Project title:	Investigating the cause and potential treatment of coriander yield decline
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Project leader:	Kate Fraser, Newcastle University
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Key staff:	Prof. Ian Singleton Prof. Anne Borland
Location of project:	Newcastle University
Industry Representative:	Dr T M Davies, Malvern View Herbs
Date project commenced:	01 October 2014
Date project completed (or expected completion date):	31 March 2017

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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.

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CONTENTS

GROWER SUMMARY

Headlines

- Coriander yield decline has been successfully induced in two commercial coriander varieties (Santos and Cruiser) in one soil type and a compost.
- A reproducible system was developed to generate yield decline-inducing soils allowing further in-depth study.
- In coriander pot experiments, yield decline was evident as a significant decrease in above ground biomass with no obvious signs of disease or reduction in root biomass.
- Soils in close association with the roots (rhizosphere) of plants exhibiting yield decline symptoms were found to have different microbial communities compared to the rhizosphere of healthy plants but no obvious microbial cause was found.

Background

Coriander (*Coriandrum sativum* L.) is an annual herb that is of high value with sales accounting for over 25% of the fresh herb market. Since the 1970s coriander has been grown commercially in the UK, however, production has been hindered in recent years by yield decline. Growers have reported yield losses of over 50% due to decline and indicate that the issue can persist for up to eight years, impacting negatively on the UK herb market. There are many factors that can cause yield decline such as toxic root exudates (autotoxicity), poor soil quality, poor soil management practices and soil microorganisms. Since coriander yield decline can persist for up to 8 years, autotoxicity is probably not the cause, as over time root exudates will degrade and bind with the soil matrix. Furthermore, preliminary work during this project showed that yield was not affected when coriander was grown in soil containing chopped coriander roots. From available evidence, it is considered that coriander yield decline most likely has a soil microbiological basis.

Summary

While growers reported coriander yield decline, the phenomenon needed experimental confirmation and had to be reproduced under controlled conditions to allow subsequent in-depth studies. The aim of this project was to determine whether yield decline could be induced in different soils and coriander varieties and to assess the impact of coriander cropping on soil microbial communities, using an Illumina Next Generation Sequencing (NGS) technique.

The following hypotheses were tested during the project:

1. Coriander yield decline can be induced in two commercial coriander varieties in soil and compost.
2. Coriander cropping causes a marked change in the soil microbial community, with bacterial communities in rhizosphere soil (soil in close association with roots) differing from those in bulk (the surrounding) soil, in soils planted with a single coriander crop.
3. The bacterial community of rhizosphere soil samples obtained from healthy coriander is different to that of rhizosphere soil obtained from coriander exhibiting yield decline symptoms.
4. The rhizosphere bacterial community obtained from yield decline plants grown in different soils is the same.

Establishing coriander yield decline in an agricultural soil

An experiment was conducted to determine if coriander yield decline could be induced under controlled conditions. This pot experiment involved two treatments: (1) coriander grown in a soil with no history of coriander cropping (sandy soil from Cockle Park Farm, Northumberland, UK); (2) coriander grown in the same soil type buy planted with coriander once before. A single coriander variety Santos was used. Plants from both treatments were harvested nine weeks after sowing for the collection of yield data.

Coriander plants grown in control soil with no history of coriander cropping, had significantly higher above ground weights (fresh weight, $p=0.001$; dry weight, $p=0.01$) compared to plants grown in previously planted soil (Figures A and B). Root fresh weight was also significantly higher in control soil ($p=0.008$).

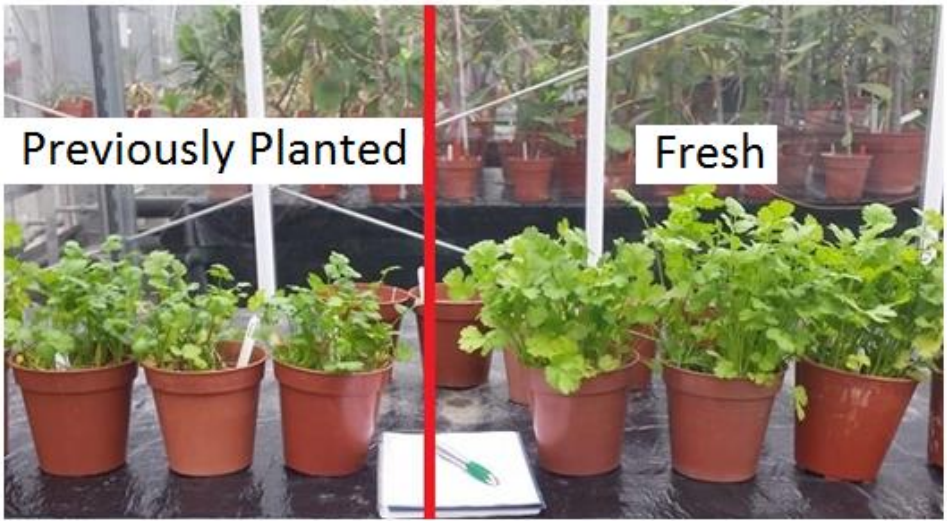


Figure A. Comparison of observable difference in yield between coriander var. Santos plants grown in control soil and soil planted with coriander once before.

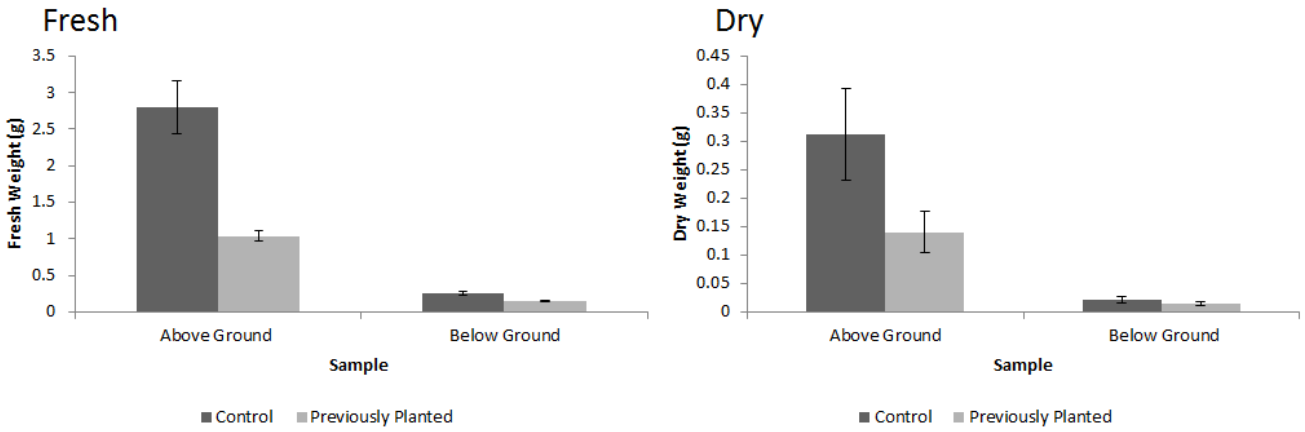


Figure B. Comparison of mean fresh and dry weights of coriander var. Santos plants grown in control and previously planted Cockle Park soil. Weights for above ground (shoot and leaf) and below ground (roots) are shown. Values plotted are ±1 Standard Error.

Inducing yield decline in compost with more coriander varieties

In a second pot experiment, John Innes No. 2 (JI) compost was used, as it could be expected to have consistent properties and have an entirely different microbiota to Cockle Park soil. Two coriander varieties, Cruiser and Santos, were each grown in both control JI compost and in JI compost sown with coriander once before. Plants were harvested eight weeks after sowing for the collection of yield data.

Both coriander var. Cruiser and Santos plants grown in control JI compost had a larger visible biomass compared to those grown in previously planted JI compost but the difference in total

fresh biomass was not significant ($p>0.05$). However, for coriander var. Santos, shoot dry biomass was significantly reduced ($p>0.05$) in compost previously planted with coriander.

These results confirmed the occurrence of the yield decline phenomenon and that this could be demonstrated in a reproducible pot assay. Results demonstrated that yield decline occurred in both an agricultural soil and a compost, and with two coriander varieties. Further work will be required to investigate the occurrence of yield decline in more soil types and coriander varieties.

Investigating soil bacterial communities using Illumina NGS

The next focus of the work was to assess the impact of coriander cropping on soil microbial communities using an Illumina Next Generation Sequencing (NGS) technique. After harvesting coriander from pot experiments with Cockle Park soil and JI compost, pots were sampled for rhizosphere soil / compost (in close association with roots) and the surrounding bulk soil / compost. DNA was subsequently extracted from the soil / compost samples and sequenced using the Illumina MiSeq platform at NU-OMICS Ltd, Northumbria University. This work focussed on sequencing and analysing the bacterial communities but fungal community analysis was also carried out (A summary is provided in Appendix 2 of the Science Section of the full report). Data received following Illumina MiSeq sequencing were processed and the bacterial species composition of each soil was analysed.

A. Comparing bacterial communities of coriander rhizosphere soils to corresponding bulk soils

For this comparison, DNA was extracted from both rhizosphere and bulk samples taken from Cockle Park soil cropped once with coriander and containing healthy plants.

Results clearly showed that rhizosphere and bulk samples from Cockle Park soil (planted with a single coriander crop) had different bacterial community compositions. The top five abundant species in bulk soil samples were *Chitinibacter* spp. (~1.7%), *Thermacetogenium* spp. (~1.6%), *Desulfovibrio* spp. (~1.4%), *Mucilaginibacter* spp. (~1.3%), and *Pyramidobacter* spp. (~1.3%). Whereas in rhizosphere soil samples, *Pseudomonas* spp. (~2.2%) replaced *Chitinibacter* spp. as the most abundant species and *Chitinibacter* spp. was no longer within the top twenty most abundant species. In rhizosphere soil, the next most abundant species after *Pseudomonas* spp. were *Lysobacter* spp. (~1.5%), *Thermacetogenium* spp. (~1.5%), *Terrimonas* spp. (~1.4%) and *Desulfovibrio* spp. (~1.4%) (detailed data are shown in the Science Section). This suggests that coriander growth has a marked effect on soil microbial communities, with bulk soil samples having a different bacterial community composition to corresponding rhizosphere samples.

B. Analysing the bacterial communities associated with the coriander rhizosphere in previously planted vs. unplanted soil

For this comparison, DNA was extracted from: (1) Cockle Park soil, cropped once with coriander, containing healthy plants exhibiting no yield decline symptoms; (2) previously planted Cockle Park soil, cropped with coriander twice, and containing plants exhibiting yield decline symptoms.

Rhizosphere samples from 'control' Cockle Park soil planted with a single coriander crop had different bacterial community compositions compared to Cockle Park rhizosphere samples in which two coriander crops had been grown. The top five most abundant species in control rhizosphere soils were *Pseudomonas* spp. (~2.2%), *Lysobacter* spp. (~1.5%), *Thermacetogenium* spp. (~1.4%), *Terrimonas* spp. (~1.4%), and *Desulfovibrio* spp. (~1.3%). In previously planted rhizosphere soil, *Pseudomonas* spp. was the most abundant (~2.5%) however it was not significantly ($p<0.05$) more abundant compared to control soils. The next most abundant species were *Sphingomonas* spp. (~2.5%), *Mycoplasma* spp. (~2.2%), *Halothiobacillus* spp. (~2.0%) and *Sphingobacteriaceae* spp. (~2.0%) (detailed data are shown in the Science Section).

