Coriander Yield Decline: Potential management options

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Declaration

I, Amanda L. Jones hereby certify that this thesis and the work presented in it are the result of my own original research. No part of this work has been submitted for any degree other than Master by Research at Edinburgh Napier University. Where other sources of information have been used, they have been duly acknowledged.

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Abstract

Crop yield decline is increasingly associated with the intensive practices of modern agriculture. It affects a wide range of crops, including Coriander (*Coriandrum sativum* L.), the UK's most economically important herb. The crop suffers from a particularly severe form of decline, which can reduce yields by 50%. Unlike other widely grown crops, the growth of coriander in the UK has not been optimised, and growers use highly variable practices. The main aim of this study was to investigate crop and soil management techniques which could reduce coriander yield decline: *e.g.*, different depths of tillage, various sowing densities, and the desiccation or sterilisation of crop soils. Glasshouse pot trials were used to assess the efficacy of these practices at reducing yield decline in successive coriander crops. Results showed reduced levels of decline when soils were: harrowed (compared to unharrowed), and sown at a 'medium' density (compared to a relatively low or high density). Coriander grown for a second cycle under a set of 'optimum growth' conditions still experienced some decline, suggesting a level of microbial involvement.

To investigate the potential involvement of soil microbes, soil desiccation and soil sterilisation were assessed as soil management techniques. Desiccation of crop soils after one cycle of crop growth prevented yield decline in a subsequent crop. Additionally, sterilisation of field soils (showing severe decline symptoms) produced 50% greater yields per pot and 70% larger plants, compared to a crop grown in non-sterilised field soils. MinION nanopore sequencing (16S and ITS barcode approach) was used to facilitate a microbial community study. Identifications were made for fungal and bacterial taxa of rhizosphere and bulk soils in a grower's field soils and in soils from the glasshouse desiccation experiment. Results showed a defined shift in fungal taxa between healthy and yield decline samples. Overall results indicated a multifactorial problem, with the likely involvement of deleterious soil microorganisms. The next stages of investigation should be to assess the efficacy of a set of management strategies and optimised growth parameters in a field trial environment. Greater replication and further study are needed to elucidate the microbiological mechanisms of coriander yield decline, including potentially identifying specific associated microorganisms.

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Abbreviations

µl	Microlitre
ANOVA	Analysis of variance
CYD	Coriander yield decline
DNA	Deoxyribose nucleic acid
g	Gram
K	Potassium
N	Nitrogen
ng	Nanogram
P	Phosphorus
UK	United Kingdom

Chapter 1: General introduction

1.1 Global and UK herb production

Globally, the herb and spice sector has been experiencing rapid growth for several decades, as growers strive to meet the demands of booming economies alongside the increasing recognition of the health and culinary merits of herbs and spices (FAO, 2018). The world-wide herb and spice trade currently represents a multi-billion-dollar industry, and one that is characterised by its vast diversity (FAO, 2018). Producers range from 'backyard' growers to huge operations. About fifty plant species are considered of global trade significance in the herb and spice sector. A great range of by-products are harvested from these plants, many of which are 'high value' commodities (Matthews and Jack, 2011). Consequently, these crops are of increasing economic importance; supporting many of the world's rural and developing economies, and also contributing significantly to larger economies. Within this global sector, the UK herb industry has seen rapid growth since the 1960s (BHTA, 2018), and consequently represents an economically important range of crops. However, the sector is highly diverse, and figures specifically for UK herbs are limited. These crops fall under the umbrella of UK horticulture, a valuable industry which employs over 100,000 full-time and seasonal workers, and contributes over three billion pounds annually to the UK GDP (Gross Domestic Product) (BGA, 2018). The economic importance of the UK herb industry is set to continue in response to country's increasing demand for herb products.

1.1.1 Coriander: an important part of the global and UK herb industries

Coriander is a globally significant herb crop. It is grown world-wide under hugely variable climactic and environmental conditions (Chaulagain *et al.* 2011). Its short life cycle allows growers to fit its cultivation into some part of the growing cycle in most regions (Lopez, *et al*., 2008), and consequently, it is grown both as a summer and a winter crop. India is considered the largest producer of coriander, with other major producers including: Morocco, Bulgaria, Romania, Canada, China, Russia, Ukraine, Argentina, Egypt, and Mexico (Sharma, *et al.*, 2014; Karvy, 2008). However, statistics on coriander crop production are limited and highly variable depending on source.

