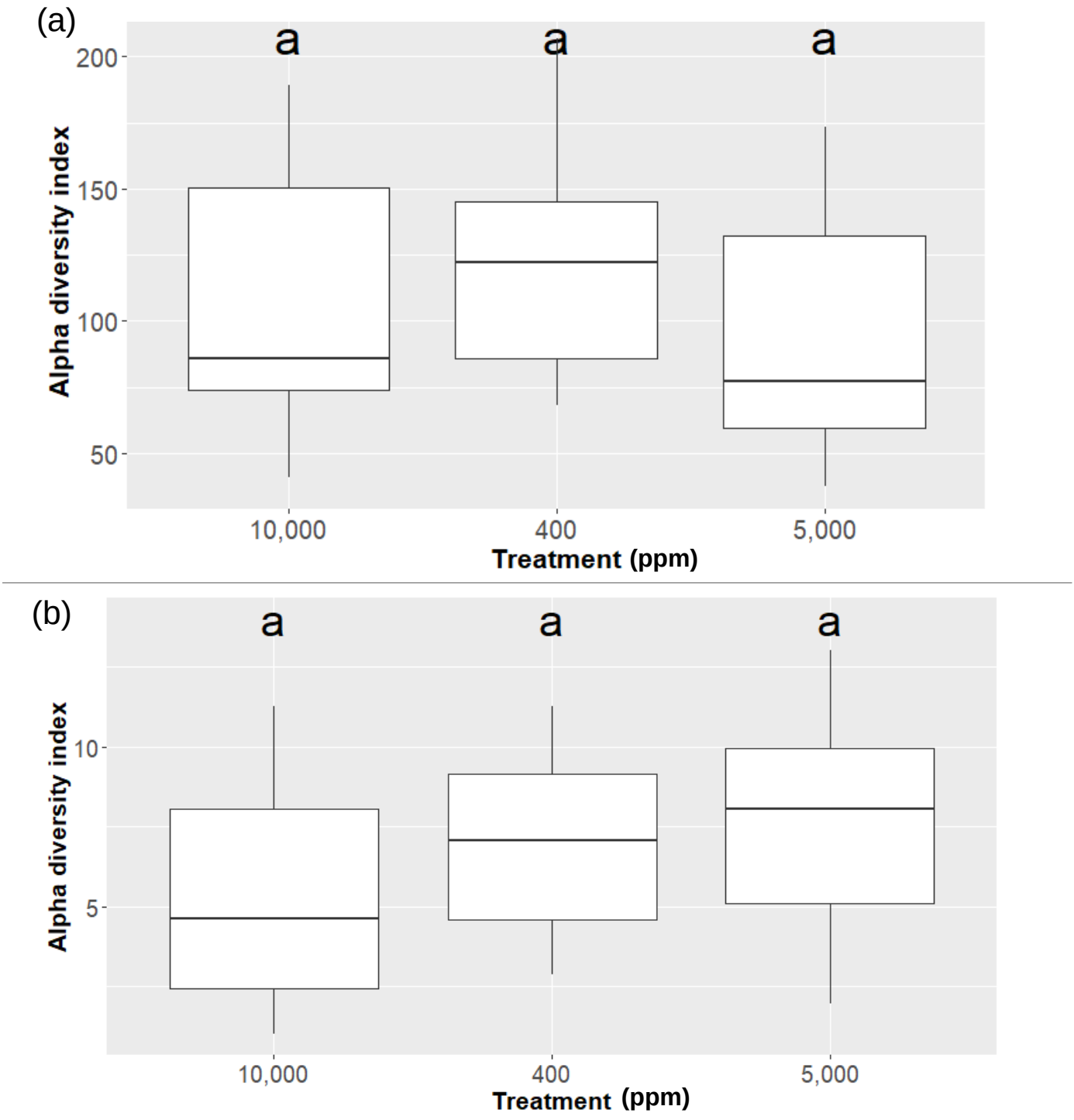

Figure 3: Boxplot of Simpson's Reciprocal Index as a measure of α diversity. Significantly different diversity is denoted by a different letter. (a) The effect of increased atmospheric CO_2 concentration measured in parts per million (ppm) on bacterial populations. (b) The effect of increased atmospheric CO_2 on fungal populations.