Plants grown in previously planted soil exhibited symptoms of yield decline; therefore, it could be that a significant change to the microbial community is associated with yield decline; however, more work will be required to pursue this theory.

C. Establishing whether coriander yield decline has the same microbial causal agent in different soil types

To establish a potential causal agent of coriander yield decline, DNA was extracted from the following soils: (1) previously planted Cockle Park soil, cropped with coriander twice, and containing plants exhibiting yield decline symptoms; (2) previously planted JI compost, cropped with coriander twice, and containing plants exhibiting yield decline symptoms (three replicates per soil type).

Comparisons between the two soil types show that previously planted Cockle Park rhizosphere soil had a different microbial community composition compared to previously planted JI rhizosphere compost. The top five most abundant species in previously planted Cockle Park rhizosphere soils were *Pseudomonas* spp. (~2.5%), *Sphingomonas* spp. (~2.5%), *Mycoplasma* spp. (~2.2%), *Halothiobacillus* spp. (~2.0%) and *Sphingobacteriaceae* spp. (~2.0%). In previously planted JI rhizosphere compost, *Flavobacterium* spp. (~2.5%)

was more abundant compared to Cockle Park rhizosphere soils. The next most abundant species were *Escherichia/ Shigella* spp. (~4.0%), *Vibrio* spp. (~2.0%), *Cytophaga* spp. (~1.7%) and *Flavobacterium* spp. (~1.7%) (detailed data shown in Science Section).

Since yield decline was obtained in soil and compost with different bacterial communities then it could be that the phenomenon is not linked to a specific causal agent. Instead yield decline could be induced by a change in the microbial community (or in microbial species not detected using the current methods) that is able to impact on coriander growth due to an overall functional change. Therefore, it should be noted that the yield decline phenomenon is very complex with different microbial communities having a similar negative impact on coriander crops.

Financial Benefits

The results suggest that coriander yield decline can be induced in soil and compost. Results from the Illumina NGS sequencing indicate that coriander cropping leads to a change in the soil microbial community composition. Hence rhizosphere soil has a different microbial community compared to surrounding bulk soil. However, current findings indicate that no single bacterial species is implicated in yield decline and it is possible that the phenomenon results from a change to the overall function of the microbial community present. This suggests that practices such as tilling after coriander growth, or adding soil amendments could help to alleviate decline by 'resetting' the soil microbial communities to change their overall function. Such remedies require further research but could potentially deliver financial benefit to the UK herb industry.

Action Points

No clear change of practice can yet be recommended.

SCIENCE SECTION

Introduction

Coriander (*Coriandrum sativum* L.) is an annual herb that is of high value with sales of coriander accounting for over 25% of the fresh herb market (Diedrichsen 1996; Segall 2015). Since the 1970s coriander has been grown commercially in the UK; however, the UK production of coriander has been recently hindered by yield decline (Tom Davies, pers. comm.). Reports of coriander yield declines of over 50% have been reported, and if coriander is re-sown in the same affected soil, the issue can persist for up to eight years impacting negatively on the UK herb market. Observational evidence suggests that microbial communities within the soil play a major role in influencing yield decline. The aim of this project is to elucidate the cause of yield decline and to determine potential remedies.

A questionnaire was distributed to growers early in the first year of the project to determine the extent and severity of the yield decline issue. Three out of four UK growers who took part in the questionnaire reported that they experienced coriander yield decline. These growers were located in Surrey, Worcester and Dundee, indicating that yield decline is seen across production areas. Yield decline was reported in a number of soil types including sandy loam, sandy clay and silty clay soils. Of the three growers that suffered from yield decline, all reported that coriander appeared more susceptible to yield decline during the early growing season when weather conditions were cooler and wetter. Furthermore, growers suffering from yield decline indicated that they try to grow coriander in ground that has not been recently cropped with coriander. Plants grown in new soil were reported to be larger and healthier than plants grown in soil that had been previously cropped with coriander.

While growers have observed the coriander yield decline phenomenon in their fields, these reports needed to be scientifically confirmed. Experiments were carried out over the course of the project to determine if coriander yield decline could be induced in a number of soil types and commercial coriander varieties. A review by Bennett *et al.* (2011) stated that yield decline is probably caused by a number of interacting factors which can include deleterious rhizosphere microorganisms; unusual behaviour of mycorrhizal fungi; autotoxicity; and soil properties. Since decline can persist for up to eight years this suggests toxic root exudates (autotoxicity) are likely not the cause as over time these will degrade and bind with the soil matrix (Tom Davies, pers. comm.). Furthermore, other crops can grow well in the same soil indicating that soil quality is not a cause. An experiment done in project year 1 showed that yield was not affected when coriander was grown in soil containing chopped coriander roots, also suggesting that autotoxins are not a contributory factor to yield decline (Appendix 1). The most likely factor is thought to have a soil microbiological basis. Therefore, microbial

communities associated with yield decline soil were examined using Illumina Next Generation Sequencing (NGS) technique and compared to the communities of healthy soils. The Illumina platform was chosen as it allows for large volumes of sequence data at a lower cost to other methods (Metzker, 2010) and can be used to analyse microbial communities in great detail (at least to genus level). It therefore aids in determining the microbial composition of a biome and how it interacts with the environment (Gloor *et al.* 2010).

The overall aim of this study was to induce yield decline in a number of soil types and coriander varieties and to subsequently assess the impact of coriander cropping on soil microbial communities. The following hypotheses were tested:

1. Coriander yield decline can be induced in two commercial coriander varieties in two soil types (Cockle Park soil and John Innes No. 2 compost).
2. Coriander cropping causes a marked change in the soil microbial community, with bulk soil bacterial communities differing from rhizosphere soil bacterial communities in soils planted with a single coriander crop.
3. The bacterial community of a control rhizosphere soil obtained from healthy coriander is different to that of a previously planted rhizosphere soil from coriander exhibiting yield decline symptoms.
4. The bacterial rhizosphere communities of coriander exhibiting yield decline are the same between different soil types (Cockle Park soil and John Innes No. 2 compost).

Materials and methods

Overview

This project focused on inducing yield decline in a number of soil types and varieties. In an initial experiment the coriander variety Santos was used (as it is commonly used by growers) along with sandy soil obtained from a field at Cockle Park Farm, Northumberland, UK. This soil was chosen as it was an agricultural sandy soil with no previous history of coriander cropping. In a second experiment two varieties commonly used by producers of coriander were chosen: Coriander var. Santos and Cruiser; and John Innes No. 2 compost was used. John Innes No. 2 compost was chosen as it could be expected to have consistent properties (and therefore useful to use in different experiments) and have an entirely different microbiota to Cockle Park soil. It was hypothesised, for both experiments, that yield decline would be observed in all varieties and soil types. Greenhouse trials began at Cockle Park in December 2014. Following the greenhouse trials, Illumina NGS was used to compare the microbial communities of yield decline vs. healthy soils.

Testing for coriander yield decline in Cockle Park soil

Experimental set-up

Approximately 30 coriander var. Santos seeds were sown in pots (~12 cm diameter) containing ~700 g air-dried sandy soil collected from a field at Cockle Park, Northumberland with no previous history of coriander cropping. To replicate UK summer growing conditions, plants were exposed to 14 hours of light per day with a maximum air temperature of 20^{o}C during the day and 16^{o}C during the night. Plants were watered on a daily basis and there was a single application of 0.85 g Scott's Peters Professional 20-20-20 fertiliser per pot at week 4 of growth.

This initial crop was harvested ten weeks after sowing. From each pot ten plants were removed fully; the above ground biomass of remaining plants was removed while their roots were left in the soil. All pots were replanted with coriander var. Santos seeds. At the same time, coriander var. Santos seeds were sown in ~700 g of control, previously unplanted, air dried sandy soil obtained from the same field. Therefore, there were two experimental treatments grown under exactly the same conditions, with three replicates per treatment: (1) coriander grown in control soils with no history of coriander cropping; (2) coriander grown in soils planted with coriander once before. All pots were sampled nine weeks after sowing and five plants per pot were randomly sampled for yield data. Fresh weights and dry weights (plants dried in oven for 24 hours at ~60^{o}C) were subsequently measured.

Testing for yield decline in another soil type with more coriander varieties

To determine whether yield decline could be established in a number of soil types and coriander varieties a further experiment was carried out. Pots were set up to contain a single soil type and variety of coriander. John Innes No. 2 compost was chosen alongside coriander var. Santos and Cruiser. These coriander varieties were chosen as they are commonly used by growers. Six pots were set up each containing ~700 g of John Innes compost, three pots were sown with ~30 coriander var. Santos seeds while the remaining three pots were sown with ~30 coriander var. Cruiser seeds. Therefore, there were three replicates of two treatments: (1) John Innes No. 2 compost sown with coriander var. Santos; (2) John Innes No. 2 compost sown with coriander var. Cruiser. Light and temperature settings; watering regime; and fertiliser application remained as above (see 'Establishing coriander yield decline in Cockle Park soil).

All John Innes pots were harvested eight weeks after sowing. From each pot ten plants were removed fully; the above ground biomass of remaining plants was removed while their roots were left in the soil. All pots were then replanted with the same variety of coriander that they

had previously contained. The initial pot design was then repeated and coriander seeds were sown in ~700 g of control John Innes compost with no history of coriander cropping. Therefore, there were four experimental treatments: either control or previously planted John Innes compost planted with either coriander var. Santos or Cruiser. Light and temperature settings; watering regime; and fertiliser applications were the same as above. All pots were harvested eight weeks after sowing and three plants per pot (from three replicate pots, therefore nine plants per treatment) were randomly sampled for yield data. Fresh weights, dry weights (plants dried in oven for 24 hours at ~60^{o}C) were subsequently measured.

Examining the microbial communities of healthy soils vs. yield decline soils

Experimental set-up

Approaches outlined above in both 'Establishing coriander yield decline in Cockle Park soil' and 'Testing another soil type and more coriander varieties for susceptibility to yield decline' were used for this experiment.

Sampling

Upon harvesting all above experiments, three pots per treatment were sampled for bulk and rhizosphere soils for the Cockle Park and John Innes soils. Only pots sown with coriander var. Santos were used for the analysis of soil microbial communities. Bulk soil was classed as soil not tightly adhering to coriander roots; ~3 g of bulk soils were collected from each pot. Rhizosphere soil was classed as soil tightly adhering to coriander roots; five plants per pot were removed for the collection of rhizosphere soil. In summary, DNA was extracted from the following bulk and rhizosphere soils: (1) control Cockle Park and John Innes soils, cropped once with coriander, containing plants exhibiting no yield decline symptoms; (2) Cockle Park and John Innes soils, cropped with coriander twice, containing plants exhibiting yield decline symptoms.

DNA Extraction

For each treatment, DNA was extracted from ~0.5 g bulk and ~0.5 g rhizosphere soil samples as described in the instruction manual of the PowerSoil DNA isolation kit from MO BIO laboratories, Inc. (except at step 5 where samples were shaken using Qiagen® TissueLyser II). Since there were three replicate pots per treatment in the original experimental set-up, this meant that three DNA extractions were performed per treatment.