Coriander has been grown commercially in the UK since the 1970s (The Telegraph, 2008). Although the country is a small producer by global standards, coriander is currently a high value UK crop. Consumer demands have continually increased with expanding global food influences and shifts towards healthier eating habits (Morales-Payan, 2011; Bashtanova and Flowers, 2011). Coriander is now the most economically important herb crop in the UK, representing over a quarter of supermarket herb sales (Hargreaves, 2014) and approximately £50 million pounds per annum, and increasing annually (Tom Davies, pers. comm., cited in Fraser, 2017). Coriander also currently accounts for the largest growing area for herbs in the UK, estimated at 1500 hectares (BHTA, 2017) (Figure 1.1).

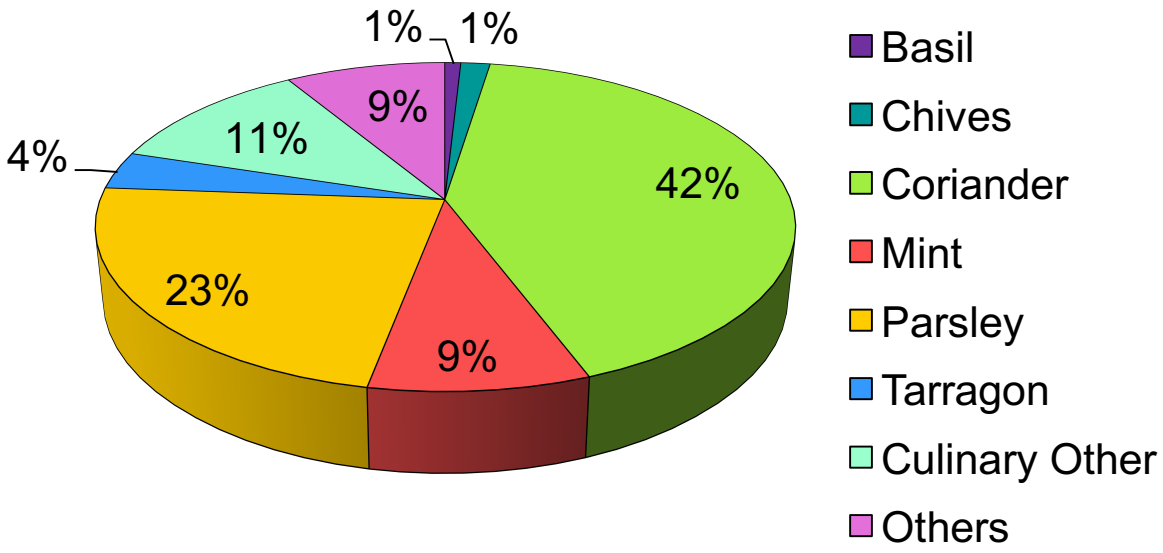

Figure 1.1: Relative growing areas for UK herbs, 2017 (BHTA, 2017)

However, in the UK, the crop suffers from a severe form of yield decline. Coriander yield decline (CYD) is a significant obstacle for growers in meeting domestic coriander demands. This problem is considered acute in that it dramatically reduces yields and its effects persist beyond the time scale of conventional crop rotations. To further compound the problem, there is a lack of growing space typically available to UK farmers. This precludes longer crop rotations which could potentially enable growers to avoid or minimise yield decline (Fraser, 2017). CYD poses a serious problem for growers of this increasingly valuable herb crop and solutions are urgently required.

1.2 Chapter overview

This chapter provides a general introduction to coriander as a botanical species and its commercial uses and the varieties found. The general phenomenon of yield decline is outlined, alongside general factors contributing to the problem, and also more specific causes and effects. Finally, the overall focus and aims of this MRes study are outlined.

1.3 Coriander (Coriandrum sativum L.)

1.3.1Taxonomic position and species description

Coriander, *Coriandrum sativum* L. belongs to the Apiaceae family (subfamily Apioideae, tribe Coriandreae) (Diederichsen, 1996). The name *Coriandrum* is derived from the Greek 'koris', meaning bedbug, referring to the characteristic foetid smell of the plant's unripe fruits (Jansen, 1981). The genus *Coriandrum* contains only two representatives: the cultivated *C. sativum*, and the wild species *C. tordylium*. The closest relative to *Coriandrum* is the small genus *Bifora*, also containing agricultural weeds, but no cultivated members (Diederichsen, 1996). *C. sativum* is a highly very variable species, and botanical literature reports many sub-classifications into subspecies, varieties and forms (CABI, 2015). These are further explored in section 1.5.