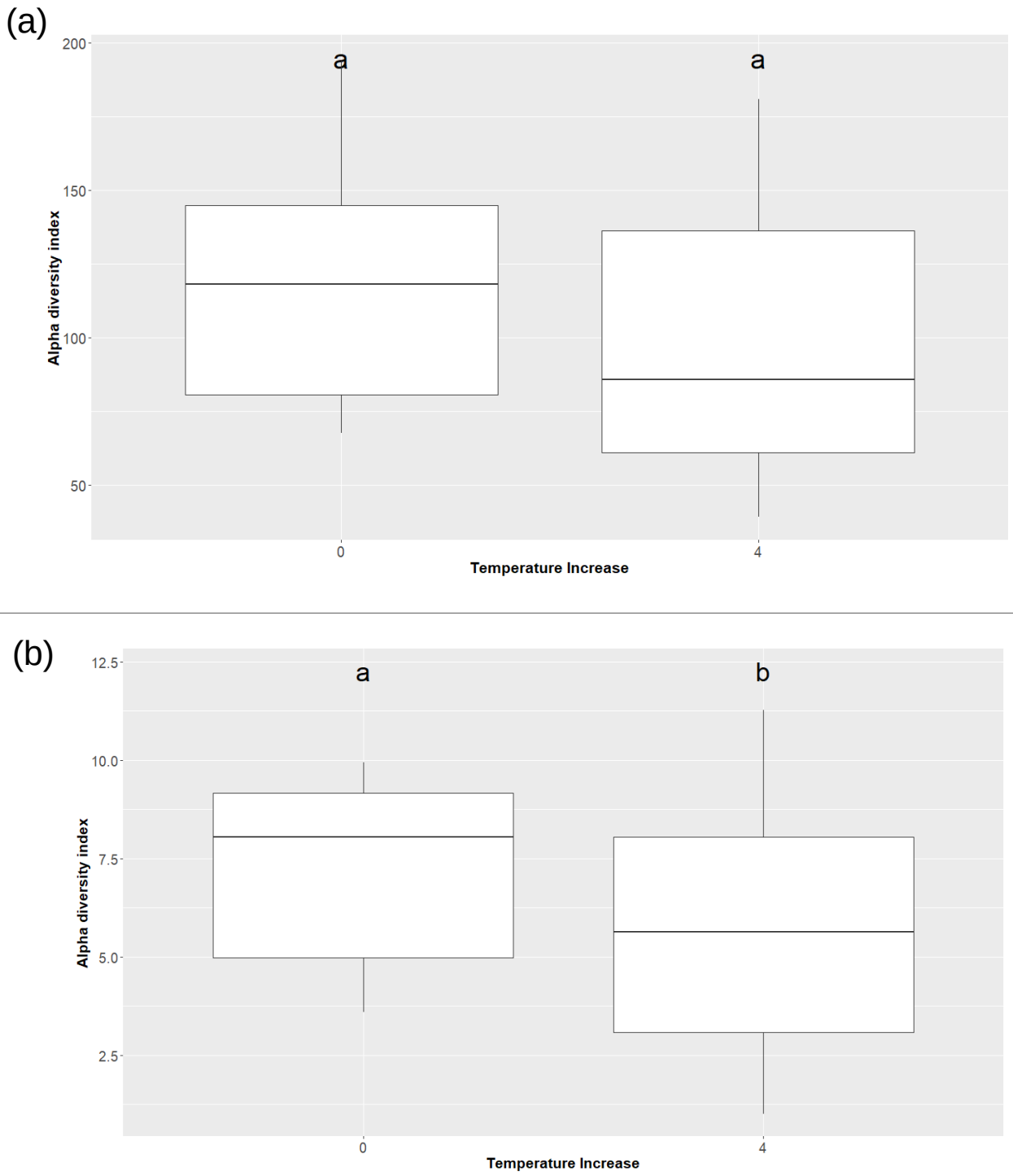

Figure 4: Boxplot of Simpson's Reciprocal Index as a measure of α diversity. Significantly different diversity is denoted by a different letter. (a) The effect of increased temperature measured in Celsius increase on bacterial populations. (b) The effect of increased temperature on fungal populations.