Three comparisons were conducted: (1) comparing the bacterial communities of coriander rhizosphere soils with their corresponding bulk soils; (2) analysing the bacterial communities associated with the coriander rhizosphere in previously planted soil vs. control soil; (3)

establishing whether coriander yield decline has the same microbial causal agent in different soil types. For clarity, to compare bacterial communities in rhizosphere vs. corresponding bulk soil, DNA was extracted from the following soils: control Cockle Park soils, cropped with coriander once, containing plants exhibiting no yield decline symptoms (three replicates per soil type were taken). To compare bacterial communities of previously planted vs. control soils, DNA was extracted from the following soils: (1) control Cockle Park soils, cropped once with coriander, containing plants exhibiting no yield decline symptoms; (2) previously planted Cockle Park soils, cropped with coriander twice, and containing plants exhibiting yield decline symptoms (three replicates per soil type). Finally, to establish a potential causal agent of coriander yield decline, DNA was extracted from the following soils: (1) previously planted Cockle Park soils, cropped with coriander twice, and containing plants exhibiting yield decline symptoms; (2) previously planted John Innes soils, cropped with coriander twice, and containing plants exhibiting yield decline symptoms (three replicates per soil type).

Illumina NGS sequencing

The DNA extractions were then sequenced using the Illumina MiSeq platform at the NU-OMICS, Northumbria University. This work focussed on sequencing and analysing the bacterial communities in the soil but we have also carried out fungal soil community analysis (detailed results are not shown in this report but a summary is provided in Appendix 2).

The highly robust and accurate UPARSE pipeline, developed by Edgar (2013), was used to analyse the reads following Illumina sequencing. FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to check the quality of forward and reverse reads from the Illumina MiSeq sequencer before proceeding to merge forward and reverse reads using the python script join_paired_ends.py. Merged reads were then quality checked using FastQC. Merged reads were found to have an acceptable sequence quality and were then processed using USEARCH (v8.0.1477_i86osx32) sequence analysis tool (http://www.drive5.com/usearch). During this process samples were labelled and Operational Taxonomic Unit (OTU) tables were created. The data were processed as paired-end reads; to ensure an even sequence depth was obtained from each sample the grep –c command was used to count the number of sequences in each sample. The lowest sequence number, that still allows a good number of sequences, was found; the lowest good sequence number was 1022. The fastq_filter command (with 1.0 error threshold and 240 minimum sequence length) was used to carry out quality filtering on the reads. Reads were then rarefied using fastax_subsample before identifying all unique sequences using the derep_fulllength command. Singletons were discarded using the sortbysize command. Clustering and chimera filtering were carried out using the cluster_otus and

uchime_ref commands, respectively. During the chimera filtering step, the gold database (drive5.com/uchime/uchime_download.html) was used as a reference. Reads were mapped back to the OTUs, using the usearch_global command, and an OTU table was produced using the python script uc2otutab.py. The utax command was used to assign taxonomy to the OTU representative species. Finally, the derep_fulllength and sortbysize commands, followed by fasta_rarify, were used for each sample to create a rarefaction graph. Data received following Illumina MiSeq sequencer was processed and species richness was examined by observing the number of different species from each sample. For clarity, soils were taken from three replicate pots per treatment (outlined in 'DNA Extraction'). Therefore, the mean species richness of three replicate samples for each treatment was analysed.

Statistical Analysis

Statistical analysis on all data was carried out using Analysis of Variance (ANOVA) on Minitab 17. Significant differences between mean values were determined using a Tukey test.

Results

Testing for coriander yield decline in Cockle Park soil

Figure 1 shows that coriander plants grown in control soil, with no history of coriander cropping, had a larger observable biomass compared to those grown in soils previously planted with coriander once before.

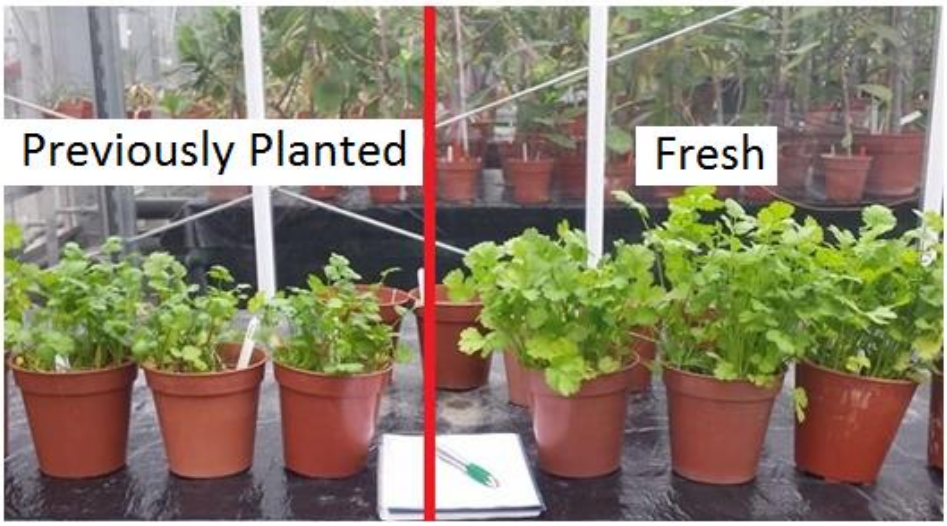


Figure 1. Comparison of observable difference in yield between coriander var. Santos plants grown in control soils and soils previously planted with coriander once before. 'Control' coriander plants were grown in air dried sandy soil collected from a field at Cockle Park farm with no history of coriander cropping. 'Previously Planted' coriander plants were grown in air dried sandy soil, collected from the same field at Cockle Park farm, then used to grow a single coriander crop.

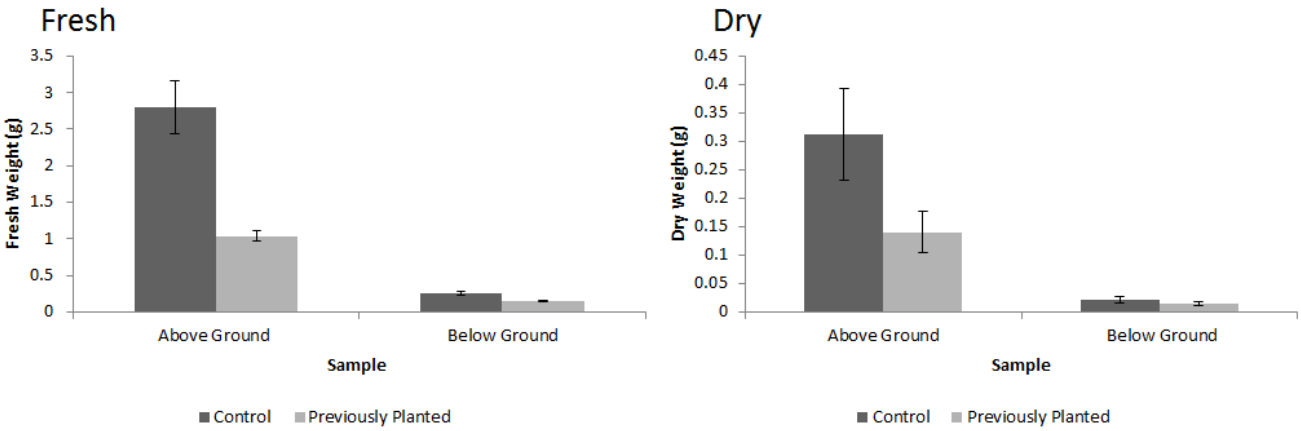


Figure 2. Comparison of mean fresh and dry weights of coriander var. Santos plants grown in control and previously planted Cockle Park soil. Weights for above ground (shoot and leaf) and below ground (roots) are shown. Values plotted are ±1 Standard Error.

Coriander plants grown in control soils, with no history of coriander cropping, had a significantly higher above ground biomass compared to those grown in soils where coriander had been grown once before; plants grown in previously planted soils exhibited symptoms of yield decline. Both fresh ($p=0.001$) and dry ($p=0.01$) above ground; and fresh ($p=0.008$) below ground weights were significantly greater in the control plants than in plants grown in

soil where coriander had been grown before (Fig. 2; see Appendix 3 for raw data). Furthermore, no visible symptoms such as foliar yellowing or root lesions were found on the foliage or roots.

Testing for yield decline in another soil type (John Innes compost) with more coriander varieties

Both coriander var. Cruiser and Santos plants grown in control John Innes compost had a larger visible biomass compared to those grown in previously planted John Innes compost (Fig. 3) but the difference in total biomass was not significantly different ($p>0.05$) (see Figure 4 and Appendix 4 for raw data). The mean above ground biomass produced by both varieties in previously planted soil was approximately half that produced in control soil but there was a high level of variability in the results. It is thought that a larger experiment, with a greater level of replication, would demonstrate a significant above ground yield decrease in previously planted soils.

Figure 3. Comparison of observable difference in yield between coriander var. Santos (above) and Cruiser (below) plants grown in control soils and soils previously planted with coriander once before. 'Control' coriander plants were grown in John Innes No. 2 compost with no history of coriander cropping. 'Previously Planted' coriander plants were grown in John Innes No. 2 compost then used to grow a single coriander crop.

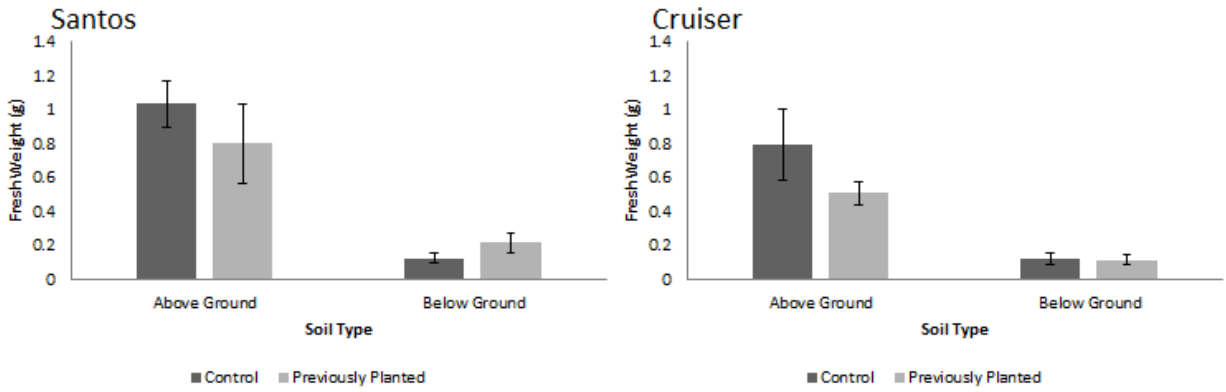


Figure 4. Comparison of mean fresh weights of coriander var. Santos and Cruiser plants grown in control and previously planted John Innes No.2 Compost. Weights for above ground (combined shoot and leaf) and below ground (roots) are shown. Values plotted are ±1 Standard Error.

However, coriander var. Santos shoots of plants grown in control John Innes soil (mean biomass of ~0.06 g) had a significantly greater dry mass compared to those grown in previously planted soils (mean biomass of ~0.03 g; p=0.018; data shown in Appendix 5) .

