

Effect on microbial communities in apple orchard soil when exposed short-term to climate change abiotic factors and different orchard management practices

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Abstract

Aim: We assessed the effect of exposing apple orchard soil to different temperatures and CO₂ levels on the resident microbiome of soils from a conventionally managed and an organically managed apple orchard. The key difference between these two orchards was that synthetic fertilizers and pesticides are routinely used in the former one.

Methods and results: To investigate the effect of CO₂ and temperature, soil samples from each site at two depths were exposed to either elevated temperature (29°C) at either 5000 or 10 000 ppm for five weeks or control conditions (25°C + 400 ppm). Both bacterial and fungal communities were profiled with amplicon-sequencing. The differences between the two orchards were the most significant factor affecting the bacterial and fungal communities, contributing to 53.7–14.0% of the variance in Bray–Curtis β diversity, respectively. Elevated CO₂ concentration and increased temperature affected organic orchard microbial diversity more than the conventionally managed orchard. A number of candidate beneficial and pathogenic microorganisms had differential abundances when temperature and CO₂ were elevated, but their effect on the plant is unclear.

Conclusions: This study has highlighted that microbial communities in bulk soils are most significantly influenced by crop management practices compared to the climate conditions used in the study. The studied climate conditions had a more limited effect on microbial community diversity in conventionally managed soil samples than in organically managed soils.

Significance and impact of study

Climate change scenarios can have a significant impact on the microbiome of soils, with agronomic factors being particularly important. This study highlights the buffering capacity of conventionally managed soils compared to organically managed soils at elevated temperatures and CO₂. It also identifies beneficial microorganisms sensitive to climate conditions as candidates for soil amendment and potential emerging pathogens. This study provides the basis for further work on the relative impacts of changes in climatic conditions and plant development influences on the soil microbiome.

Keywords: soil, climate change, apple replant disease, microbiome, top fruit, apple

Introduction

To maximize the efficiency of high-intensity fruit production, reducing or minimizing disease on fruit and trees is essential. In apple orchards, soilborne pathogens are of particular concern as, due to the perennial nature of the trees, soilborne diseases are difficult to prevent and treat. Apple replant disease (ARD) is an example of an important soilborne disease in apple production. ARD is a disease caused by a complex of pathogens in the soil that arises in orchards when young trees are planted in an established orchard without crop rotation or land rest. Symptoms of ARD include stunted growth, reduced yields, a reduction in fruit quality, and a reduction in root biomass and root health (Mazzola and Manici 2012).

The soil microbiome is important for affecting disease development in apples. One example is the number of pathogenic microbes present in the rhizosphere of ARD trees associated with the disease. The fungal pathogens *Rhizoctonia*, *Cylindrocarpon*, and *Fusarium*, along with the oomycetes *Pythium* and *Phytophthora*, have all been associated with

ARD when their abundance is increased, particularly in synergy (Tewoldemedhin et al. 2011). *Pratylenchus penetrans*, a root-lesion nematode, can exacerbate ARD by creating root lesions for pathogen entry into roots (Mai and Abawi 1981, Mazzola and Manici 2012). Similarly, beneficial microorganisms play a critical role in promoting plant growth by nutrient exchange and biocontrol action against pathogens in the rhizosphere. Plants recruit these microbes to the rhizosphere through root exudation (Haichar et al. 2008). Recent sequence data have demonstrated a positive correlation between plant growth-promoting rhizobacteria (PGPR) species and apple tree development, such as increased plant height, root length, and dry weight, as well as in many other important crops such as tomato, cucumber, maize, and wheat (Bhat-tacharyya and Jha 2012, Nicola et al. 2017).

Large scale differences in soil microbiome are primarily down to soil-physiochemical properties. Bacterial communities are strongly defined by the pH of the soil in that particular location (Rousk et al. 2010). Smaller scale differences are attributed to the management practices applied to the soils, with

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organic systems tending to have higher microbial richness and exhibiting both pathogen suppression and increased abundance of beneficial microbes (Peltoniemi et al. 2021, Suyal et al. 2021). Crop disease, storage, and production are all tightly controlled by climate conditions, and the uncertainty around the effect of the changing climate makes the development of mitigation strategies essential for global food security (Chakraborty and Newton 2011). The geographic distribution of plant pathogens is expected to be altered as the dispersal of climatic regions changes with the overall temperature increase, leading to the emergence or increased severity of plant pathogens (Shaw and Osborne 2011). Apple microbiome ecological studies in the face of climate change are needed to understand the aetiology of soilborne diseases.

It is difficult to pinpoint the effect on soil microbiome due to changing climate factors separately as elevated CO₂ is often coupled with warming and subsequently drying of soils (Jansson and Hofmockel 2020). Elevated atmospheric CO₂ removes the C-limitations on the microbiome as more carbon is available for growth, whereas increased temperatures can increase the efficiency of enzymatic activity. Elevated CO₂ and temperature should thus be beneficial; however, studies have shown increases, decreases, or no effect on microbial biomass and activity (Drigo et al. 2008). Increase in plant biomass in elevated CO₂ conditions has also been positively correlated with increased pathogen biomass such as *Fusarium pseudo-graminearum* (Eastburn et al. 2011).

Previous studies suggest that, in conditions of elevated atmospheric CO₂, soil organic carbon may remain the same, indicative of the inability of soils to naturally stabilize or reduce atmospheric CO₂ (Carney et al. 2007). Elevated atmospheric CO₂ initially increased soil bacterial diversity but decreased exponentially as concentrations exceeded 10 000 ppm (Ma et al. 2017). Exposure of soil cores collected from grassland to an elevated CO₂ concentration (20% increase above ambient) showed no effect on the nitrogen cycling communities, but N₂O fluxes were doubled, indicating that there may be a transient increase in N-cycling microbial community functionality rather than population shifts (Brenzinger et al. 2017). In addition, elevated CO₂ had little or no direct effect on the fungal community diversity in soil but increased total fungal biomass, although it did not affect fungal activity (Kandeler et al. 1998, Klamer et al. 2002, Phillips et al. 2002). However, rhizosphere populations of PGPR and fungi, particularly AMF, can play an important role in nitrogen fixation and cycling as N availability is reduced under elevated CO₂ (Drigo et al. 2008). The potential sensitivity of fungal communities to elevated CO₂ is important as they have a lower demand for N than bacteria, and thus, the fungal composition could be more important for nutrient exchange with roots in elevated CO₂ conditions, which may be N-limiting for beneficial bacteria (Hu et al. 2001).

Changes in microbial communities could be further influenced by an increase in the release of root exudates correlated with increasing CO₂ and photosynthesis (Phillips et al. 2006), providing additional carbon sources that can stimulate the activity of specific components of microbial communities (Kapoor and Mukerji 2006). Many plants rely on beneficial microorganisms such as AMF and nitrogen-fixing PGPRs (Mekala and Polepongu 2019); thus, the increased abundance of these organisms would likely translate into increased plant growth. To date, however, there is little knowledge on what effects/impacts elevated CO₂ concentration and increased tem-

perature may have on soil microbial communities and the functionality of these populations.

Soil warming generally causes shifts in both bacterial and fungal populations in soils (Hayden et al. 2012, Xue et al. 2016), with warming soils showing a positive correlation with *Fusarium* wilt control in strawberries (Hewavitharana et al. 2021). Studies have, however, also shown there is no effect of warming on soil microbiome structure or function with a +3°C increase (Macdonald et al. 2021). As soils warm, water availability changes leading to more drought conditions, which along with the root exudation effect of the plant may explain how microbiome composition and activity are controlled by the interaction between plant, drought, and warming (Jansson and Hofmockel 2020). Recent studies have suggested that a 30% reduction in water holding capacity is sufficient to cause a shift in the dominant fungal community members (Mekala and Polepongu 2019). During drought conditions, AMF colonization is generally reduced, but most plants benefit from mycorrhizal symbiosis during drought stress (Boyer et al. 2015, Mekala and Polepongu 2019).