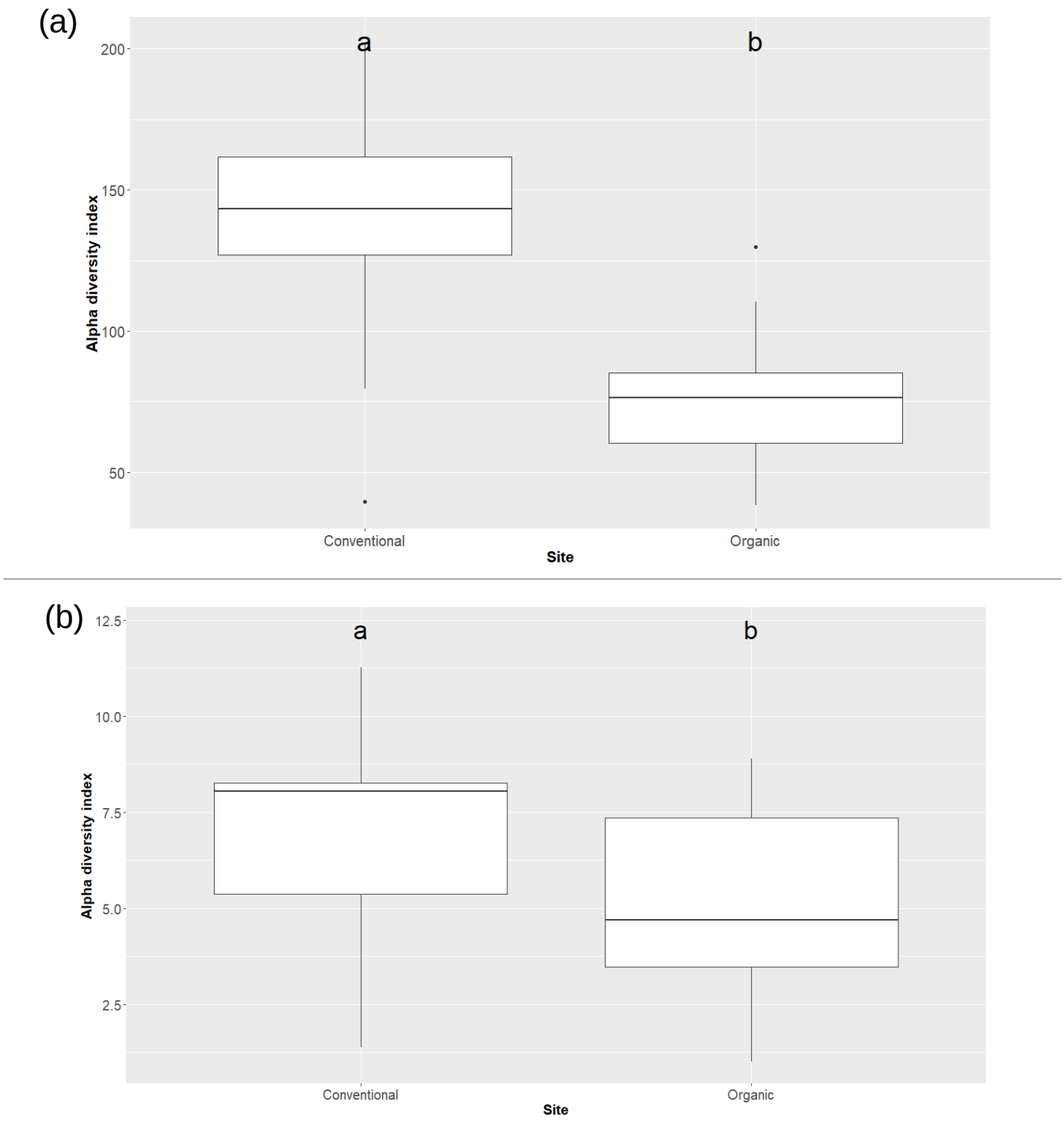

Figure 5: Boxplot of Simpson's Reciprocal Index as a measure of α diversity. Significantly different diversity is denoted by a different letter. (a) The effect of site management (organic vs conventional) bacterial populations. (b) The effect of site management on fungal populations.