Coriander (Figure 1.2) is a soft herbaceous annual which typically forms a tap root and slender, branching stems. The plant's compound leaves are variably shaped and generally broadly lobed at the base of the plant and increasingly incised and filiform towards the top of the plant (particularly with maturity/flowering). The inflorescences of coriander are typical of Apiaceae, borne in compound umbels comprised of both bisexual and staminate flowers. Its fruit (typically known as a 'seed') is a dry, globose, schizocarp up to 6mm in size and containing two seeds (Diederichsen, 1996).

Figure 1.2: Coriander plant showing two distinct leaf morphologies
Figure shows two leaf types, flowers forming compound umbels, and mature fruits (encircled and enlarged) (Hennings, 1993).

1.3.2 Origins and history of coriander

Coriander has been widely cultivated since ancient times, with the earliest archaeological evidence of fruits dating to Nahal Hemar cave, Israel 6000 BC (Zohary and Hopf 1993). The centre of origin *C. sativum* is unclear, but is likely the Near East, given the origins and distribution of other members of the tribe Coriandreae (Diederichsen, 1996). Depending on author, the plant's native range includes: the Mediterranean, the Middle East and Near East, North Africa, and the Caucuses (Jansen, 1981; Vavilov, 1992; Diederichsen, 1996; Lopez, *et al.,* 2008; Bashtanova and Flowers, 2011; Balasubramanian, *et al*., 2012). The plant is also now naturalised in many tropical and subtropical regions around the world (CABI, 2015). Coriander has become a widely grown, highly variable and broadly adapted plant which tolerates a range of environmental conditions (Diederichsen and Hammer, 2003; Purseglove, *et al.,* 1981; Lopez, *et al.,* 2008).

Unlike many cultivated plants, the existence of a wild relative of coriander is unclear (Diederichsen, 1996). It has historically been considered both a cultivated annual

herb and a spontaneous agricultural weed, the latter of which was proposed as early as the 18th Century by Linnaeus. (Diderichsen, 1996; Diederichsen and Hammer, 2003). Supporting the suggestion of its status as a secondary agricultural crop (and also potentially contributing to its vast range), coriander exhibits some strategies typically used by weeds to ensure reproduction and dispersal. Its umbels shatter easily, and while its growth cycle can be very short, its flowering and fruiting period are often extended. Additionally, coriander fruits can endure for considerable lengths of time in agricultural fields, frequently emerging amongst other crops and along roadsides/disturbed places (Diederichsen, 1996).

1.4 Uses of coriander

1.4.1 Food

Coriander is cultivated worldwide and has a long history of uses, including medicinal, culinary, and industrial applications. The plant is typically grown for two diverse food products depending on the part of the plant used: vegetative parts or fruits. Fresh leaves and stems are harvested from young plants for use as a herb/vegetable to add flavour to culinary dishes around the world (Balasubramanian *et al*. 2012; Sharangi and Roychowdhury 2014). Roots are also sometimes consumed as a vegetable in parts of China and Southeast Asia (Diederichsen and Hammer, 2003). The dried fruits of coriander are grown and harvested for use as a spice and flavouring (either ground or whole), used in sweet and savoury foods in many countries (Diederichsen, 1996).

1.4.2 Coriander oil

Coriander is one of the most widely used Apiaceae species for oils, which are extracted from the dried fruits. The plant's oils are contained in specialised channels which start at the roots and form in all parts of the plant, with the highest concentration of oils found in the fruits (Diederichsen, 1996). These oils contain a range of organic compounds, which change as the plant matures. The characteristic smell of coriander is caused by the dominance of aldehydes present in volatile oils of the green plant and immature fruits (Telci, and Hişil, 2008; Mandal and Mandal, 2015). This is in contrast to pleasant-smelling linalool and other monoterpenes which dominate oils present in the mature fruits (Potter and Fagerson, 1990).