The objective of this study was to assay the short-term effect of exposing soils to extreme increases in both temperature (25 and 29°C) and CO₂ concentrations (400, 5000, and 10 000 ppm). The direct effect of the increased temperature and CO₂ increases would provide information on the resilience of the community structure of the resident microbiota (pathogenic, beneficial, or neither) in bulk soils available for recruitment by a replanted apple tree in both a conventional and organically managed orchard.

Materials and methods

Soil nutrient analysis

Soil from an organic plot and a conventionally managed (chemical application) plot at NIAB East Malling, Kent, UK (51°17'9.5"N 0°27'12"E) were sent for nutrient analysis in January 2020 and July 2021, respectively to NRM Laboratories, Winklefield Row, Berkshire, UK. The soil type, pH, soil organic matter, P, K, Mg, and Cu content were measured for a sub-sample of soil from each site.

Soil core collection

Soil cores were collected from two orchards: one conventionally managed using industry-standard chemical applications of fertilizers, pesticides, and herbicides, and the other organically managed without the input of these synthetic fertilizers, pesticides, and herbicides at NIAB East Malling in March 2019. The conventionally managed orchard usually receives between 10 and 15 fungicide sprays per year for disease control that the organic orchard did not receive. The organic orchard complied with the organic farming standards in the UK and had no organic or chemical fungicides, insecticides, foliar nutrients, or fertilizers applied. Apple trees that had been planted in 2009 were grubbed in October 2018; soil cores were collected from the original tree stations where the previous trees had been. A 15-cm-deep soil corer (diameter 3 cm) was used to take the samples. The top 10 cm of soil was discarded, and two soil cores at different depths (10–17.5 cm and 17.5–25 cm) were then collected from the same core (the core was divided into two using a sterilized knife). A core from each depth was collected from 12 separate tree stations on each site. There were a total of 24 samples per site, or 48

samples total. Each sample was placed into a separate polythene bag and immediately stored at 4°C until further processing.

Growth incubator CO₂ calibration and setup

Soil samples were transported to Cranfield University, Cranfield, Bedfordshire, UK. Growth incubators were used to create climate change conditions. Incubators were set to two conditions simulating climate change scenarios: 29°C (+4°C above ambient) at either 5000 or 10 000 ppm CO₂ concentration using a CO₂ gas cylinder. A tray of water was placed at the bottom of the incubator and refreshed once per week during the experiment to prevent CO₂ from drying out the air in the incubator. Pipes were placed at the base of the incubator to prevent CO₂ accumulation and increase the resident CO₂ concentration. Gas chromatography was used to test the peak area (pA) of CO₂ in each of the incubators, which was converted to target ppm values. A standard curve was produced for CO₂ in the range of 0.10–5% and then used to check whether the target %CO₂ level was achieved with air extracted from the closed incubation chamber using a syringe. In Chamber 1, 0.3% CO₂ (pA = 0.52) equalled 5000 ppm and in Chamber 2, 0.5% CO₂ (pA = 0.98) equalled 10 000 ppm. As a control treatment, soil samples were placed in a climate-controlled growth room set at 25°C with ambient atmospheric CO₂ conditions (~400 ppm).

Soil exposure to interacting climate change-related abiotic stresses of temperature × CO₂ concentration

For each site and depth combination, 4 of the 12 samples were randomly assigned to one of the three conditions, including the control. The two layers from the same core were exposed to the same climate condition. Soil cores were placed in surface-sterilized glass jars which were closed with lids containing a microporous layer allowing air and water vapour exchange.

The jars were placed in a random 4 × 4 lattice design in the two climate change incubators: 5000 ppm CO₂ + 29°C (Condition A) or 10 000 ppm CO₂ + 29°C (Condition B). The remaining control soils were distributed randomly in a 4 × 4 lattice in the climate-controlled growth room set at 25°C and ambient CO₂ concentration (Control). Beakers of water were placed in the control growth room to maintain high humidity throughout the experiment. The soil samples were all incubated for five weeks.

Next-generation sequencing of the soil cores exposed to different interacting climate-related abiotic factors for analyses of bacterial and fungal populations

After five weeks of exposure, soils were removed from each glass jar and the outer edges were scraped off carefully with a spatula from the centre of the sample along the whole length of the core. A 2-ml Eppendorf was fully filled with the soil sample to ensure sufficient soil was collected for each test. After taking each sample, the spatula was washed in 70% ethanol and dried. The soil samples were transported to NIAB EMR in East Malling, Kent, UK, at 4°C and subsequently stored at –20°C for two weeks until used for DNA extraction.

Soil DNA extraction and PCR amplification

Amplicon sequencing and sequence processing followed the method used previously (Deakin et al. 2018). In summary, genomic DNA was extracted from a sub-sample of 0.25 g soil sample using the DNeasy PowerSoil Kit (Qiagen, Carlsbad, USA) in conjunction with a bead-beating benchtop homogenizer (Fastprep FP120, Qbiogene, Carlsbad, USA). DNA concentration and quality were determined using a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Cambridge, UK). DNA was diluted to 2 ng/μl for PCR amplification.

The regions and primer pairs used for PCR amplification used were: for bacteria the 16S V4 region was amplified with the primer pair Bakt_341F/Bakt_805R (Herlemann et al. 2011); for fungi, the ITS1 and ITS2 regions were amplified with the primer pair EkITS1F/Ek28R(= 3126T) (Gardes and Bruns 1993, Sequerra et al. 1997). PCR conditions for ITS and 16S reactions are described in Supplementary Table S1. Nextera transposase adapters (Illumina, San Diego, USA) were appended to each amplicon. All PCR reactions were performed in 25 μL reaction mixtures comprising 1 × PCR buffer containing 50 mM MgCl₂ and 1 U Platinum *Taq* DNA Polymerase (Invitrogen, Life Technologies, Carlsbad, USA), 5 mM dNTP (Fisher Bioreagents, Thermo Fisher Scientific, Pittsburgh, USA), 2 μM forward and reverse primers each (Integrated DNA Technologies, Coralville, USA), 2 ng/μl template DNA, and molecular water (Sigma, St Louis, USA). PCR reaction success was checked by gel electrophoresis.

DNA clean-up 1

The DNA was library prepared following Illumina MiSeq manufacturer's protocols. PCR plates for both ITS and 16S amplified regions were centrifuged for 30 s. A total of 50 μl of PCR product was combined with 32 μl of solid-phase reversible immobilization beads (Agencourt XP Ampure beads; Beckman Coulter, Brea, USA), gently mixed using a pipette, and incubated at room temperature for 5 min. Tubes were placed on a magnet to attract the beads. The supernatant, cleared of beads, was then removed; 200 μl of 80% ethanol was added to each tube and incubated for 30 s, after which the clear supernatant was removed. The 80% ethanol step and supernatant removal were repeated, taking care to completely remove any excess ethanol in the tubes. Beads were air-dried until beads cracked (10 + min) and then removed from the magnet. A volume of 52.5 μl of 10 mM Tris pH 8.5 was added to the beads, which were gently pipetted, mixed, and incubated for 2 min. The supernatant was then cleared from the beads again using the magnet, and 50 μl of the supernatant was collected on a fresh PCR strip and stored at –20°C.

Index PCR and DNA clean-up 2

Nextera XT DNA Library Preparation Kit (Illumina) was used to barcode amplicon libraries. Nextera index 1 primer corresponded to columns 1–12 and Nextera index 2 primers corresponded to rows A–H with 5 μl of each added to each well creating a unique primer pair barcode for each sample. A total of 35 μl KAPA HiFi Hotstart Ready Mix (Roche Sequencing Solutions Inc., Pleasanton, CA, USA) was added to each sample, together with 5 μl of the corresponding Clean-up 1 DNA sample. Samples were gently mixed and centrifuged at 1000 g for 1 min. The index PCR reaction was then performed using the conditions described in Supplementary Table S2. Samples

were then immediately cleaned up a second time as described using the Clean-up 1 procedure with 56 μ l of beads initially and 27.5 μ l 10 mM tris pH 8.5 added to the dried beads to make a final volume of 25 μ l of cleaned DNA that was transferred to fresh tubes and then stored at -20°C .