Comparing the bacterial communities of coriander rhizosphere soils with their corresponding bulk soil

Sequence depth (rarefaction analysis)

Yield decline was observed in the Cockle Park initial soil experiment (above under 'Testing for coriander yield decline in Cockle Park soil'). Work progressed to determine whether coriander growth caused any marked changes to the soil microbial community. To do this the bacterial community of control rhizosphere soils was compared to their corresponding bulk soils taken from the same pot (which had only contained a single coriander crop). A rarefaction curve (Fig. 5) was produced to show whether thorough sampling had occurred. The curve steadily increases before flattening out, indicating that there are no more unique sequences within the sample. Therefore, even if more sequences were included in the analysis, there would be no more unique sequences found. This shows that good sampling has occurred.

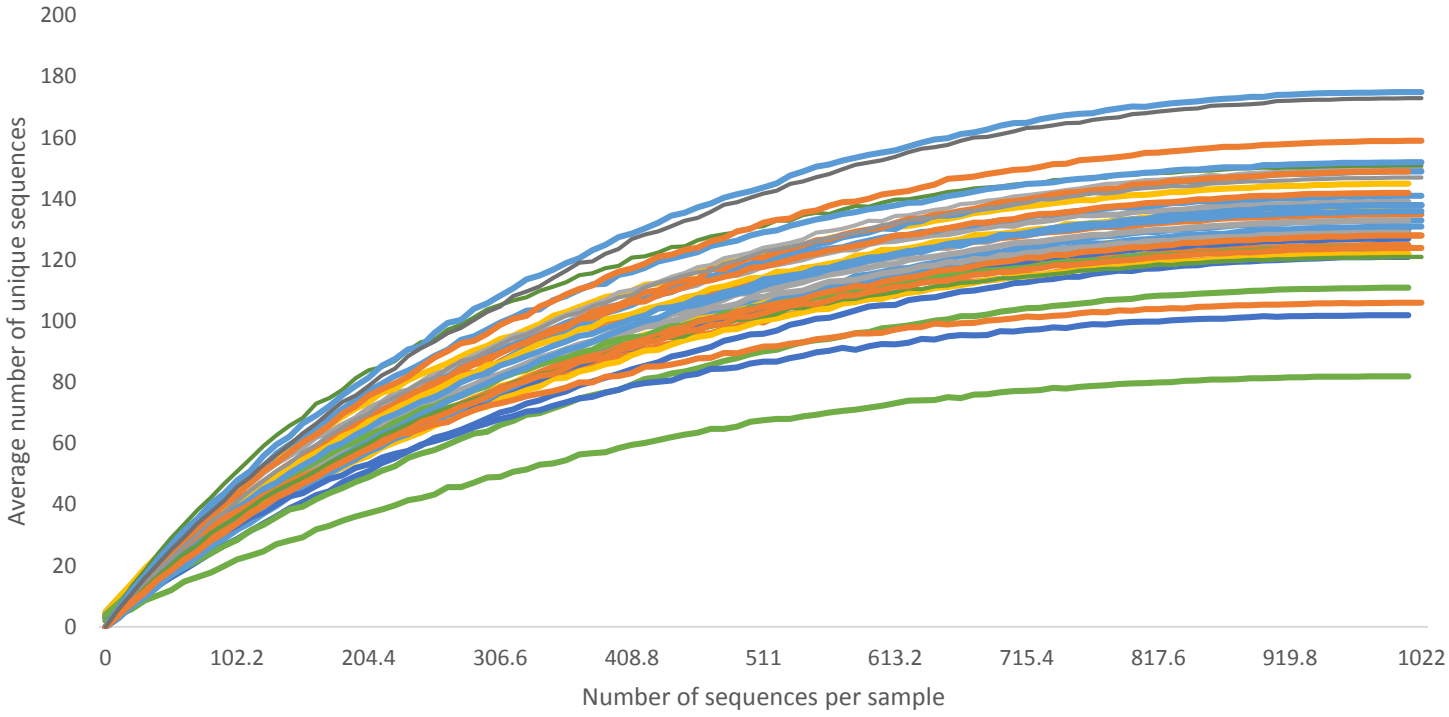


Figure 5. Rarefaction curve of each sample at even sequence depth. Each sample was dereplicated and singletons were discarded using USEARCH (v8.0.1477_i86osx32) before using the fasta_rarify command to produce a rarefaction curve of OTUs for the sample.

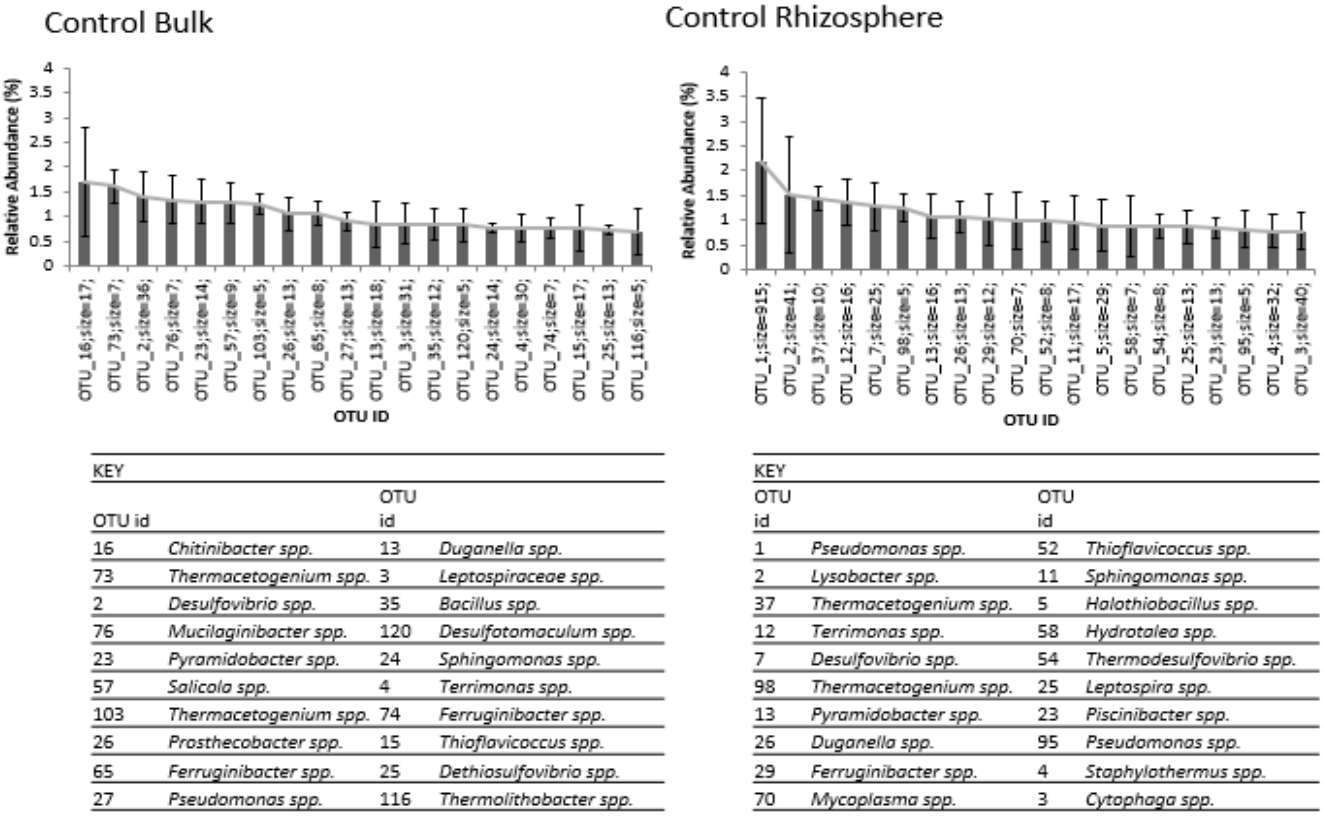
Species richness

We hypothesised that bacterial species richness (number of species found) of control bulk soil would be greater than that of control rhizosphere. However, in the Cockle Park soil, planted only once with coriander, there was no significant difference ($p>0.05$) between the species richness of the healthy rhizosphere soils (183 species) and their corresponding bulk soils (182 species).

Species evenness

Species evenness is a measure of biodiversity used to quantify the relative abundance of species within a community. If a microbial community becomes dominated by one or a few species, its species evenness decreases. A community with lower species evenness is less diverse than a community that contains several species of a more similar abundance. A relative abundance curve of species in a sample can provide an insight into species evenness. This study hypothesised that species evenness is lower in control rhizosphere Cockle Park soils vs. their corresponding bulk. The following relative abundance curves show

the mean relative abundance for three soil samples (rhizosphere samples taken from a replicate pot). Fig. 6 shows that control Cockle Park rhizosphere soil has a similar species evenness compared to corresponding bulk soil. In control rhizosphere soils *Pseudomonas* spp. is the most abundant in the community (~2.2%) with the next most abundant species being *Lysobacter* spp. (~1.5%). In corresponding control bulk soils *Chitinibacter* spp. is the most abundant species (~1.7%) followed by *Thermacetogenium* spp. (~1.6%) and *Desulfovibrio* spp. (~1.4%). Overall, there is no significant difference ($p>0.05$) in abundance of the top three OTUs within any of the soil samples.



KEY			
OTU id		OTU id	
16	*Chitinibacter spp.*	13	*Duganella spp.*
73	*Thermacetogenium spp.*	3	*Leptospiraceae spp.*
2	*Desulfovibrio spp.*	35	*Bacillus spp.*
76	*Mucilaginibacter spp.*	120	*Desulfotomaculum spp.*
23	*Pyramidobacter spp.*	24	*Sphingomonas spp.*
57	*Solicola spp.*	4	*Terrimonas spp.*
103	*Thermacetogenium spp.*	74	*Ferruginibacter spp.*
26	*Prosthecobacter spp.*	15	*Thioflavicoccus spp.*
65	*Ferruginibacter spp.*	25	*Dethiosulfovibrio spp.*
27	*Pseudomonas spp.*	116	*Thermolithobacter spp.*

KEY			
OTU id		OTU id	
1	*Pseudomonas spp.*	52	*Thioflavicoccus spp.*
2	*Lysobacter spp.*	11	*Sphingomonas spp.*
37	*Thermacetogenium spp.*	5	*Halothiobacillus spp.*
12	*Terrimonas spp.*	58	*Hydrotalea spp.*
7	*Desulfovibrio spp.*	54	*Thermodesulfovibrio spp.*
98	*Thermacetogenium spp.*	25	*Leptospira spp.*
13	*Pyramidobacter spp.*	23	*Piscinibacter spp.*
26	*Duganella spp.*	95	*Pseudomonas spp.*
29	*Ferruginibacter spp.*	4	*Staphylothermus spp.*
70	*Mycoplasma spp.*	3	*Cytophaga spp.*

Figure 6. Relative abundance of the top twenty bacterial OTUs in bulk Cockle Park soil and rhizosphere Cockle Park soil. Cockle Park soil was cropped once with coriander, upon harvest the coriander plants did not exhibit yield decline symptoms. Values plotted are ±1 Standard Error.