Specific chemical compounds of coriander were identified during industrialisation and have since become raw materials for various industrial uses (Diederichsen, 1996). Essential oil weight of dried coriander fruits is between .03%-2.7%, with linalool the primary constituent (approximately 50%). Fatty acids including petroselenic, linoleic, palmitic, and stearic acids are the other major components of the fruits (Purseglove, *et al*., 1981; Diederichsen, 1996; Lopez, *et al.,* 2008). Fruits are used for the extraction of steam distilled essential oils and solvent-extracted oleoresins for the cosmetic, pharmaceutical, and flavour industries (Purseglove, *et al*., 1981; Lopez, *et al.,* 2008).

1.5 Varieties and genotypes

Due to its long history as a crop and frequent escapes from cultivation, coriander has a wide distribution and similarly large genetic diversity. Extensive collections of coriander have been preserved in genebanks and herbaria, particularly in the former Soviet Union, Canada, and Germany (Diederichsen and Hammer, 2003). Because the existence of a wild relative of coriander is unknown, it is these collections that provide sources of new genes for breeders of the plant (Bashtanova and Flowers, 2011). Unlike major crop species, coriander has only been bred on a commercial scale since the twentieth century (Ivanova and Stoletova, 1990; Diederichsen, 1996). Proposed subspecies and varieties of coriander are strongly correlated with morphological characters linked to geographical origins; features which are ultimately dependent on the end use of the crop (Ivanova and Stoletova 1990; Diederichsen and Hammer 2003; Bashtanova and Flowers, 2011). Outside of the currently accepted infraspecific groupings (Tropicos, 2017), further divisions have been suggested by various authors (Ivanova and Stoletova 1990; Diederichsen and Hammer, 2003). However, the most widely used distinction within the species is that of two varieties, *C. sativum* var. *vulgare* Alef. and *C. sativum* var. *microcarpum* (DC) Hegi, which are based on fruit size. As its name suggests, the latter variety has distinctly small fruits (usually less than 3mm) compared to the former. These groups exhibit further differences, such as duration of flowering, leaf morphology and biomass, height, and branching pattern (CABI, 2015; Diederichsen and Hammer, 2003; Bashtanova and Flowers, 2011). It is these morphological distinctions that have formed the basis for breeding genotypes which are suited to either ‘fruit’ or

'vegetable' production of coriander (Diederichsen and Hammer, 2003; Burdock and Carabin, 2009) (Figure 1.3).

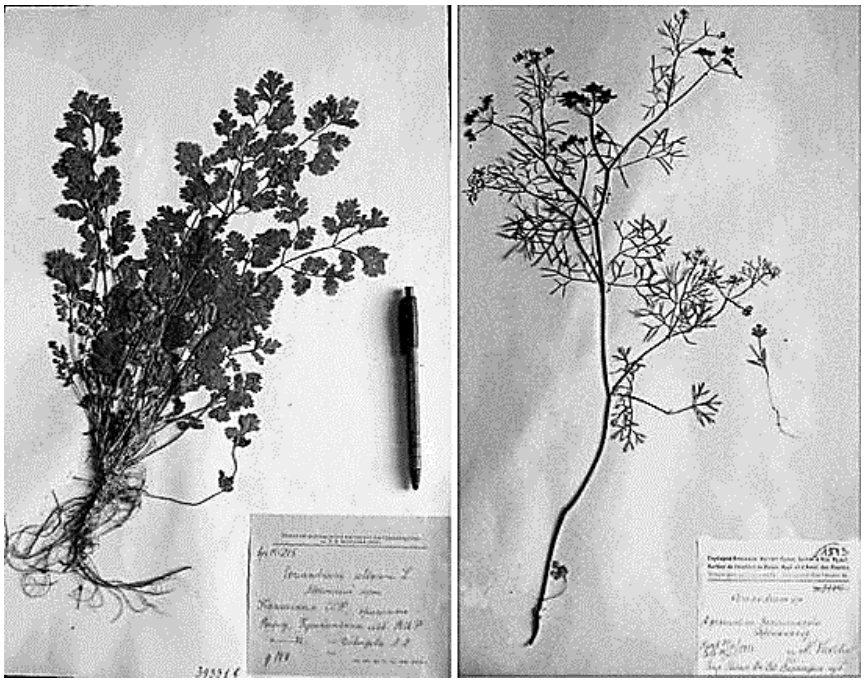

Figure 1.3: Genotypes of coriander
Figure shows diverse morphologies for fruit and vegetable genotypes of coriander. The specimen on the left is a vegetable genotype with a profusion of basal leaves (collected by A. Davydova in Kazakhstan, 1975). The specimen on the right is an extreme example of a fruit genotype, with just one basal leaf (collected by N. Vavilov in Afghanistan, 1924). (Specimens housed in the Vavilov Institute of Plant Industry, St. Petersburg, Russia) (Bashtanova and Flowers, 2008).