Library quantification, normalization, and pooling

DNA quality and concentration were checked using a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Cambridge, UK) and a fluorometer (Qubit 2.0, Life Technologies, Carlsbad, USA). DNA sample of 5 μ l was from each extraction and then pooled into a 1.5 ml Eppendorf tube. The pooled DNA was stored at -20°C .

Library—PhiX control denaturation and MiSeq loading

A total of 5 μ l of the pooled library (4 nM) was added to 5 μ l of 0.2 nM NaOH and mixed briefly by vortexing. A volume of 990 μ l of HT1 solution (MiSeq Reagent Kit v3, Illumina) was added to the tube, resulting in a concentration of 20 pM. In a separate tube, the pooled library was diluted to a final concentration of 10 pM. PhiX control (Illumina) was denatured and diluted before use to the same concentration as the denatured library (10 pM). PhiX control is spiked in at 20% with a 10 pM denatured library. A total of 120 μ l 10 pM PhiX control was combined with a 480 μ l 10 pM denatured library and stored on ice. Immediately before loading onto the MiSeq, heat denaturation of the combined PhiX-Library was incubated on a hot block at 96°C for 2 min and then mixed by inverting the tube twice. The tube was then incubated in an ice water bath for 5 min and immediately loaded onto the MiSeq cartridge and sequenced using the manufacturer's protocol. Raw reads were used for sequence read processing.

Sequence read processing

Sequence data was submitted to the NCBI database (Project PRJEB52533). Sequence processing followed previously reported methods/pipelines (Deakin *et al.* 2018). FASTQ reads were demultiplexed into 16S and ITS fungal read datasets based on their primer sequences. Any ambiguous reads that did not match the forward and reverse read primers for 16S and ITS were removed before sequences were processed further.

Bacterial and fungal reads were processed separately to create separate representative OTUs for bacterial and fungal reads. ITS forward and reverse reads were aligned with a maximum difference in overlap of 10% threshold and 16S reads aligned similarly with a threshold of 5%. Forward and reverse primers were removed from both 16S and ITS reads. Merged reads with adaptor contamination or fewer than 150 nucleotides for ITS reads or fewer than 300 nucleotides for 16S reads were removed. Merged reads were then quality filtered using a maximum expected error threshold of 0.5.

OTU generation

All analyses were performed using UPARSE 11.0 (Edgar 2013) unless otherwise specified. Sequences were dereplicated to find unique sequences, with any unique sequence with fewer than four reads being discarded from generating OTUs. Unique sequences were then sorted by decreasing frequency and clustered at 97% similarity into OTUs, with a representative sequence for each OTU generated. Then an OTU table was gen-

erated by mapping all the merged reads against the representative OTU sequences. Taxonomy was predicted for generated OTUs using the SINTAX algorithm (https://www.drive5.com/usearch/manual/sintax_algo.html) by aligning ITS OTU representative sequences to the reference database “UNITE v7.1” (Nilsson *et al.* 2019) and 16S OTU representative sequences to the RDP training set v16 (Cole *et al.* 2014).

Statistical analysis

All statistical analyses were conducted in R V4.0.2 (R Core Development Team 2021). The vegan package V2.5.7 (Dixon 2003) was used to produce rarefaction curves to identify outliers. The metacoder package V0.3.4 (Foster *et al.* 2017) was used to indicate the differences in microbial groups at different taxa levels between treatments. Counts of 2 or less were zeroed, and OTUs with no reads were subsequently removed from further analysis. The count data were normalized by the rarefaction calculated in the Vegan package for Metacoder analysis.

Alpha diversity (α) indices (Chao1, Shannon, Simpson, and invSimpson) were calculated using the Phyloseq package V1.34 (McMurdie and Holmes 2013) from the rarefied counts. Alpha diversity indices were subject to ANOVA analysis and significance determined by permutation testing using the package LmPerm package V2.1 (Wheeler 2016) to assess the effect of orchard management, soil core depth, climate condition (5000 ppm $+4^{\circ}\text{C}$, 10 000 ppm $+4^{\circ}\text{C}$, and control) and the interaction between site management (conventional vs organic), depth, and condition. Alpha indices were visualized in the ggplot2 package v3.3.2 (Wickham 2011).

Beta diversity (β) index (Bray–Curtis) was calculated in the Vegan package from the rarefied OTU count data and plotted using a non-metric multidimensional scaling (NMDS) plot to visualize sample locations concerning the study factors. A permutation MANOVA (multivariate analysis of variance) using *F*-tests based on sequential sums of squares (ADONIS) from 1000 permutations was used to determine statistical significance. Principal components (PC) were calculated in the DESeq2 package V1.30.1 (Love *et al.* 2014). ANOVA analysis was used to determine the relative contribution of each experimental factor to the total variability in the top six PCs.

Differential OTUs were determined by the DESeq2 package to see the effect of each experimental factor on individual taxa. Log fold change (LFC) was shrunk within DESeq2 when extracting results from the model (Zhu *et al.* 2019). DESeq2 adjusted *P*-value threshold was set to 0.1 for the significantly higher or lower abundance of OTUs. Taxonomy was assigned to each OTU with significantly different abundance with a SINTAX confidence score ≥ 0.65 at the lowest assignable taxonomic rank. For large differences in abundance, the Metacoder package was used to display a phylogeny of bacterial and fungal taxonomy, coloured by different abundance due to experimental factors. Differences in Metacoder are calculated using the Wilcoxon Rank Sum test corrected for multiple comparisons with only significant differences in taxonomy displayed on the phylogenetic tree. The fungi tree was only labelled with taxonomy with a Wilcox *P*-value < 0.05 . The Bacteria tree was labelled up to rank Phylum due to the complexity of the tree.

Table 1. Summary of the bacterial and fungal representative operational taxonomic units (OTUs) after quality filtering and removal of low counts.

	Total counts	Total OTUs	Number of OTU per sample		Number of reads per sample		Number of reads per OTU	
			Min	Max	Min	Max	Min	Max
Bacteria	2 828 947	11 722	61	3397	5552	197 191	2	14 392
Fungi	338 920	990	11	237	31	27 039	1	8407

OTUs were generated using 97% sequence similarity.

Table 2. Percentage of the variability in alpha diversity indices accounted for by site (organic or conventionally managed), depth of the soil core (10–17 cm; 18–25 cm), the simulated climate condition the core was exposed to (Condition A: 5000 ppm CO₂ + 29°C; Condition B: 10 000 ppm CO₂ + 29°C; Control: 400 ppm CO₂ + 25°C), and the interaction between management, depth, and climate condition.

Measure	Site		Depth		Condition		Interaction		Residual
	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value	%	%
Bacteria									
Chao1	0.27	1.39	0.001	13.02	0.003	17.82	0.233	3.71	64.06
Shannon	$<2 \times 10^{-16}$	23.92	0.003	6.95	$<2 \times 10^{-16}$	18.70	0.335	2.85	47.58
Simpson	$<2 \times 10^{-16}$	49.75	0.179	1.14	0.045	6.48	0.0486	2.08	40.55
InvSimpson	$<2 \times 10^{-16}$	49.75	0.375	1.14	0.031	6.48	0.402	2.08	40.55
Fungi									
Chao1	0.495	0.57	0.922	0.01	0.845	0.49	0.568	2.75	96.18
Shannon	0.548	0.65	0.232	3.39	0.554	3.87	0.182	7.43	84.66
Simpson	0.623	0.91	0.136	3.34	0.357	5.40	0.116	10.05	80.30
InvSimpson	0.505	0.88	0.231	3.41	0.239	5.47	0.108	10.11	80.13

Results

Soil nutrient analysis

The soil from the conventional site was classified as sandy silt loam, and the soil from the organic site was classified as sandy loam. pH on both sites was 6.9. Soil organic matter content was also similar between the conventional orchard (2.5% loss on ignition) and the organic orchard (3.4% loss on ignition). *P* content was similar on both sites, with 31.4 mg/l available on the conventional site and 31.8 mg/l available on the organic site. K levels both fell within the target index at 151 mg/l available on the conventional site and 272 mg/l available on the organic site. Excessive levels of Mg were available on the organic site at 252 mg/l, compared to 63 mg/l on the organic site. Inversely, Cu levels were excessive on the organic site at 18.4 mg/l and were at target levels on the conventional site at 9.6 mg/l.