Microbial community composition

Furthermore, Figure 6 shows that control Cockle Park (planted with a single coriander crop) bulk and rhizosphere soils have different bacterial community compositions. The top five most abundant species in control bulk soils are *Chitinibacter* spp. (~1.7%), *Thermacetogenium* spp. (~1.6%), *Desulfovibrio* spp. (~1.4%), *Mucilaginibacter* spp. (~1.3%), and *Pyramidobacter* spp. (~1.3%). Whereas in control rhizosphere soil *Pseudomonas* spp.

(~2.2%) has replaced *Chitinibacter* spp. as the most abundant species and *Chitinibacter* spp. is no longer within the top twenty most abundant species. In control rhizosphere soil the next most abundant species after *Pseudomonas* spp. are *Lysobacter* spp. (~1.5%), *Thermacetogenium* spp. (~1.5%), *Terrimonas* spp. (~1.4%) and *Desulfovibrio* spp. (~1.4%).

Analysing the bacterial communities associated with the coriander rhizosphere in yield decline soil vs. healthy soil

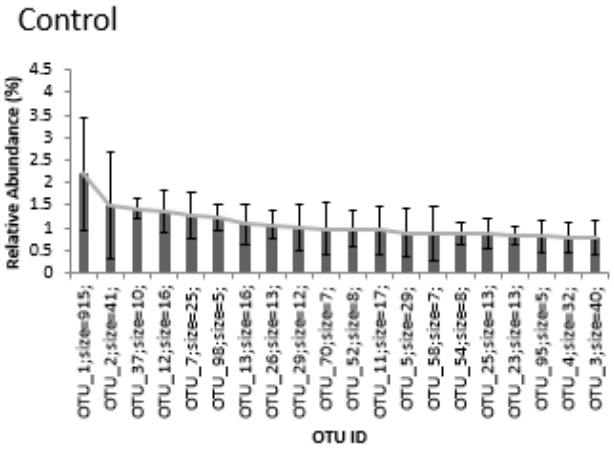
The above work established that coriander cropping causes changes in the bacterial communities of soil, with coriander rhizosphere soils containing a different bacterial community composition compared to their corresponding bulk soils. Subsequent work progressed to determine differences in the microbial community of healthy vs. yield decline rhizosphere soils.

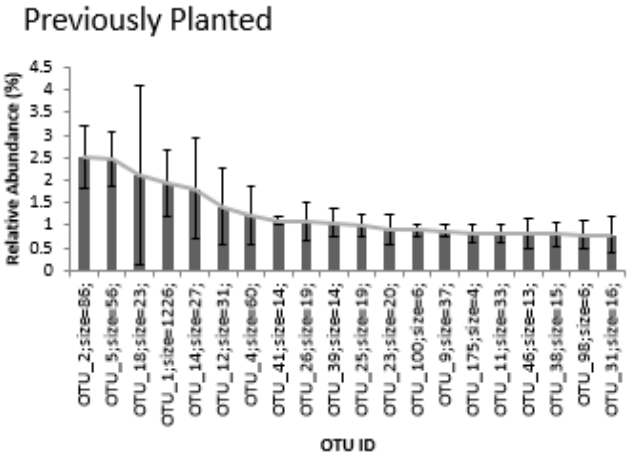
Species richness

Here, the soil bacterial communities of previously planted rhizosphere soils cropped twice vs. control rhizosphere soils cropped once were compared. It was hypothesised that previously planted soils would contain fewer species than control soils and would therefore have decreased species richness. Results obtained show that in Cockle Park soils, the control rhizosphere soils do not have a significantly greater ($p>0.05$) mean number of species (183 species) vs. previously planted soils (177 species).

Species evenness

This study hypothesised that species evenness is lower in previously planted rhizosphere soils vs. control rhizosphere Cockle Park soils. The following relative abundance curves show the mean relative abundance for three soil samples (rhizosphere samples taken from a replicate pot). By viewing Figure 7 it can be seen that control Cockle Park rhizosphere soil has a visibly similar species evenness compared to previously planted Cockle Park rhizosphere soil. In control soils the bacterial relative abundance curve decreases steadily; *Pseudomonas* spp. is the most abundant in the community (~2.2%), *Lysobacter* spp. is the next most abundant species and represents less than 1.5% of the total soil bacterial community. However, there is no significant difference ($p<0.05$) in abundance of *Pseudomonas* spp. and *Lysobacter* spp. Similar to control soils, in previously planted soil *Pseudomonas* spp. is the dominant species (~2.5%).





KEY			
OTU id		OTU id	
1	*Pseudomonas spp.*	52	*Thioflavicoccus spp.*
2	*Lysobacter spp.*	11	*Sphingomonas spp.*
37	*Thermacetogenium spp.*	5	*Halothiobacillus spp.*
12	*Terrimonas spp.*	58	*Hydrotalea spp.*
7	*Desulfovibrio spp.*	54	*Thermodesulfovibrio spp.*
98	*Thermacetogenium spp.*	25	*Leptospira spp.*
13	*Pyramidobacter spp.*	23	*Piscinibacter spp.*
26	*Duganella spp.*	95	*Pseudomonas spp.*
29	*Ferruginibacter spp.*	4	*Staphylothermus spp.*
70	*Mycoplasma spp.*	3	*Cytophaga spp.*

KEY			
OTU id		OTU id	
2	*Pseudomonas spp.*	25	*Caldalkalibacillus spp.*
5	*Sphingomonas spp.*	23	*Leptospira spp.*
18	*Mycoplasma spp.*	100	*Thermacetogenium spp.*
1	*Halothiobacillus spp.*	9	*Caulobacter spp.*
14	*Sphingobacteriaceae spp.*	17	*Mucilaginibacter spp.*
12	*Stenotrophomonas spp.*	11	*Naxibacter spp.*
4	*Lactobacillus spp.*	46	*Lysobacter spp.*
41	*Lysobacter spp.*	38	*Sphingomonas spp.*
26	*Geobacter spp.*	98	*Piscinibacter spp.*
39	*Thioflavicoccus spp.*	31	*Rhizobium spp.*

Figure 7. Relative abundance of the top twenty bacterial OTUs in fresh, control Cockle Park soil and previously planted Cockle Park soil. Control Cockle Park soil was planted with coriander once while previously planted Cockle Park soil was planted with two successive coriander crops. Values plotted are ±1 Standard Error.

Microbial species composition

Figure 7 also shows that control Cockle Park (planted with a single coriander crop) rhizosphere soils have different bacterial community compositions compared to previously planted Cockle Park rhizosphere soils. The top five most abundant species in control rhizosphere soils are *Pseudomonas* spp. (~2.2%), *Lysobacter* spp. (~1.5%), *Thermacetogenium* spp. (~1.4%), *Terrimonas* spp. (~1.4%), and *Desulfovibrio* spp. (~1.3%). In previously planted rhizosphere soil *Pseudomonas* spp. is the most abundant (~2.5%) however it is not significantly ($p>0.05$) more abundant compared to control soils. The next most abundant species are *Sphingomonas* spp. (~2.5%), *Mycoplasma* spp. (~2.2%), *Halothiobacillus* spp. (~2.0%) and *Sphingobacteriaceae* spp. (~2.0%).

Establishing whether coriander yield decline has the same microbial causal agent in different soil types

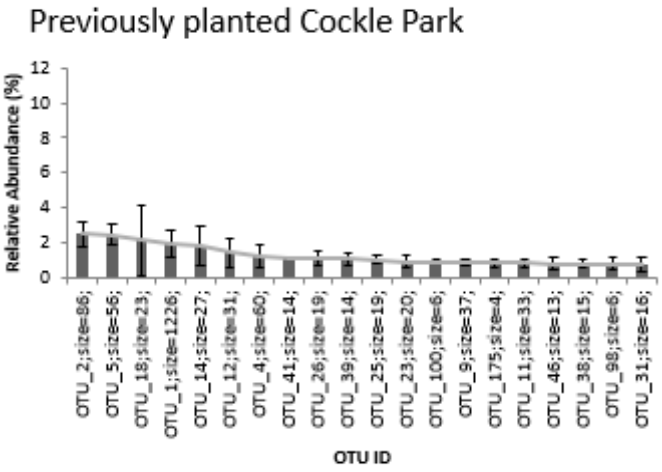
The aim of this section of work was to compare the soil bacterial communities of yield decline rhizosphere soils from different sources (Cockle Park and John Innes soil planted with coriander var. Santos). This was done to investigate whether the same microbe/microbial community are associated with yield decline in different soil types.

Species richness

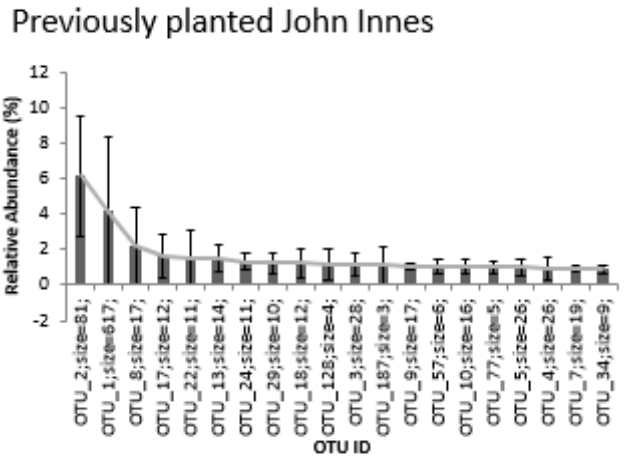
Here, the soil bacterial communities previously planted Cockle Park and John Innes rhizosphere soils were compared. It was found that the Cockle Park rhizosphere soils do not have a significantly greater ($p>0.05$) mean number of species (177+/- 27 species) vs. John Innes rhizosphere soils (158 +/- 19 species).

Species evenness

This study hypothesised that species evenness would be the same in previously planted rhizosphere Cockle Park soils vs. John Innes soils. The following relative abundance curves show the mean relative abundance for three soil samples (rhizosphere samples taken from a replicate pot). Figure 8 shows that previously planted Cockle Park rhizosphere soil has a visibly higher species evenness compared to previously planted John Innes rhizosphere soil probably reflecting the different microbial communities present in these contrasting soil types.



KEY			
OTU id		OTU id	
2	*Pseudomonas spp.*	25	*Caldalkalibacillus spp.*
5	*Sphingomonas spp.*	23	*Leptospira spp.*
18	*Mycoplasma spp.*	100	*Thermacetogenium spp.*
1	*Halothiobacillus spp.*	9	*Caulobacter spp.*
14	*Sphingobacteriaceae spp.*	17	*Mucilaginibacter spp.*
12	*Stenotrophomonas spp.*	11	*Naxibacter spp.*
4	*Lactobacillus spp.*	46	*Lysobacter spp.*
41	*Lysobacter spp.*	38	*Sphingomonas spp.*
26	*Geobacter spp.*	98	*Piscinibacter spp.*
39	*Thioflavicoccus spp.*	31	*Rhizobium spp.*



KEY			
OTU id		OTU id	
2	*Flavobacterium spp.*	3	*Anaerolinia spp.*
1	*Escherichia/ Shigella spp.*	187	*Asanoa spp.*
8	*Vibrio spp.*	9	*Pyramidobacter spp.*
17	*Cytophaga spp.*	57	*Prosthecobacter spp.*
22	*Flavobacterium spp.*	10	*Cytophaga spp.*
13	*Thiobacillus spp.*	77	*Prosthecobacter spp.*
24	*Desulfotomaculum spp.*	5	*Polyangiaceae spp.*
29	*Alterococcus spp.*	4	*Steroidobacter spp.*
18	*Pseudomonas spp.*	7	*Devosia spp.*
12	*Pseudomonas spp.*	34	*Rugmonas spp.*

Figure 8. Relative abundance of the top twenty bacterial OTUs in previously planted Cockle Park and John Innes rhizosphere soil. Previously planted Cockle Park and John Innes soils were planted with two successive coriander crops. Values plotted are ±1 Standard Error.