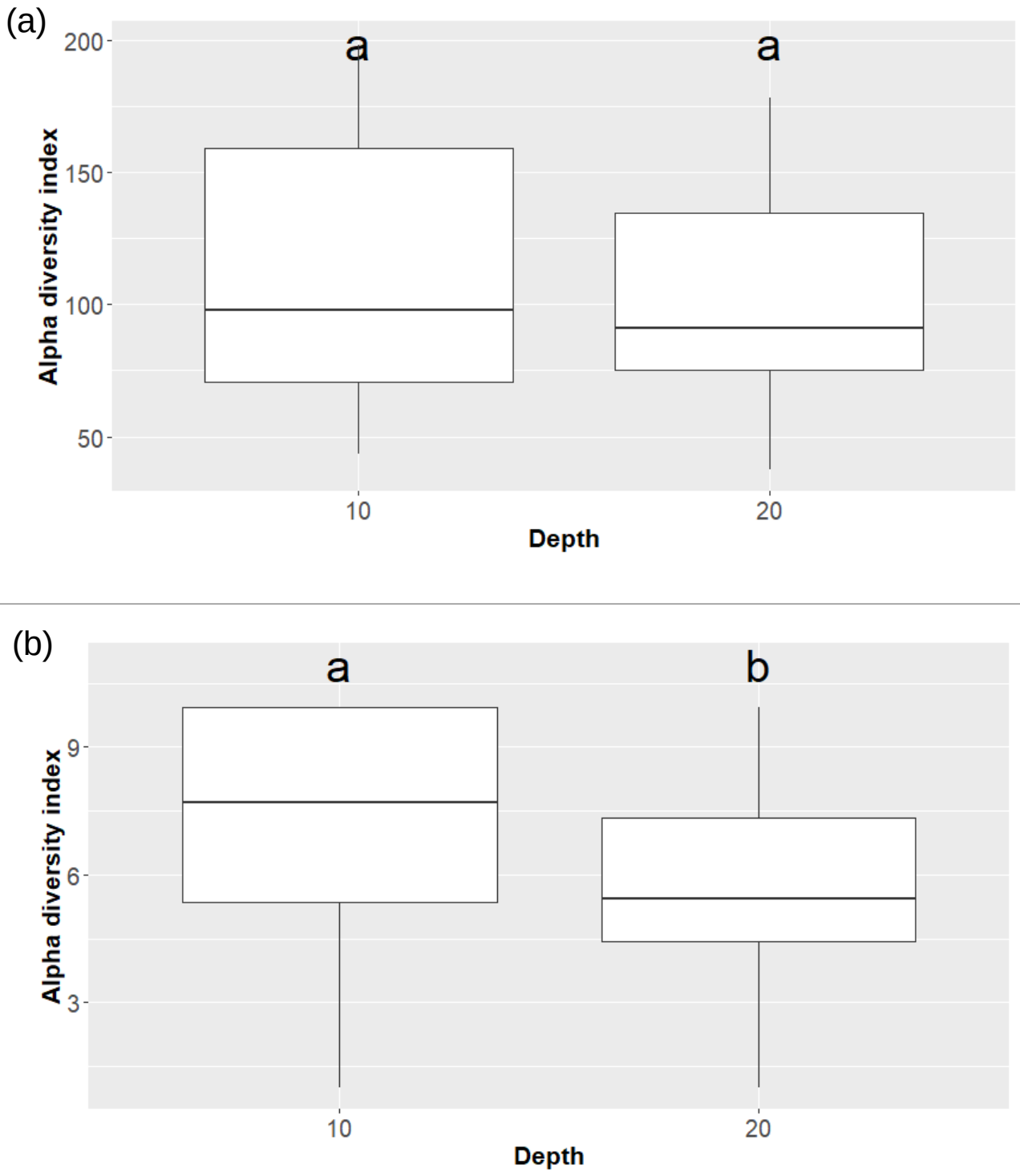

Figure 6: Boxplot of Simpson's Reciprocal Index as a measure of α diversity. Significantly different diversity is denoted by a different letter. 10 = 10-17cm depth, 20 = 18-25cm depth. a) The effect soil depth on bacterial populations. (b) The effect of soil depth on fungal populations.

Discussion

This report presents a portion of the data of a larger experiment on the impacts of climate change on soil microbiome. This work shows the impact of climate change stresses on alpha diversity in soils as a measure of only local diversity within samples. The results show that diversity of bacterial populations in bulk apple soil is much higher than the fungal diversity in the soils. CO_2 elevation to extreme levels much higher than atmospheric concentrations will reach had no significant impact on diversity in this experiment for either bacteria or fungi. Increasing temperatures by 4°C lowered the fungal diversity but had no effect on the bacterial diversity, implying fungal pathogenic and beneficial microorganisms may be more temperature sensitive than the bacterial populations. Similarly, deeper depths showed a reduction in fungal diversity but no effect on bacterial populations, highlighting the differences that 10cm of difference in depth within soil can have on the populations present. Higher depths of soil will therefore have richer and more diverse microbiome for utilisation of fine root dispersed within these upper layers.