Summary of sequencing data

Total bacterial counts were higher than fungal counts across all samples, with 2 828 947 and 338 920 reads, respectively. Overall OTU data for both bacteria and fungi are summarized in Table 1. The mean number of reads per sample was 58 936 for bacteria and 7061 for fungi. Sample 30 (a lower-depth organic soil core exposed to 5000 ppm at 29°C) had the lowest number of reads (5552 for bacteria and 808 for fungi) and was therefore removed from subsequent statistical analysis for both bacterial and fungal analysis. Samples 22 (lower depth conventional soil core incubated at 5000 ppm and 29°C), 41 (higher depth organic soil core exposed to 10 000 ppm and 29°C), and 42 (lower depth organic soil core exposed to 10 000 ppm and 29°C) all had low reads per sample for the fungal reads (538, 31, and 67 reads, respectively) so were removed from the further fungal analysis.

Diversity indices

Four alpha diversity measures (Chao1, Shannon, Simpson, and InvSimpson) were calculated. Bacterial diversity was higher than for fungi across all samples. Each of the indices showed that fungal diversity was predominantly unaffected by site management, depth, or climate condition, with ~80% of the total variability unexplained for the Shannon, Simpson, and InvSimpson indices and 96% for the Chao1 index (Table 2). Shannon, Simpson, and InvSimpson indices for bacteria were significantly greater in the conventional orchard than in the organic orchard ($P < 2 \times 10^{-16}$) (Fig. 1). All bacterial alpha diversity indices were lower in the samples subjected to the two climate condition treatment samples than in the control samples (Table 2, Fig. 1).

Bray–Curtis indices were used to represent differences in microbial communities between samples. ADONIS analysis highlighted Bray–Curtis values that were significantly influenced by site management, contributing 53.7% and 14.0% of the variability for bacteria and fungi, respectively (Table 3, Fig. 2). In the conventional orchard alone, the soil depth the core was taken from contributed to 14.9% of the total variability in bacterial communities and 5.6% in fungal communities. The effect of depth on variability was similar in the organic orchard, contributing 13.3% for bacteria and 5.2% for fungi. The depth effect was only statistically significant for bacterial populations. The conditions the soils were exposed to had a statistically significant effect on both bacterial and fungal populations in the organic orchard, but this was not observed in the conventional orchard soils (Table 3, Fig. 3a and c). The climate condition effect contributed 12.0–13.9% of the total variability in bacterial and fungal communities from the organic orchard, whereas for communities in the soil from the conventional orchard, the climate condition contributed 9.2% and 8.7% for bacterial and fungal groups, respectively. Bacterial communities for the control soils from the organic orchard were dissimilar to bacterial communities for both the climate-treated soils but the communities did not

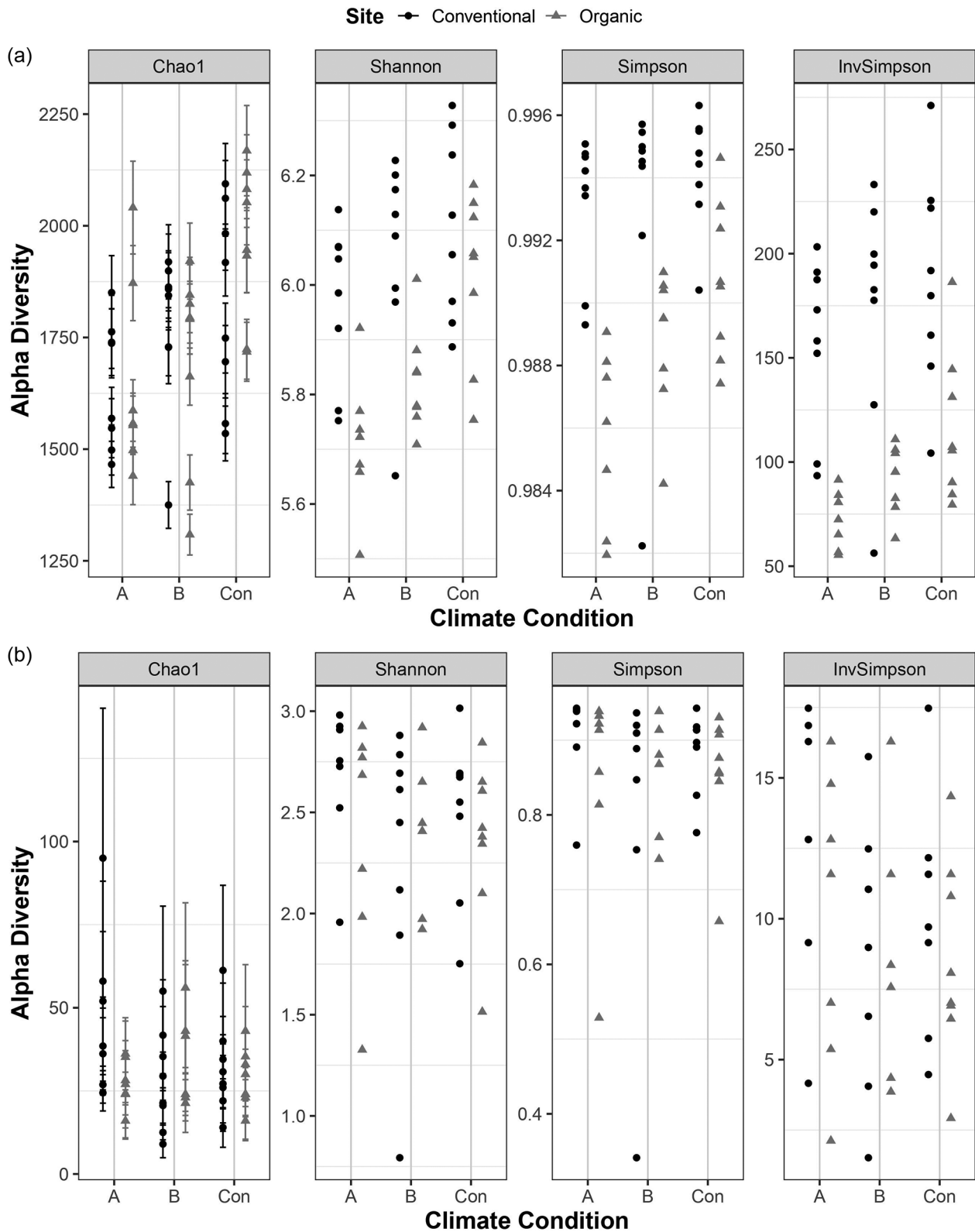


Figure 1. Alpha (α) diversity measures: Chao1, Shannon, Simpson, and InvSimpson for (a) bacteria and (b) fungi. The x-axis indicated the simulated climate conditions the cores were exposed to: A—5000 ppm CO₂ + 29°C, B—10 000 ppm CO₂ + 29°C, and Con—400 ppm CO₂ + 29°C. The shape of the point indicates site management, both organically (▲) and conventionally (●) managed.

Table 3. Percentage of the variability in Bray–Curtis dissimilarity and the scores from principal component analysis (PCA) are accounted for by site (organic or conventionally managed), depth of the soil core (10 to 17 cm; 18 to 25 cm), the climate condition the core was exposed to (Condition A—5000 ppm CO₂ + 29°C; Condition B—10 000 ppm CO₂ + 29°C; Control—400 ppm CO₂ + 25°C), and the interaction between management, depth, and climate condition.