Microbial species composition

Comparisons between the two soil types show that previously planted Cockle Park rhizosphere soil has a different microbial community composition compared to previously planted John Innes rhizosphere soil. The top five most abundant species in previously planted Cockle Park rhizosphere soils are *Pseudomonas* spp. (~2.5%), *Sphingomonas* spp. (~2.5%), *Mycoplasma* spp. (~2.2%), *Halothiobacillus* spp. (~2.0%) and *Sphingobacteriaceae* spp. (~2.0%). In previously planted John Innes rhizosphere soil *Flavobacterium* spp. (~2.5%) has become more abundant compared to Cockle Park rhizosphere soils. The next most abundant species are *Escherichia/ Shigella* spp. (~4.0%), *Vibrio* spp. (~2.0%), *Cytophaga* spp. (~1.7%) and *Flavobacterium* spp. (~1.7%; Fig. 8).

Discussion

Coriander yield decline successfully shown to occur in Cockle Park soil

Coriander yield decline is an observable phenomenon in growers' fields with producers of coriander reporting declines of up to 50%. Until recently, it was unknown whether it would be possible to establish yield decline under controlled greenhouse conditions. In this study, an experiment was conducted to determine whether yield decline could be induced under controlled conditions using coriander var. Santos and soil collected from a field at Cockle Park, Northumberland (not previously cultivated with coriander). This study successfully managed to induce yield decline under controlled conditions, with a significant reduction in the fresh weight of above ground biomass being observed in the initial Cockle Park trial.

Yield decline successfully induced in more soil types and varieties

To establish yield decline, this study compared the above and below ground biomass of plants grown in control soils vs. previously planted soils. Visual observations (see Grower Summary for photographs), taken before the plants were harvested, indicate that yield decline did occur in the John Innes compost in both varieties of coriander. In general, both the fresh and dry *total* above and below ground biomass of plants grown in control John Innes compost were greater than plants grown in previously planted soils, though not significantly (Fig. 3). For instance, the dry above ground biomass for both varieties of coriander grown in fresh soil was around double the biomass found in plants in previously planted soils (Fig. 4). The lack of significant difference in total above ground biomass may be due to high variation within the coriander plants and this study would need to be repeated with greater levels of replication. However, results looking at *shoot*s alone did demonstrate a significant reduction in biomass production in plants grown in soils previously planted with coriander. This demonstrates that yield decline is occurring and was reproduced in John Innes compost.

Yield decline soils appear to be associated with changes in microbial community composition

This study has shown that cropping coriander repeatedly in the same soil can lead to changes in the bacterial community of that soil. One crop of coriander was grown in Cockle Park soil and upon harvest exhibited no signs of yield decline. Subsequent microbial community analysis showed that the bacterial communities of the rhizosphere soil (soil in close association with coriander roots) were different to the communities associated with bulk soil. For instance, the most abundant species in the Cockle Park (planted once with coriander) bulk soils was *Chitinibacter* spp. whereas in the corresponding rhizosphere soil the most

abundant species was *Pseudomonas* spp.. Furthermore, when this control Cockle Park rhizosphere soil (soil only cropped once with coriander) is compared to previously planted Cockle Park rhizosphere soil (soil cropped twice successively with coriander) it can be seen that the soil bacterial communities are once more altered. *Pseudomonas* spp. remains the most abundant species in both healthy and yield decline Cockle Park rhizosphere soils, yet the remaining top four species differ between the two treatments. Overall it was shown that coriander has a marked effect on soil microbial communities, with bulk soil samples having a different community composition to corresponding rhizosphere samples. It could be that a significant change to the microbial community is associated with yield decline; however, more work will be required to pursue this theory.

This study also analysed the bacterial communities of two different soil types to see if a common microbe/microbial community could be linked to yield decline. The previously planted rhizosphere of Cockle Park and John Innes soil was compared and it was found that they contained different bacterial communities. In Cockle Park rhizosphere soil the most abundant species were *Pseudomonas* spp. and *Sphingomonas* spp., whereas in John Innes rhizosphere soil *Flavobacterium* spp. and *Escherichia/ Shigella* spp. were the most abundant. Similar results were also observed with fungal communities in yield decline compared to healthy soils i.e. no single causal agent could be determined (see Appendix 2 for data). Since yield decline was obtained in different soil types with different microbial communities then it could be that the phenomenon is not linked to a specific causal agent. Instead yield decline could be induced by a change in the soil microbial community that is able to impact on coriander growth due to an overall functional change. Therefore, it should be noted that the yield decline phenomenon is very complex with different microbial communities having a similar negative impact on coriander crops.

After an extensive literature search it was found that there is no published data on the effect of cropping coriander on soil microbial communities. However, Hilton *et al*. (2013) demonstrated that continuously cropping oil seed rape had a significant effect on rhizosphere fungal communities. In particular, the study found that this change in fungal community was mostly due to an increase in abundance of two fungal species. Bever *et al.* (2012) state in their review, that an individual plant species can alter the microbial community composition. Plants influence the soil microbial communities through the release of plant exudates which alter soil chemistry and provide nutrient sources; these factors in turn influence the microbial composition of the soil. These changes to microbial composition can have important consequences on plant ecology, for instance the plant could inadvertently select for a microbial composition that has a negative effect on its growth. Such deleterious microbes

can restrict plant growth via production of chemicals that interfere with root development or by competing for nutrients or preventing delivery of plant growth substances (Bennet *et al*., 2012). Therefore, continuous cropping of coriander is likely altering the soil microbial composition. As discussed, coriander crops may be selecting for deleterious microorganisms or even groups of microorganisms that function in a way that inhibits growth.

Future work

This project has demonstrated that yield decline does occur in multiple soil types and varieties, with Illumina NGS results suggesting that an underlying cause could be changes in microbial communities. Future work should aim to determine potential methods to alter microbial communities and examine any subsequent effects on yield decline. Practices such as tilling or drying out the soil could alter the microbial community and alleviate yield decline. Bennet *et al*. (2011) suggest that full tillage could alleviate the effects of overwintering deleterious microorganisms. Furthermore, Fierer *et al*. (2003) conducted an experiment that demonstrated how drying and rewetting a soil can cause changes to soil bacterial community composition. Since the microbial community influences soil processes, any changes to soil microbial composition could alter soil functional capacity and alleviate yield decline. Future work could involve tilling and dry-rewetting experiments to examine if such practices could be implemented to alleviate yield decline. Such experiments would need to determine the depth at which tilling and soil drying are most effective at alleviating decline. It should also be noted that soil drying would need to take place in-between successive crops to prevent coriander bolting due to unevenness of soil moisture during growth. Also, soil amendments such as composts, soil fumigation and organic matter could be used to alleviate yield decline. Marschner e*t al.* (2003) found that the use of organic and inorganic soil amendments significantly affected soil bacterial community structure. Therefore, soil amendments may alter the soil bacterial community composition and effectively reset the soil and alleviate yield decline. Furthermore, it may be useful for further experiments to be conducted to determine an appropriate crop rotation protocol. Such experiments could determine the length of time required before coriander can be replanted in a field and not suffer from yield decline; and the best intermediate crop to use.

The next stage of investigation should also aim to determine the function of the microbial communities in yield decline soils. For instance, Xie *et al.* (2013) carried out Illumina sequencing on samples before aligning genes against a protein database. This study was then able to determine the top three functions of the microbial communities from their samples. However, there is a limitation; since most of the soil microbiota is unknown this means that the majority of their data contained microorganisms with an unknown function.

Therefore, the community may have a collective function that was not detected by this analysis. Understanding the function of microbial communities and how they subsequently influence plant growth could shed light on how to manage such populations to alleviate yield decline.

Conclusions

- Coriander yield decline was replicated under controlled conditions in two soil types (one field soil, one compost) and two varieties (Santos and Cruiser).
- The yield decline phenomenon is a complex one; it seems that separate incidences of yield decline do not share a single microbial cause. Therefore, different microbial community compositions can lead to similar effects on a coriander crop.

Knowledge and Technology Transfer

Networking at the AHDB Student Conference 2015

Site visit to Red Deer Farm to collect soil samples

Networking at the British Herb Trade Association (BHTA) Conference 2015

Newcastle University Postgraduate Poster Conference 2015

Newcastle University Postgraduate Conference 2016

Presentation by supervisor (I. Singleton) at BHTA Technical Conference, November 2016

Glossary

Bulk soil – the soil that is not in a close association, or under the direct influence of, coriander roots.

Control soil – a soil used for the growth of coriander which has no previous history of coriander cropping.

FastQC – an analysis module that enables quality control checks on raw sequence reads following Illumina NGS.

Illumina Next Generation Sequencing (NGS) – a modern high-throughput sequencing method that is used in this study to sequence microbial DNA.

Operational Taxonomic Unit (OTU) – is a group of closely related individuals whose sequences are highly similar to one another (usually 97% similarity).

Previously planted soil – a soil used for the growth of coriander which has already contained at least one crop of coriander before being immediately replanted.

Rhizosphere soil – the soil that adheres tightly to, and is influenced by, coriander roots

Rarefaction curve – a plot of the number of species obtained against the number of samples. This allows for subsequent calculations of species richness for a set number of samples.

USEARCH – a sequence analysis tool used in this study to process and analyse raw sequence reads following Illumina NGS.

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Appendices

Appendix 1 – Discounting autotoxicity as a cause for coriander yield decline

An experiment was carried out during year 1 to determine the effect of adding chopped coriander roots to soil on coriander growth. Approximately 30 coriander var. Santos seeds were sown in pots (~12 cm diameter) containing ~700 g air-dried sandy soil collected from a field at Cockle Park Farm, Northumberland with no previous history of coriander cropping. Three root treatments were used, with three replicate pots per treatment: (1) soil with sterilised coriander roots added; (2) soil with non-sterilised coriander roots added; (3) control, soil with no coriander roots added. Both sterile and non-sterile root treatments were included as this would allow us to determine whether any subsequent effects on plant growth were due to microbes that the roots may harbour, or to the roots themselves. Sterilised coriander roots had been sterilised via a treatment of 1% sodium hypochlorite for 6 min, followed by treatment of 70% ethanol for 30 seconds. Light and temperature settings; watering regime; and fertiliser application remained as above (see 'Establishing coriander yield decline in Cockle Park soil). All pots were sampled eight weeks after sowing and three plants per pot were randomly sampled for yield data. Fresh weights and dry weights (plants dried in oven for 24 hours at ~60^{o}C) were subsequently measured.