1.6 Summary of coriander: an important UK crop with a serious problem

The economic importance of coriander in the UK is undeniable, and its status as such is likely to continue in the future. The specific growing practices of this relatively new crop are explored in Chapter 2. Generally speaking, the current production of coriander reflects its relatively recent arrival as a UK crop. Even so, its popularity has resulted in it comprising the biggest growing area for a UK herb. Along with increases in consumer demands, come increasing production pressures, including land constraints for UK growers. The globally significant but ill-defined problem of yield decline is an important issue facing modern agriculture as a whole. It is also one that extends to UK horticulture, herbs, and specifically, to the subject of this study: coriander.

1.7 The phenomenon of yield decline

1.7.1 Defining yield decline

Whilst feeding more people than ever, modern agricultural systems can result in serious environmental impacts including: soil and water course degradation, diminished ecosystem services, and great losses to biodiversity (Tilman, *et al*., 2002). Intensive production practices can also negatively impact on the crops themselves, with 'yield decline' creating significant impacts on crops (Bennett, *et al*., 2012). This phenomenon can be defined as a reduction in crop yield associated with continuous cropping in the same soils (Bennett, *et al*., 2012). Although increasingly linked to modern cropping systems, references to yield decline, also referred to as 'soil sickness' and 'replant disease', date back to early agriculture. Theophrastus mentioned the problem in ca. 300 BC (Huang, *et al*., 2013). Examples of yield decline are particularly frequent where crops are grown in monoculture and shortened rotations (Bennett, *et al*., 2012). Irrespective of the impact of this phenomenon, agricultural intensification is likely to continue in order to meet the demands of a population expected to reach 8.9 billion by 2030 (UN 2017).

1.7.2 Factors shaping current cropping systems and contributing to yield decline

Post-industrialisation, there have been successive increases in mechanisation, chemical, and biotechnological advances in crop production (Mingay, 1963; Buckwell and Armstrong-Brown, 2004). The Mid-20th Century enabled further improvement in farming technologies linked to the 'Green Revolution'. These included additional advancements in mechanisation; greater inputs of water (through irrigation infrastructure); affordable access to chemical pesticides and fertilisers; and new, high-performance crop strains (Knox, *et al*., 2011; Tilman*, et al.,* 2002). As a result, arable crop yields have increased almost four-fold since 1945 (Robinson and Sutherland, 2002), with global cereal output doubling in the last 40 years (Tilman, *et al.*, 2002). Modern cropping systems have become highly specialised, efficient, high-output operations which are now capable of feeding more than the world's six billion plus inhabitants (Tilman, *et al.,* 2002).

Apart from the advancements mentioned above, a range of additional factors have shaped current cropping systems in the UK. These include government incentives

and legislation, such as the Common Agricultural Policy (CAP) (Karlen, *et al*., 1994). Likewise, wide-ranging economic pressures, shortages in arable land, and the consumer demands of a rapidly growing population have all been identified as sources of pressure on crop producers (Pimentel, 1996; Buckwell and Armstrong-Brown, 2004). Not surprisingly, conventional agriculture is now characterised by intensification and a tendency to grow in monoculture or shortened rotations (Bennett, *et al*., 2012). Consequently, crop yields have increased, and the use of traditional cultural practices has reduced. Techniques such as rotation and tillage traditionally functioned in the management of all aspects of crop production, including control of pests, disease and weeds (Katan, 2010; Robinson and Sutherland, 2002). These practices are known to be intimately linked to soil health, crop yields, and the wider environment (Tilman, *et al*., 2002). Whilst feeding increasing populations, intensive cropping systems have been identified as major causal agents in the degradation of the natural environment, particularly soils (Tilman, *et al.,* 2002; Knox, *et al*., 2011). In the last few decades, increases in yields of many grain crops have stagnated, and yield declines are increasingly common (Tilman, *et al*., 2002; Bennett, *et al.,* 2012).