	Site		Depth		Condition		Interaction		Residual
Measure	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value	%	%
<i>Bacteria</i>									
Bray–Curtis	0.0001	53.7	0.008	4.2	0.155	2.7	0.371	1.9	37.6
PCA*		35.4		3.9		3.6		2.7	46.4
<i>Organic</i>									
Bray–Curtis			9×10^{-4}	13.3	0.019	12.0	0.123	9.5	65.2
PCA*				9.7		11.0		9.6	69.8
<i>Conventional</i>									
Bray–Curtis			9×10^{-4}	14.9	0.174	9.2	0.686	6.7	69.2
PCA*				11.1		8.9		7.1	72.9
<i>Fungi</i>									
Bray–Curtis	0.0001	14.0	0.005	4.0	0.324	4.3	0.790	3.3	74.4
PCA*		12.0		3.0		3.8		3.77	67.3
<i>Organic</i>									
Bray–Curtis			0.308	5.2	0.033	13.9	0.432	9.5	71.4
PCA*				4.4		14.0		10.1	71.4
<i>Conventional</i>									
Bray–Curtis			0.141	5.6	0.561	8.7	0.479	9.0	76.7
PCA*				5.8		7.9		8.0	78.3

The depth, climate condition, and interaction are also scored for each site management separately.

*Based on the top six principal components.

appear to clearly differentiate between the two climate conditions (Fig. 3b). Fungal communities from the organic orchard appeared to differ between the control condition and the higher 10 000 ppm CO₂ condition, whereas the lower 5000 ppm treatment was more similar to the control than the higher CO₂ condition but was not clearly different from either condition (Fig. 3d). Interaction between the depth and climate condition was not significant in the ADONIS analysis and contributed to between 6.7 and 9.5% of the total variability in bacterial and fungal communities across soils from both sites (Table 3). The relative contribution of each experimental factor was quantified for the top six principal components (PC) scores. The percentage contribution of each experimental factor was similar to those from the ADONIS analysis (Table 3).

Differential OTUs

To see the effect of the climate conditions on individual taxa, DESeq2 was used to identify OTUs with differential relative abundance between climate conditions. The effect was limited for both bacterial and fungal taxa, with <1% of OTUs showing significant differences in the relative abundances (expressed as log₂ fold change—LFC) (Table 4). In the organic soils, bacterial relative OTU abundance was more influenced by the two climate conditions than fungal relative OTU abundance. For bacteria, 42 OTUs were identified with different abundance between Condition A (5000 ppm CO₂ + 29°C) and the control in organic soils; and 39 OTUs between Condition B (10 000 ppm CO₂ + 29°C) and the Control. No bacterial OTUs were different between the two climate conditions in the organic or conventional soil samples. Only four OTUs were different between either climate condition and the control for fungal OTUs in organic soils, and one OTU was more abundant in Condition B than in Condition A. In the conventional soils, only 12 bacterial OTUs differed between

either climate condition and the control (11 of which differed between Condition A and the control). Only three fungal OTUs had a differential abundance between the climate conditions and the control, and one OTU differed between both conditions.

In contrast, 24% of bacterial OTUs differed significantly in their relative abundance between the two orchards: half of these OTUs had decreased abundance and the other half increased in the organic orchard, compared to the conventionally managed orchard. About 14.4% of fungal OTUs differed in their relative abundance between the two orchards, with a higher number of OTUs abundant in the organic orchard (9.2%) than those abundant in the conventional orchard (5.2%).

Table 5 shows bacterial and fungal OTUs with differential abundance from DESeq2 analysis (Table 4) and their associated SINTAX taxonomy predictions. Only bacterial OTUs at rank genus and below with a SINTAX confidence threshold of 0.65 were used for increased accuracy of taxonomy assignments. All of the bacterial OTUs were identified by rank genus. The fungus *Trichoderma evansii* was identified as more abundant after treatment with Condition A or Condition B than in the control soils. Two *Trichosporon* OTUs, *T. porosum* and *T. loubieri*, were more abundant in Condition B for both taxa; *T. porosum* was also more abundant in Condition A than in the controls.

Supplementary Fig. S1 shows the differences in the relative OTU abundance between the two sites according to the rank order for bacteria and fungi. Differences in bacteria were distributed across the taxa, with a higher abundance in most taxa in the conventional soils compared to the organic soils. Node labels in Supplementary Fig. S1 are labelled for taxa with a differential abundance with a Wilcoxon *P*-value < 0.05. Differences in taxa were restricted more to specific taxa, namely higher relative abundances for *Saccharomycetales* and *Leotiomycetes* in the conventional orchard, and the opposite

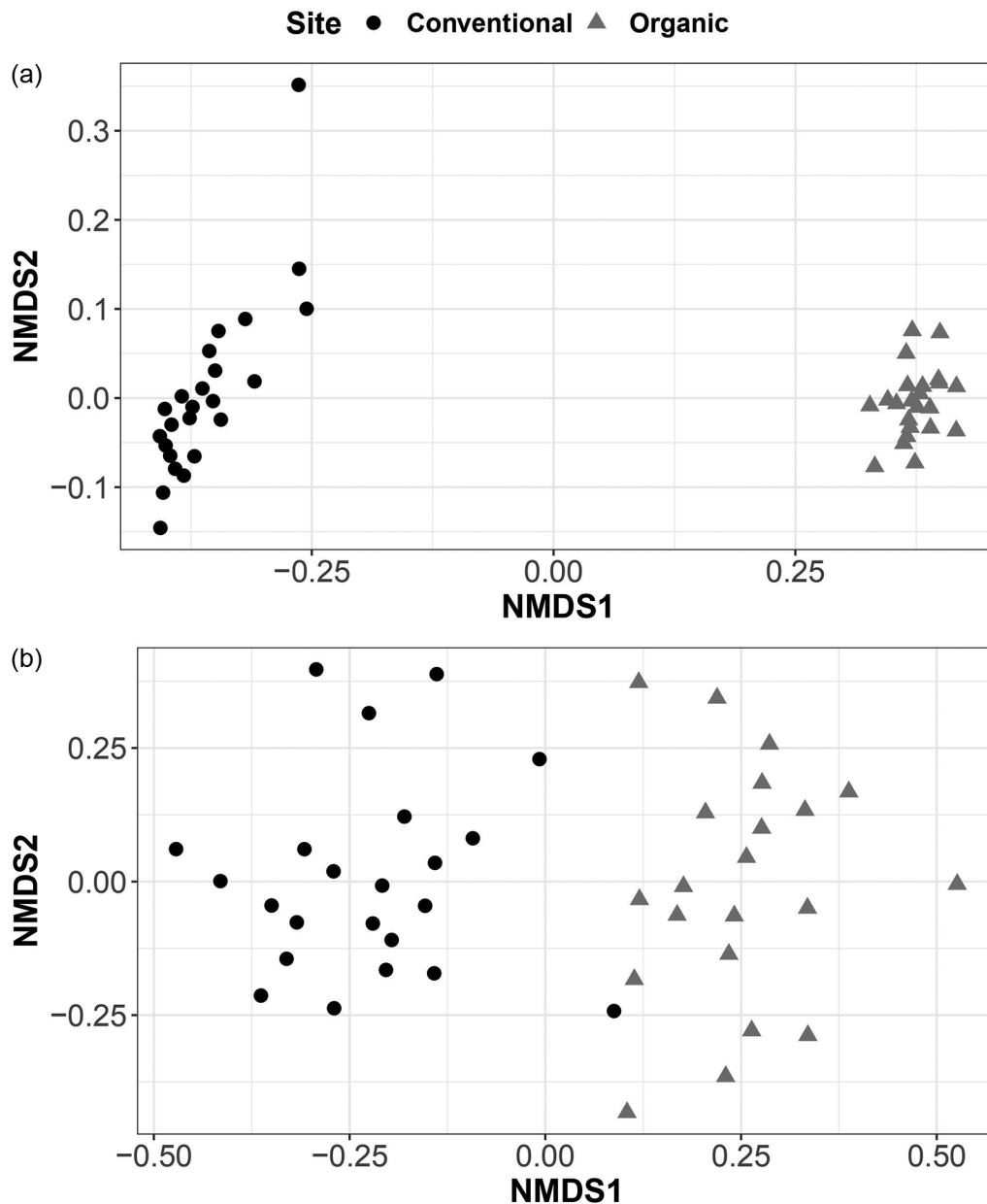


Figure 2. The first two dimensions of the NMDS analysis of the beta (β) diversity indices (Bray–Curtis dissimilarity) for (a) bacteria and (b) fungi. Closer the distance between the points indicates more similarity in the microbial communities between the samples. The shape of the point indicates site management, both organically ($\blacktriangle$) and conventionally ($\bullet$) managed.

was true for *Glomeromycetes* and *Agaricomycetes*. The order *Paraglomerales* within the class *Glomeromycetes* was also shown to be significantly more abundant in the organic orchard soil.