Coriander plants grown in soils containing sterilised roots were not significantly larger ($p>0.05$) compared to plants grown in soils with no added roots (Figure 9). Therefore, yield was not reduced when coriander was grown in soil containing chopped coriander roots; this suggests that autotoxins are not a contributory factor to yield decline**.** Furthermore, coriander plants grown in soils with sterilised roots added had a significantly higher above ground biomass compared to those grown in soils where non-sterilised roots had been added ($p=0.047$). This could provide more evidence for a microbial cause of coriander yield decline, and suggest that while the root residues themselves do not contribute to yield decline, the microbes they harbour might have a deleterious effect on plant growth.

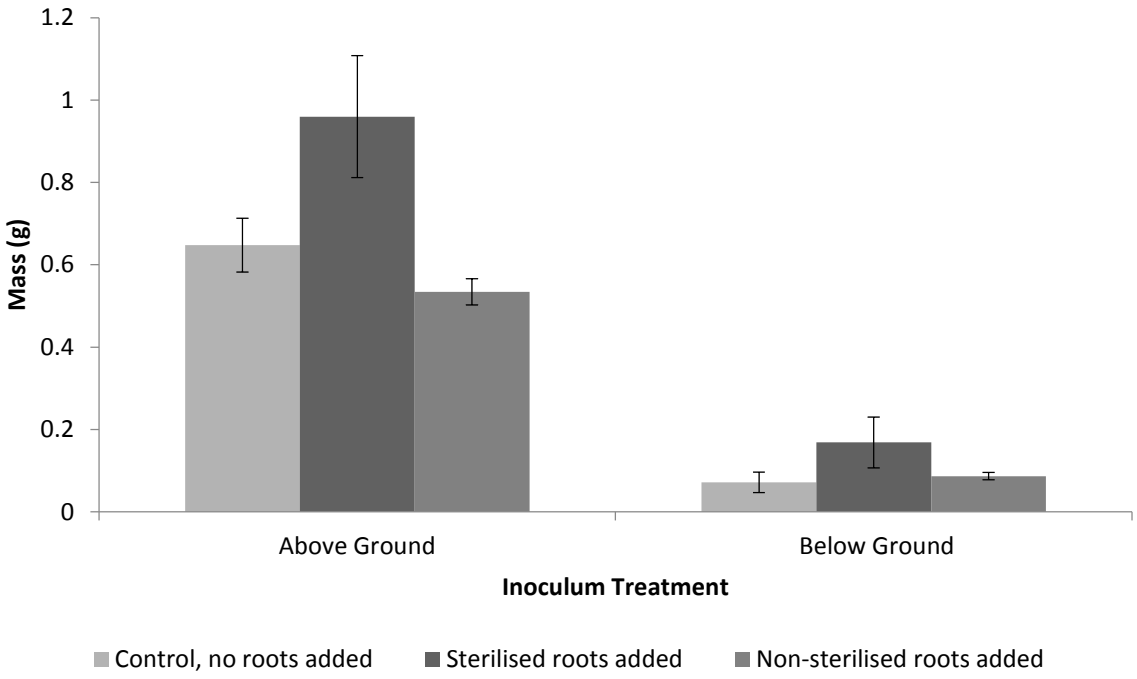
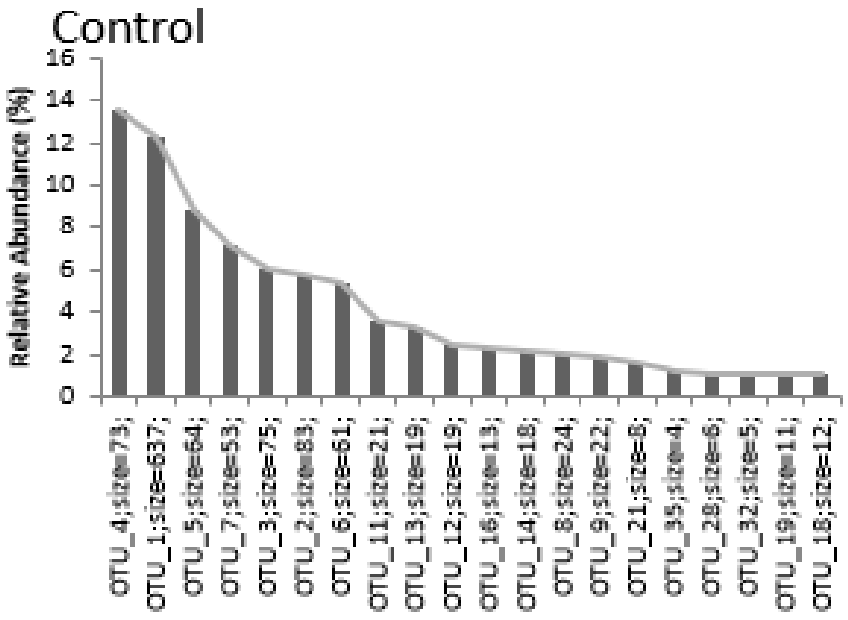


Figure 9. Comparison of mean fresh weights of coriander var. Santos plants grown in soils with various root treatments: (1) control, no roots added; (2) sterilised roots added, (3) non-sterilised roots added. Weights for above ground (combined shoot and leaf) and below ground (roots) are shown. Values plotted are ±1 Standard Error.

Appendix 2 - Summary of fungal communities associated with yield decline soils

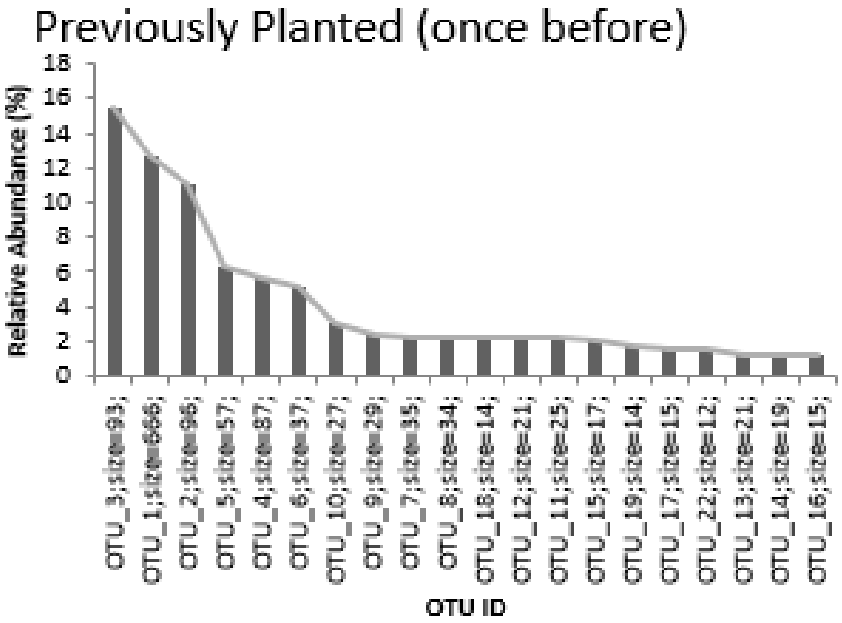
The experiment described under 'Establishing coriander yield decline in Cockle Park soil' was carried out. This experiment was then extended; all soils were replanted with coriander var. Santos and at the same time coriander var. Santos was sown in fresh Cockle Park soils with no history of coriander cropping. Therefore, there were 3 treatments (with 3 replicates per treatment): (1) control, coriander var. Santos grown in fresh Cockle Park soil with no history of coriander cropping; (2) coriander var. Santos grown in Cockle Park soil that had been previously cropped with coriander once before; (3) coriander var. Santos grown in Cockle Park soil that had been previously cropped with coriander twice before. Pots were then sampled as mentioned in 'Materials and methods' under 'Examining the microbial communities of healthy soils vs. yield decline soils.'

The fungal communities of rhizosphere soils taken from control (no history of coriander cropping); previously planted with coriander once before; and previously planted with coriander twice before were analysed. Fungal species evenness was greatest in the control soils; by viewing Figure 10 it can be seen that the relative abundance curve in this soil decreases steadily. In control soils the most abundant species is *Tilletia* spp. (~14%) followed by *Lecythophora* spp. (~12%), *Tomentellopis* spp. (~9%) and *Gliocladium* spp. (~7%). Fungal species evenness is subsequently lower in soils previously planted with coriander once and twice before. In soils planted with coriander once before three species dominate: *Lophodermium* spp. (~15%) and two *Hypocrea* spp. (~13% and 11%), the next most abundant species is *Tilletia* spp. which only accounts for ~6% of the fungal community. In soils planted with coriander twice before, two species dominate: *Zopfiella* spp. (~16%) and *Ramalina* spp. (~12%), the next most abundant species is *Pseudaleuri* spp. which only accounts for 5% of the fungal community (Figures 10-12).



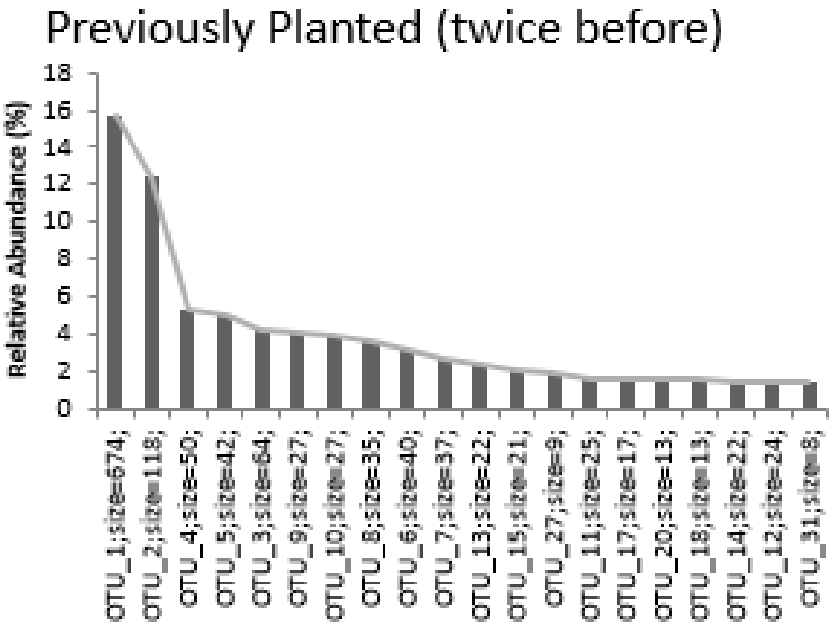
KEY (Control)	
OTU id	OTU id
4 Tilletia spp.	16 Thelebolus spp.
1 Lecythophora spp.	14 Cryptococcus spp.
5 Tomentellopsis spp.	8 Zopfiella spp.
7 Gliocladium spp.	9 Hypocrea spp.
3 Waitea spp.	21 Cryptococcus spp.
2 Lophodermium spp.	35 Tilletia spp.
6 Ramalina spp.	28 Tricladium spp.
11 Sistotrema spp.	32 Leucosporidiella spp.
13 Gibberella spp.	19 Zopfiella spp.
12 Physcia spp.	18 Coprinellus spp.

Figure 10. Relative abundance of the top twenty fungal OTUs in control Cockle Park soil. Control Cockle Park soil was planted with coriander once.