1.7.3 Specific causes and effects of yield decline

Not all crops grown in monoculture/shortened rotations suffer from yield decline. The situation is highly dependent on the individual cropping situation (Bennett, *et al*., 2012). The effects or symptoms of yield decline vary depending on plant species, soil, and environmental factors. However, the exact causes of this problem appear to be complicated and have not been clearly defined (Huang, *et al*., 2013). Hypotheses of the (potentially interdependent) causal agents of this problem include: build-up of plant pathogens, pests, weeds, and nematodes; deleterious rhizosphere microorganisms; negative mycorrhizal associations; autotoxicity; poor land management; and nutrient depletion (Bennett, *et al*., 2012). Examples of the more widely researched potential causes of yield decline are described in more detail below.

Allelopathy and autotoxicity

Allelopathy in plants can be defined as processes involving the release of secondary metabolites that influence the growth and development of biological and agricultural

systems (Macías, *et al*., 2007). These processes are known as 'autotoxicity' when they are infraspecific, resulting in self-inhibition of germination and growth of the same plant species. Regarding yield decline, autotoxicity has been identified as a potential causal agent, and is particularly associated with shortened crop rotations (Bennett, *et al.,* 2012). Specifically, phytotoxins may be produced by a preceding crop, and subsequently build up in the soils and negatively affect plant growth and yields (Singh, *et al.,* 1999). Autotoxicity is thought to be an evolutionary mechanism involved in regulating plant populations over space and time; a means of avoiding intra-species competition, self-perpetuation, and improved geographical distribution (Singh, *et al*., 1999). Some of the major crops thought to suffer from autotoxicity include: sugarcane, wheat, corn, and rice, and a range of orchard crops (Bennett, *et al.,* 2012). Various studies have isolated specific autotoxic compounds that can negatively affect crops. However, these have largely been laboratory/glasshouse based investigations, isolating compounds which may not accurately represent chemical interactions of plants in the field (Bennett, *et al*., 2012). The transformation of phytotoxic compounds over time in field soils is not well-understood, particularly regarding interactions with soil microorganisms (Wu *et al*., 2001). Consequently, the role of autotoxicity in the yield decline of crops is still largely unclear (Bennett, *et al.,* 2012).

Soil properties

The quality and health of soils is essential for sustaining high crop yields. Nonetheless, intensive agriculture has dramatically altered the properties of many soils. Particularly associated with crops grown in monoculture or shortened rotations, the degradation of soil physical, chemical and biological properties has been implicated in yield decline (Bennett, *et al*., 2012). Although soil health has been well-studied, it is difficult to pin-point the factors directly associated with yield decline. Soil systems are highly complex; they affect plants directly, but also indirectly by influencing associated soil microorganisms (Bennett, *et al.,* 2012). It is well known that growing crops in diverse rotations is important to maintaining soil health (reviewed in more detail in Chapter 2). Not surprisingly, shortened rotations and monoculture of crops are linked with degradation of soils and consequently a suite of factors which may negatively affect crops and result in yield declines.

Microbial causes

While early research on yield decline primarily focused on phytotoxins of root exudates (*e.g.* Börger, 1960), since the 1960s, many soil and root microbes have been isolated and characterised in association with yield decline (Huang, *et al*., 2013). Microbial aspects of yield decline are an increasing focus of crop research. This coincides with a growing understanding of the complex biotic nature of soils. Many cases of yield decline are attributed to the presence of deleterious rhizosphere microorganisms (DRMOs), which are distinct from pathogens, and may increase in monocropping situations (Bennett, *et al.,* 2012). Cropping practices, such as tillage and rotation, along with many other environmental influences, are thought to modify the composition and activities of rhizosphere microbial communities (Smalla, *et al*., 2001; Weiland *et al*., 2001). While the effects are difficult to quantify, it appears that microorganisms, including DRMOs, can strongly affect plant growth through numerous interrelated mechanisms (Smalla, *et al*., 2001; Bennett, *et al*., 2012).

Interconnected factors

A prevailing theme in the study of yield decline is the difficulty encountered in elucidating precise causes. Single causal agents rarely account for yield decline in a crop species in diverse locations or cropping systems (Bennett*, et al*., 2012). This is due to the fact that causes implicated in this phenomenon are part of the wider complexity of plant environment interactions. Even if one cause is identified in inducing yield decline, it is likely that the overall effect is caused by a combination of interacting factors which limit crop growth and yields (Bennett, *et al*., 2012).