Discussion

Next-generation sequencing allowed for in-depth analysis of differences between microbial communities in apple orchard bulk soil and highlighted that site management (i.e. organic vs conventional) was the most significant factor affecting the microbiome in apple orchard soils. A high percentage of the variance in the microbial diversity was attributed to site differences. Microbiomes differ between conventionally and organically managed soils, with the latter possessing more heterogeneous microbial communities (Lupatini *et al.* 2017). This

study has shown that soils in an orchard managed with conventional strategies (including the use of synthetic chemical products) had a higher alpha diversity for bacteria than an orchard managed organically (without using synthetic chemical products). Previous studies have shown that bacterial species richness increases in organic farming systems compared to conventional farming systems (Acharya *et al.* 2021). We, however, found the inverse, the reasoning for which is yet unclear and requires further studies.

One soil was sandy silt loam and the other was sandy loam, which may suggest differences in property independent of the management effect, which may explain some of the large difference in communities between the organic and conventional sites. Excessive Mg was available on the organic site, but increased concentrations of Mg more strongly affect the functionality of the soil microbiome than the taxonomic

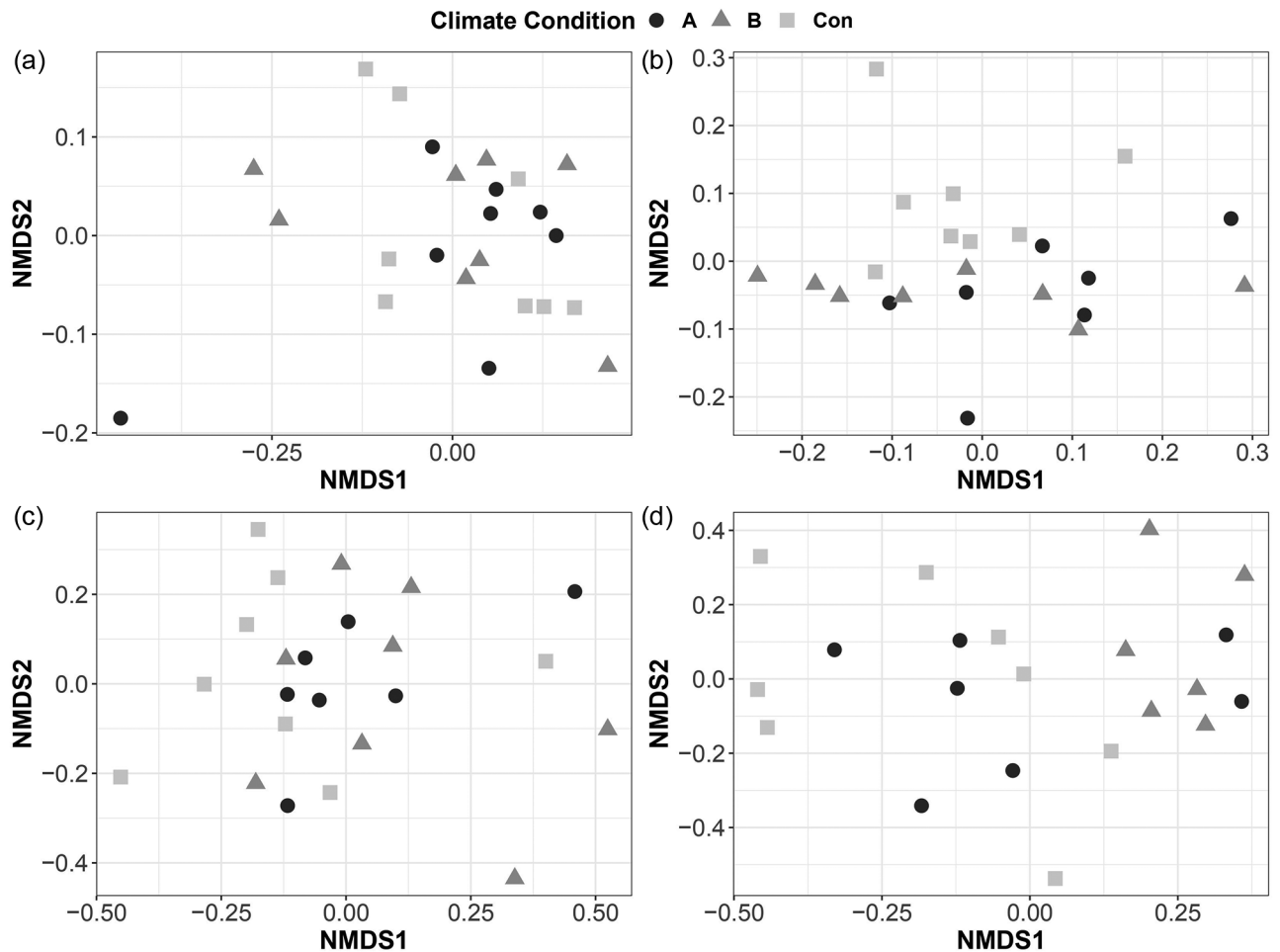


Figure 3. The first two dimensions of NMDS analysis are the beta (β) diversity indices (Bray–Curtis dissimilarity) for bacterial communities in conventionally managed (a) and organically managed (b) sites, and for fungi in conventional (c) and organic (d) sites. Closer the distance between the points indicates more similarity in the microbial communities between the samples. Shape of the point indicates the climate condition treatment of the soils: A = 5000 ppm CO₂ + 29°C (●), B = 10 000 ppm CO₂ + 29°C (▲), and Con = Control, 400 ppm + 25°C (■).

structure, which is more driven by the availability of organic nutrients such as C and N than inorganic nutrients (Nicolitch et al. 2019). Cu concentration was also excessive in the organic soils, and increased Cu availability has been shown to be negatively correlated with bacterial local diversity in soils (Nunes et al. 2016), but the concentrations required for a significant reduction in diversity (~100–500 mg/l) are much higher than the concentration observed in this study (18.4 mg/l). It is unlikely that nutrient differences between the two orchards lead to the differences in community structure observed between the two sites, but it could be a possibility that individual populations could respond to the difference in nutrients, leading to changes in abundance in individual OTUs.

Fungi had similar alpha diversity between sites. The differences in the microbial communities were spread evenly across bacterial taxa but were more restricted to specific fungal taxa. The order *Paraglomerales* within the class *Glomeromycetes*, a group of beneficial AMF species in soil, is an example of one such taxon of significance with higher abundance in the organic orchard (Schüßler et al. 2001). This highlights that there may be more natural symbiosis with mycorrhizae in this particular organic orchard and perhaps indicates the potential for the use of AMF inoculants in conventionally managed

orchards. A recent review has discussed AMF such as *Glomeromycetes* as an amendment to mitigate ARD through benefits such as greater root system architecture, increased nutrient exchange, and regulation of root endophytes and rhizosphere ARD pathogens (Lü and Wu 2018). We have shown that the microbiomes of organic and conventional orchards are different, which may impact the onset and subsequent development of ARD in these two systems, but the management effect could also amalgamate within the overall effect of site and environment. Some other studies have also shown differences in microbiomes in organic and conventionally managed orchards (Hartmann et al. 2015), but not with elevated CO₂ concentrations and increased soil temperature.