The most influential factor investigated in this experiment was the difference due to the site management of the plot they were taken from. The conventionally managed plot managed using chemical products had a much more diverse population compared to the organic plot showed a roughly 50% reduction in diversity for both bacterial and fungal populations. The trees on the organic plot therefore have a much less diverse microbiome in the soil, which ultimately could result in differences in growth solely due to microorganism availability to the rhizosphere on each site. This report has focused on just one aspect of the research project whilst I concurrently conduct the following experiments:

- Long term growth analysis of replanted apple trees amended with biological soil amendments at the time of planting to alleviate ARD stress.
- Soil microbial community analysis of amended orchards compared to see interaction of treatments with native population and presence absence of ARD causal pathogens.
- Functionality of inoculated soils compared to see differences in carbon source usage between treatments.

Conclusions

- Extreme levels of CO_2 increase (5,000 and 10,000ppm) did not affect bacterial of fungal microbiome alpha diversity compared to ambient concentrations (400ppm).
- Temperature increase of 4°C lowered fungal alpha diversity but did not significantly affect bacterial alpha diversity.
- Alpha diversity was approximately 50% lower for both bacterial and fungal populations on the organic site compared to the conventional site.
- Depth increase from 10-17cm to 18-25cm significantly reduced fungal diversity but not bacterial diversity.
- Future work will include:
    - Beta diversity analysis to compare diversity between samples rather than local sample specific alpha diversity.
    - Calculate relative abundance of OTUs between samples to see if important OTUs (pathogenic or beneficial) very due to climate change stresses.