KEY (Previously Planted (once before))			
OTU id		OTU id	
3	Lophodermium spp.	18	Pseudallescheria spp.
1	Hypocrea spp.	12	Ramalina spp.
2	Hypocrea spp.	11	Trichophaea spp.
5	Tilletia spp.	15	Cryptococcus spp.
4	Sclerostagonospora spp.	19	Cryptococcus spp.
6	Exophiala spp.	17	Fusicolla spp.
10	Gliocladium spp.	22	Arachnopeziza spp.
9	Tilletia spp.	13	Thelebolus spp.
7	Tricladium spp.	14	Pseudaleuria spp.
8	Tilletia spp.	16	Thanatephorus spp.

Figure 11. Relative abundance of the top twenty fungal OTUs in previously planted (once before) Cockle Park soil.



KEY (Previously Planted (twice before))

OTU id		OTU id	
1	Zopfiella spp.	13	Lophodermium spp.
2	Ramalina spp.	15	Gliocladium spp.
4	Pseudaleuria spp.	27	Coprinopsis spp.
5	Physcia spp.	11	Sporomiella spp.
3	Physcia spp.	17	Fusicolla spp.
9	Exophiala spp.	20	Cryptococcus spp.
10	Pseudallescheria spp.	18	Ramalina spp.
8	Ramalina spp.	14	Trichophaea spp.
6	Pseudallescheria spp.	12	Thelebolus spp.
7	Trichophaea spp.	31	Centraria spp.

Figure 12. Relative abundance of the top twenty fungal OTUs in previously planted (twice before) Cockle Park soil.

Appendix 3 – Raw data from 'Testing for coriander yield decline in Cockle Park soil'

Raw data for fresh and dry coriander var. Santos plant weights during the experiment to establish yield decline in Cockle Park soil (all values measured in grams). The treatments were control (coriander grown in soils with no previous history of coriander cropping) and previously planted (coriander grown in soils previously used to grow coriander once).

Control, Fresh Treatment Fresh Weights	Plant Replicate	Tissue Type: Shoot	Leaves	Root
Pot 1	1	1.3364	0.2794	0.182
	2	2.2651	1.653	0.315
	3	0.9649	0.8585	0.0993
	4	2.728	1.8594	0.3125
	5	0.5583	0.5784	0.059
Pot 2	1	1.5795	1.3245	0.1989
	2	3.3768	2.1684	0.5221
	3	1.8119	1.181	0.3957
	4	1.3367	0.8774	0.2279
	5	1.877	1.4579	0.2727
Pot 3	1	2.8097	2.0033	0.4792
	2	1.4293	1.1328	0.2107
	3	0.9313	0.7905	0.2203
	4	0.8906	0.7642	0.1623
	5	0.7093	0.5139	0.165

Previously Planted Treatment Fresh Weights	Plant Replicate	Tissue Type: Shoot	Leaves	Root
Pot 1	1	0.2953	0.2678	0.1132
	2	0.4199	0.4337	0.1184
	3	0.4107	0.4079	0.1835
	4	0.4829	0.4436	0.1129
	5	0.3995	0.4847	0.1134
Pot 2	1	0.8966	0.7239	0.2646
	2	0.6996	0.4442	0.2176
	3	0.9134	0.5303	0.2615
	4	0.5765	0.5394	0.1048
	5	0.4725	0.3996	0.173
Pot 3	1	0.6099	0.5092	0.1233
	2	0.7201	0.4053	0.1449
	3	0.6713	0.5488	0.1231
	4	0.5795	0.5019	0.1362
	5	0.3859	0.325	0.0511

Control, Fresh Treatment Dry Weights	Plant Replicate	Tissue Type: Shoot	Leaves	Root
Pot 1	1	0.166	0.172	0.048
	2	0.272	0.308	0.032
	3	0.133	0.183	0.049
	4	0.742	0.429	0.034
	5	0.25	0.165	0.017
Pot 2	1	0.461	0.32	0.036
	2	0.709	0.307	0.086
	3	0.484	0.184	0.08
	4	0.396	0.176	0.045
	5	0.509	0.335	0.056
Pot 3	1	0.744	0.471	0.098
	2	0.485	0.267	0.053
	3	0.252	0.153	0.04
	4	0.23	0.109	0.039
	5	0.177	0.096	0.036

Previously Planted Treatment Dry Weights	Plant Replicate	Tissue Type: Shoot	Leaves	Root
Pot 1	1	0.127	0.098	0.022
	2	0.163	0.126	0.025
	3	0.131	0.094	0.042
	4	0.169	0.106	0.04
	5	0.207	0.146	0.026
Pot 2	1	0.33	0.178	0.069
	2	0.289	0.214	0.053
	3	0.313	0.168	0.067
	4	0.197	0.14	0.027
	5	0.201	0.15	0.045
Pot 3	1	0.156	0.065	0.028
	2	0.212	0.119	0.032
	3	0.155	0.155	0.034
	4	0.189	0.105	0.026
	5	0.109	0.075	0.03

Appendix 4 – Raw data from 'Testing for yield decline in another soil type with more coriander varieties'

Raw data for fresh and dry coriander var. Santos plant weights during the experiment to establish yield decline in John Innes compost (all values measured in grams). The treatments were control (coriander grown in soils with no previous history of coriander cropping) and previously planted (coriander grown in soils previously used to grow coriander once).

Control, Fresh Treatment		Tissue Type		
Fresh Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.7702	0.4929	0.078
	2	0.2812	0.1645	0.0326
	3	0.6209	0.3162	0.0751
Pot 2	1	0.4557	0.2105	0.0991
	2	1.1311	0.5864	0.2685
	3	0.8793	0.1881	0.2144
Pot 3	1	0.6771	0.4524	0.1336
	2	0.8636	0.5884	0.1861
	3	0.3643	0.2859	0.062

Previously Planted Treatment		Tissue Type		
Fresh Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	1.596	0.9211	0.6499
	2	0.5558	0.2978	0.1216
	3	0.6268	0.3718	0.0818
Pot 2	1	0.3159	0.3098	0.2447
	2	0.2166	0.139	0.3482
	3	0.1525	0.0669	0.1368
Pot 3	1	0.4563	0.3074	0.1817
	2	0.1392	0.079	0.0625
	3	0.4604	0.2096	0.1273

Control, Fresh Treatment		Tissue Type		
Dry Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.0439	0.102	0.0023
	2	0.0255	0.0524	0.0106
	3	0.0573	0.0974	0.0308
Pot 2	1	0.0342	0.0906	0.0122
	2	0.0839	0.2604	0.0841
	3	0.1292	0.1107	0.0566
Pot 3	1	0.097	0.124	0.0467
	2	0.1225	0.1426	0.0635
	3	0.0517	0.0811	0.0284

Previously Planted Treatment		Tissue Type		
Dry Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.072	0.2476	0.0815
	2	0.0257	0.0653	0.0107
	3	0.0489	0.0714	0.014
Pot 2	1	0.0272	0.1176	0.0303
	2	0.0122	0.0759	0.0422
	3	0.0052	0.0413	0.003
Pot 3	1	0.0506	0.082	0.0484
	2	0.0208	0.0141	0.0079
	3	0.0373	0.0173	0.0077

Raw data for fresh and dry coriander var. Cruiser plant weights during the experiment to establish yield decline in John Innes compost (all values measured in grams). The treatments were control (coriander grown in soils with no previous history of coriander cropping) and previously planted (coriander grown in soils previously used to grow coriander once).

Control, Fresh Treatment		Tissue Type		
Fresh Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.6414	0.5268	0.2955
	2	0.6434	0.5608	0.1343
	3	1.3209	0.7849	0.2678
Pot 2	1	0.256	0.2014	0.0846
	2	0.2968	0.186	0.0547
	3	0.1394	0.0692	0.0367
Pot 3	1	0.2003	0.1708	0.0989
	2	0.613	0.2668	0.0928
	3	0.1799	0.0934	0.0589

Previously Planted Treatment		Tissue Type		
Fresh Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.2532	0.1869	0.0641
	2	0.4057	0.2282	0.0683
	3	0.4187	0.2678	0.0369
Pot 2	1	0.0991	0.1073	0.0473
	2	0.3178	0.2711	0.3317
	3	0.4141	0.2654	0.1828
Pot 3	1	0.3885	0.3488	0.1179
	2	0.2156	0.156	0.1258
	3	0.1522	0.1017	0.0872

Control, Fresh Treatment		Tissue Type		
Dry Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.0465	0.099	0.0436
	2	0.0537	0.1383	0.0293
	3	0.1369	0.1781	0.0317
Pot 2	1	0.0264	0.0561	0.0171
	2	0.0304	0.0529	0.0133
	3	0.0224	0.0259	0.0153
Pot 3	1	0.0331	0.0465	0.0252
	2	0.0805	0.1036	0.0362
	3	0.0077	0.0204	0.0075

Previously Planted Treatment		Tissue Type		
Dry Weights	Plant Replicate	Shoot	Leaves	Root
Pot 1	1	0.0153	0.0535	0.0098
	2	0.0394	0.0756	0.0323
	3	0.0465	0.0586	0.0267
Pot 2	1	0.0145	0.038	0.0173
	2	0.0345	0.1046	0.0518
	3	0.0164	0.0985	0.0249
Pot 3	1	0.0173	0.0544	0.0138
	2	0.0126	0.0393	0.0119
	3	0.0182	0.0128	0.0121

Appendix 5 –Dry John Innes shoot data from 'Testing for yield decline in another soil type with more coriander varieties'

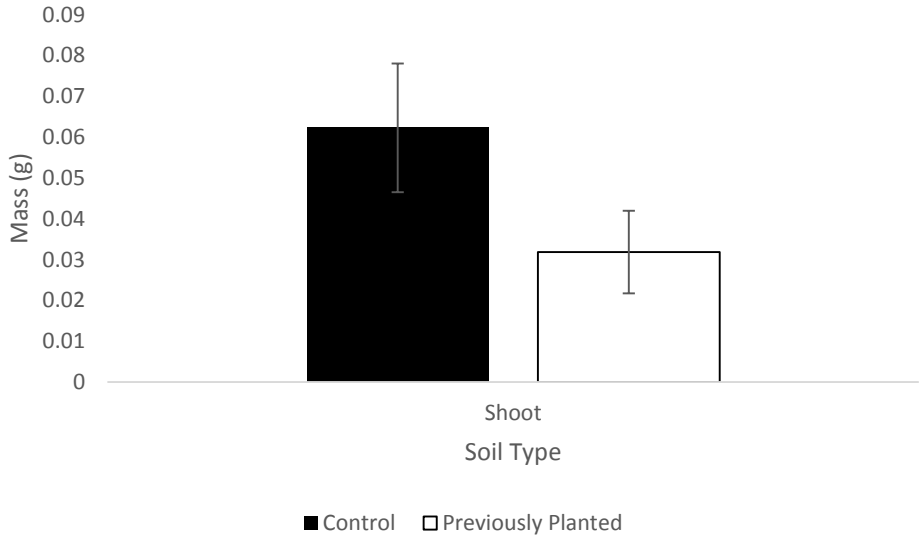


Figure 13. Comparison of mean dry shoot weights of coriander var. Santos plants grown in control and previously planted John Innes No.2 Compost. Values plotted are ±1 Standard Error.