Both of the two climate change scenarios used in the study significantly reduced bacterial alpha diversity. Bacterial diversity has previously been reported to increase with elevated CO₂ levels up to 10 000 ppm (Ma et al. 2017). The elevated temperature was predicted to contribute to this increasing diversity. The present study showed opposite results and suggested that other environmental factors and/or the nature of specific soils (including the history of crop production) (Powell et al. 2015) may have caused the reduction in alpha diversity in conjunction with climatic conditions, which would require further investigation in future work. Bacterial and fungal

Table 4. DESeq2 results summary for all differential OTUs. No. OTUs indicates the number of OTUs after DESeq2 filtering (compared between factors); Log2 Fold Change (LFC) > 0 indicates OTUs higher in the first condition/treatment than the second; LFC < 0 indicates OTUs higher in the second condition/treatment than the first.

DESeq2 model	No. OTUs	LFC > 0 (higher)	LFC < 0 (lower)	Low counts
Bacteria				
Organic vs Conventional	11 698	1386, (12%)	1357, (12%)	7545, (64%)
<i>Organic</i>				
Condition A vs Control	7300	18, (0.25%)	24, (0.33%)	5627, (77%)
Condition B vs Control	7300	13, (0.18%)	26, (0.36%)	5490, (75%)
Condition B vs Condition A	7300	0, (0%)	0, (0%)	15, (0.21%)
<i>Conventional</i>				
Condition A vs Control	8149	3, (0.037%)	8, (0.098%)	6517, (80%)
Condition B vs Control	8149	0, (0%)	1, (0.012%)	41, (0.5%)
Condition B vs Condition A	8149	0, (0%)	0, (0%)	41, (0.5%)
Fungi				
Organic vs Conventional	898	83, (9.2%)	47, (5.2%)	678, (76%)
<i>Organic</i>				
Condition A vs Control	589	0, (0%)	1, (0.17%)	13, (2.2%)
Condition B vs Control	589	0, (0%)	3, (0.51%)	13, (2.2%)
Condition B vs Condition A	589	1, (0.17%)	0, (0%)	13, (2.2%)
<i>Conventional</i>				
Condition A vs Control	658	1, (0.15%)	1, (0.15%)	63, (9.6%)
Condition B vs Control	658	1, (0.15%)	0, (0%)	63, (9.6%)
Condition B vs Condition A	658	1, (0.15%)	0, (0%)	63, (9.6%)

LFC was shrunk using DESeq2. Condition A = 5000 ppm CO₂ + 29°C; Condition B = 10 000 ppm CO₂ + 29°C; Control = 400 ppm CO₂ + 25°C. Organic vs conventional refers to the crop management site of the orchard from which the samples were obtained. Low counts are OTUs filtered out by DESeq2 independent filtering using the mean of normalized counts as a filter statistic.

microbial communities appeared more sensitive to the two climate change conditions in organic soils than in conventionally managed soils. The pressure of chemical application, in particular inorganic fertilizers, herbicides, and pesticides, has likely altered both bacterial and fungal communities historically to be more tolerant of environmental conditions. Short-term increases in CO₂ concentration and temperature are therefore unlikely to significantly affect the diversity of fungal or bacterial communities, but the impact on the functionality of microorganisms in soils would require further investigation to identify their impact on host plant establishment.

Bacterial communities in organic soils that were not exposed to the climate conditions had different communities under both conditions. It is well understood that host plants play a major role in the recruitment of microorganisms to the rhizosphere through root exudation (Haichar et al. 2008, Burns et al. 2015, Guyonnet et al. 2018). The shift in bacterial communities was rapid (five weeks), which could alter the recruitment of bacteria to the rhizosphere if bacteria become unavailable, which may lead to the sub-optimal establishment of plant species and lead to disorders like ARD. Of course, the effect of such microbial recruitment on plant development will depend on functional redundancies in the soil microbiome. Fungal populations were more resilient to the climate conditions, but communities were different when CO₂ conditions reached 10 000 ppm. The lack of effect on fungal diversity at the lower concentration agrees with previous studies that found long-term CO₂ elevation did not alter fungal diversity (Klamer et al. 2002). We suggest a value between 5000 and 10 000 ppm CO₂ is a critical threshold for the resilience of fungal populations in organic soils. What the critical value for CO₂ concentration is for fungal resilience and whether soils can reach such a value for a short period of five weeks would require further investigation in future works.

Only a small number of individual taxa were affected by the two climate change conditions. All OTUs identified by

DESeq2 were investigated, despite the low LFC for many of the OTUs, which may make the change inconsequential, as the study was investigative in nature. In conventional soils, a number of OTUs that are believed to have important biological functions were less abundant in soil with elevated CO₂ concentrations and increased temperatures conditions compared to control soils. *Pseudoxanthomonas* was one example and is described as a biocontrol exhibiting nematostatic function but is also closely related to *Xylella* containing known pathogens, particularly *X. fastidiosa* causing disease in citrus, grape, and olives (Hu et al. 2019, Bansal et al. 2021). *Janthinobacterium* and *Nocardioides* also had lower relative abundance in the climate condition treated soils than the control and both contain species that produce anti-fungal suppression against pathogenic *Fusarium* spp. *Trichoderma evansii* had higher abundance in both climate conditions than in the control, and whilst *T. evansii* is not well described in soil, *Trichoderma* species are well-known biocontrol agents of plant pathogens in soil (Mukherjee et al. 2012).

In the organic soils, a number of bacterial genera associated with beneficial PGPRs had a higher relative abundance in the control soils than soils exposed to elevated CO₂ and temperature. These genera included *Pseudomonas*, *Variovorax*, *Massilia*, *Streptomyces*, and *Mucilaginibacter*. *Pseudomonas*, well established as containing PGPR species of numerous crops but also some important plant pathogens such as *P. syringae* (Sivasakthi et al. 2014, Xin et al. 2018), was significantly more abundant in control soils than the 5000 ppm condition. Similarly, *Streptomyces* species were more abundant in the control soils than in the 5000 ppm condition only and have been described as both beneficial PGPRs, biocontrols, and pathogens in crops such as potatoes (Li et al. 2019, Suárez-Moreno et al. 2019). As *Streptomyces* and *Pseudomonas* genera were not detected in the DESeq2 analysis for the 10 000 ppm vs Control model, this suggests they are tolerant to the temperature change, but the relative effect of CO₂

Table 5. Differential OTUs from DESeq2 analysis.