Knowledge and Technology Transfer

AHDB Student Industry Visit – Dundee - July 2019

Presented Poster at Fruit Focus 2019 – July 2019

Thatchers Cider Orchard Visit – August 2019

AHDB Soft Fruit Day Poster Runner-Up – November 2019

AHDB PhD student conference Nottingham – January 2020

NACM Parliament Cider Tasting – February 2020

Glossary

ARD – Apple Replant Disease

IPCC – Intergovernmental Panel on Climate Change

RLC – Root length colonisation

AMF – Arbuscular Mychorrizal Fungi

ppm – parts per million

PCR – Polymerase chain reaction

OTU – Operational taxonomic unit

α – alpha diversity

References

Augé RM, 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* **84**, 373–381.

BÁRCENAS-MORENO G, GÓMEZ-BRANDÓN M, ROUSK J, BÅÅTH E, 2009. Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. *Global Change Biology* **15**, 2950–2957.

Carney KM, Hungate BA, Drake BG, Megonigal JP, 2007. Altered soil microbial community at elevated CO(2) leads to loss of soil carbon. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4990–5.

Deakin G, Tilston EL, Bennett J *et al.*, 2018. Spatial structuring of soil microbial communities in commercial apple orchards. *Applied Soil Ecology* **130**, 1–12.

Edgar RC, 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**, 996–998.

Foster ZSL, Chamberlain S, Grünwald NJ, 2018. Taxa: An R package implementing data standards and methods for taxonomic data. *F1000Research* **7**, 272.

Hoestra H, 1968. Replant diseases of apple in The Netherlands. *Agriculture University Wageningen*.

Intergovernmental Panel on Climate Change, 2014. *Climate Change 2014 Synthesis Report - IPCC*.

JACKSON JE, 1979. Soil Fumigation against Replant Disease of Apple. *Developments in Agricultural and Managed Forest Ecology* **6**, 185–202.

Jaffee BA, 1982a. Fungi Associated with Roots of Apple Seedlings Grown in Soil from an Apple Replant Site. *Plant Disease* **66**, 942.

Jaffee BA, 1982b. Role of Soil Microflora and *Pratylenchus penetrans* in an Apple Replant Disease. *Phytopathology* **72**, 247.

LIU E, WANG G, LI Y *et al.*, 2014. Replanting Affects the Tree Growth and Fruit Quality of Gala Apple. *Journal of Integrative Agriculture* **13**, 1699–1706.

Ma J, Wang S, Xue L *et al.*, 2017. Research of the Impact of Elevated CO2 on Soil Microbial Diversity. *Energy Procedia* **114**, 3070–3076.

Mai WF, Abawi GS, 1981. Controlling replant diseases of pome and stone fruits in Northeastern United States by preplant fumigation. *Plant Disease*.

Mazzola M, Manici LM, 2012. Apple Replant Disease: Role of Microbial Ecology in Cause and Control. *Annual Review of Phytopathology*.

Nicola L, Insam H, Pertot I, Stres B, 2018. Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of

extended model of disease development. *Applied Soil Ecology*.

Robinson-Boyer L, Grzyb I, Jeffries P, 2009. Shifting the balance from qualitative to quantitative analysis of arbuscular mycorrhizal communities in field soils. *Fungal Ecology* **2**, 1–9.

van Schoor L, Denman S, Cook NC, 2009. Characterisation of apple replant disease under South African conditions and potential biological management strategies. *Scientia Horticulturae*.

Simon M, Lehndorff E, Wrede A, Amelung W, 2020. In-field heterogeneity of apple replant disease: Relations to abiotic soil properties. *Scientia Horticulturae* **259**, 108809.

Zhu Y, Fazio G, Mazzola M, 2014. Elucidating the molecular responses of apple rootstock resistant to ARD pathogens: Challenges and opportunities for development of genomics-assisted breeding tools. *Horticulture Research*.

Zolla G, Badri D V., Bakker MG, Manter DK, Vivanco JM, 2013. Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. *Applied Soil Ecology* **68**, 1–9.