DESeq2 model	Species/Taxa*	Base mean	LFC	P-value
Bacteria				
<i>Conventional</i>				
Condition A vs Control	<i>Pseudonocardia</i> (g)	4.64	2.49×10^{-6}	0.04
	Acidobacteria—Gp7 (g)	54.92	3.25	0.04
	<i>Pseudoxanthomonas</i> (g)	6.73	-2.33×10^{-6}	0.04
	<i>Janthinobacterium</i> (g)	8.34	-4.63×10^{-6}	0.04
	<i>Flavobacterium</i> (g)	4.22	-3.14×10^{-6}	0.04
	<i>Nocardioidea</i> (g)	26.16	-9.19×10^{-6}	0.09
Condition B vs Control	<i>Flavobacterium</i> (g)	35.96	-5.67×10^{-6}	0.02
<i>Organic</i>				
Condition A vs Control	<i>Sphingomonas</i> (g)	1132.97	0.98	0.05
	Acidobacteria—Gp3 (g)	151.13	0.67	1.23×10^{-3}
	<i>Pseudonocardia</i> (g)	7.93	4.96	5.44×10^{-4}
	<i>Pseudomonas</i> (g)	304.60	-5.28×10^{-6}	0.10
	<i>Variovorax</i> (g)	35.84	-6.18×10^{-6}	0.05
	<i>Flavobacterium</i> (g)	8.70	-3.86×10^{-6}	5.44×10^{-4}
	<i>Chryseolinea</i> (g)	46.12	-2.34×10^{-5}	0.02
	Acidobacteria—Gp6 (g)	37.55	-1.04×10^{-5}	0.10
	<i>Massilia</i> (g)	14.22	-7.36×10^{-6}	0.01
	<i>Streptomyces</i> (g)	9.34	-5.78×10^{-6}	0.05
	<i>Nonomuraea</i> (g)	3.97	-1.94×10^{-6}	0.10
	<i>Janthinobacterium</i> (g)	14.55	-2.87	5.44×10^{-4}
	<i>Aeromicrobium</i> (g)	11.43	-4.60×10^{-6}	0.01
	<i>Pseudonocardia</i> (g)	6.60	-4.60×10^{-6}	0.02
	<i>Methylobacter</i> (g)	10.15	-2.22×10^{-6}	0.06
Condition B vs Control	Acidobacteria—Gp6 (g)	17.60	1.36	1.89×10^{-3}
	<i>Burkholderia</i> (g)	51.13	2.77	0.04
	Acidobacteria—Gp1 (g)	28.31	1.52	0.06
	<i>Chryseolinea</i> (g)	23.78	1.93	7.29×10^{-4}
	<i>Pseudonocardia</i> (g)	7.93	5.25	1.18×10^{-4}
	<i>Variovorax</i> (g)	35.84	-7.15×10^{-6}	0.17
	<i>Flavobacterium</i> (g)	8.70	-4.66×10^{-6}	7.41×10^{-4}
	<i>Chryseolinea</i> (g)	46.12	-1.94	7.85×10^{-4}
	<i>Variovorax</i> (g)	29.69	-1.77	0.04
	<i>Massilia</i> (g)	14.22	-2.75	1.05×10^{-5}
	<i>Janthinobacterium</i> (g)	14.55	-3.52	9.62×10^{-6}
	<i>Mucilaginibacter</i> (g)	6.51	-2.94×10^{-6}	0.01
	<i>Pseudonocardia</i> (g)	6.60	-4.96×10^{-6}	6.91×10^{-3}
	<i>Methylobacter</i> (g)	10.15	-2.73×10^{-6}	2.29×10^{-3}
Fungi				
<i>Conventional</i>				
Condition A vs Control	<i>T. evansii</i>	7.47	3.45×10^{-7}	2.86×10^{-9}
Condition B vs Control	<i>T. evansii</i>	7.47	2.32×10^{-7}	3.01×10^{-4}
<i>Organic</i>				
Condition A vs Control	<i>T. porosum</i>	44.71	-6.50×10^{-7}	4.80×10^{-2}
Condition B vs Control	<i>T. loubieri</i>	759.96	-6.97×10^{-7}	0.05
	<i>T. porosum</i>	44.71	-5.58×10^{-7}	0.03
	<i>Conocybe</i> (g)	41.70	-2.34×10^{-7}	2.50×10^{-6}
Condition B vs Condition A	<i>Conocybe</i> (g)	41.70	-2.14×10^{-7}	4.15×10^{-10}

Positive Log2 Fold Change (LFC) indicates higher OTU abundance in the first condition/treatment, and vice versa for negative values. P values are Benjamini and Hochberg corrected. Condition A = 5000 ppm CO₂ + 29°C; Condition B = 10 000 ppm CO₂ + 29°C; Control = 400 ppm CO₂ + 25°C.

*The lowest assignable taxonomic rank at genus or below with a SINTAX confidence ≥ 0.65 .

concentration on their abundance is unclear and would require further investigation.

Burkholderia genera have similarly been described as both beneficial bacteria as a N-fixing PGPR and as an opportunistic pathogen (Angus et al. 2014) and were more abundant in the 10 000 ppm CO₂ condition than in the control soils. *Burkholderia* could become an emerging pathogen causing disease in organic orchards if conditions become sufficient or, inversely, a new candidate soil amendment if the strain is beneficial. Identification to species rank would be required to identify whether *Pseudomonas*, *Streptomyces*, and *Burkholderia*

are beneficial or pathogenic in the organic soils, making them either emerging plant pathogens if climate conditions become sufficient in the case of *Burkholderia* or candidate soil amendments if the species are beneficial to plant growth. This highlights the need for identification to species level where possible when interpreting the cited function of OTUs with different abundances.

Variovorax genera are known PGPRs and often used as a model organism for plant-microbe interactions, with strains such as *V. paradoxus* shown to aid stress tolerance and disease resistance and improve nutrient availability to the plant

for growth (Han *et al.* 2011, Sun *et al.* 2018). Similarly, the genus *Massilia* has been reported to show *in vitro* attributes of plant growth promotion and successfully colonize cucumber roots and seeds (Ofek *et al.* 2012). Both *Variovorax* and *Massilia* had lower relative abundance in the organic soil samples subjected to both climate change conditions than in the control soils. *Mucilaginibacter* similarly exhibits plant growth-promoting traits when isolated from the rhizosphere of cotton (Madhaiyan *et al.* 2010) and was more abundant in control soils than in the 10 000 ppm condition but not differentially abundant comparing the 5000 ppm condition to the control. *Mucilaginibacter* is likely more sensitive to the CO₂ concentration than the temperature increase, whereas both *Variovorax* and *Massilia* were sensitive to both in organic soils. This highlights the limited resilience of these beneficial bacterial groups in organic soils to climate change, which needs to be taken into consideration should these microbial groups be considered for use in commercial agriculture as a soil amendment.

These results indicated both pathogenic and beneficial microbial populations may change in bulk soils of both conventional and organic soils when extreme climate change conditions occur. The implications for the host plant are unclear. Climate change conditions (such as elevated CO₂ concentration) may likely occur irregularly and unpredictably, thus attention needs to be paid to both high and low extreme climate events. Future studies should focus on the effect of fluctuating climate perturbations in the natural environment or expose soils to both constant and varying climate conditions for longer periods known to affect microbiome populations (Guo *et al.* 2018) to better understand which microbial components are affected by climate changes.

This study focused solely on native microorganisms in bulk soils. Soilborne diseases such as ARD can be caused by the build-up of pathogens in the rhizosphere of roots (Mazzola and Manici 2012). This work examined the effect of just two specific climate change scenarios on native microbial communities in bulk soils from recently grubbed apple orchards and thus did not focus on the specific aspect of the rhizosphere where pathogens will interact with the roots. Studies have shown that increases in root exudation may be linked to increasing CO₂ (Phillips *et al.* 2006), thus the effect of changing climate conditions may be more pronounced in the rhizosphere microbiome. Microbial communities are also influenced by water availability in both soils and plants. A 30% reduction in the soil water holding capacity is sufficient to shift the dominant fungal communities (Mekala and Polepongu 2019). In this study, an attempt was made to maintain high moisture content, but over time there was a slight drying of the soils, which may have influenced microbial communities. Future work should aim to maintain moisture levels comparable to those in field conditions or include water availability as a study factor.

In summary, the present results highlighted that microbial communities in bulk soils are most influenced by the soil location, likely due to differences in the use of synthetic chemical products in organic and conventional systems but also the overall location and environmental effect. Moreover, the location and management practices appear to influence bacteria more than fungi. We also noted a weaker but still significant impact of the two simulated climate conditions on bacterial and fungal diversity in organic soils. Conventionally managed soils seemed to have more similar bacterial and fungal com-

munities when comparing the communities in the two simulated climate conditions with untreated soils. Chemical management seems to have applied selection pressure on the bulk soil microbiome, leading to more tolerance to climate effects than organic soil microbiome communities. Several potential beneficial organisms, as well as plant pathogens, may be influenced by climate change factors in both organic and conventional soils. Further studies need to examine the influence of fluctuations in temperature, CO₂ concentration, and water availability to quantify the temporal changes in the soil and rhizosphere microbiome in apple production systems.

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Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest

No conflict of interest declared.

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Author contributions

Chris Cook (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing), Naresh Magan, (Conceptualization, Supervision, Writing – review & editing), Louisa Robinson-Boyer (Conceptualization, Supervision, Writing – review & editing), and Xiangming Xu (Conceptualization, Supervision, Writing – review & editing)

Data availability

The datasets generated during and/or analysed during the current study are available in the NCBI repository (Project PRJEB52533).

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Effect on microbial communities in apple orchard soil when exposed short-term to climate change abiotic factors and different orchard management practices

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