1.7.4 Main affected crops

Many crops have been well-studied in terms of yield decline. As mentioned previously, hypothesised mechanisms for this loss in productivity are attributed to a range of potential causes, which are increasingly found to be microbial in nature. A prime example is the study by Hilton, *et al*. (2013), in which oilseed rape (*Brassica napus*) was found to yield approximately 25% less when re-cropped. Correlating with this decline, rhizosphere fungal communities of continuously cropped oilseed rape were shown to be significantly different to crops grown within other rotations (Hilton, *et al*., 2013). Further examples of similarly affected (and well-studied) crops include: barley, corn, potatoes, rice, rye, soybeans, sugarcane, cucumber, peach,

apple, and strawberries (Bennett, *et al*., 2012; Bhandari, *et al*., 2002; Pankhurst *et al*., 2003; Pankhurst *et al*., 2005; Wu, *et al*., 2009; Beniziri, *et al*., 2005; Yim, *et al*., 2012; Xu, *et al*., 2015).

Although the phenomenon of yield decline has been studied extensively in major crops, there has been little investigation of its occurrence in herb crops. This is not to say that the problem does not exist. The aforementioned factors and pressures surrounding current cropping systems also influence other crop sectors. As mentioned previously, global herb production has increased dramatically in recent decades, with UK herbs following suit (FAO, 2018; BHTA, 2018). It is not surprising, therefore, that the UK's most popular herb has become yet another example of a crop species suffering from yield decline.

1.7.5 Coriander yield decline

Coriander suffers from a particularly severe form of yield decline when replanted on the same soils. Needless to say, there is no research specifically on coriander yield decline (CYD), which growers have observed as stunted growth and reduced emergence, rather than caused by a particular pathogen (Fraser, 2017). This issue was first brought to the attention of the Agriculture and Horticulture Development Board (AHDB) in 2010 (Kim Parker, pers. comm., 2017), but has likely existed for some time. CYD can be considered acute in its effects as it can reduce productivity by 50%; in some cases, affecting subsequent crops for up to eight years (Tom Davies pers. comm. cited in Fraser, 2017).

According to results of a survey completed by growers, coriander yield decline occurs throughout the UK and in conjunction with a range of agronomic practices (Fraser, 2017). Because of the disparate nature of coriander production in the UK, no single factor has been definitively implicated in causing CYD. It is likely a multi-faceted problem with interdependent causes both in the short term and longer term (Ian Singleton, pers. comm., 2017). Yield decline likely depends on factors beyond monoculture and shortened rotations, including the individual cropping situation, soil type, and climate (Bennett, *et al.*, 2012). This likely applies to the particular persistence of CYD, amongst a wide range of cultural practices. Adding further

intrigue and complexity to this issue, some UK coriander growers do not suffer from CYD.

1.7.6 Similarly affected Apiaceae crops

Also of relevance to the problem of CYD, other members of the Apiaceae family have demonstrated yield decline, or at least phytotoxic effects which may inhibit growth. The medicinal herb *Angelica sinensis* was found to experience a superficially similar yield decline to that of coriander (Zhang, *et al*., 2013; Zhang *et al*., 2015; Zhang, *et al*., 2016). Re-cropping studies of this herb propose both autotoxicity and microbial community changes as influences potentially reducing yield. Of further relevance to the present study, autotoxicity has been reported in coriander and other members of Apiaceae (*Cachrys pungens*, parsley, and parsnip) (Araniti, *et al*., 2014; Gog, *et al*., 2005). Also of note is the fact that, along with several other Apiaceae species (fennel, cumin, carum, and carrot), coriander produces phytotoxins which inhibit seed germination (Chaturvedi and Muralia, 1975).

1.7.7 Previous agronomic research of coriander

Coriander is not a 'developed' crop, in the sense of major crop species like cereals (David Kenyon, pers. comm., 2018), and research on the crop is limited. This is due to the fact that commercial scale breeding is relatively recent; and agronomic practices vary widely depending on the individual grower, growing region, environment, genotype, and the end use of the plant. Existing research on coriander crops has primarily been carried out to improve seed yield. Historically, most studies were conducted in the former Soviet Union, however, no research program currently exists (Flowers and Bashtanova, 2008). Several studies have been carried out in tropical/sub-tropical India (*e.g*. Sharangi and Roychowdhury, 2014) and semi-arid Mediterranean regions (*e.g*. Telci and Hisil, 2008; Carrubba *et al*., 2001; Carrubba *et al*., 2006). These primarily cover topics such as general phenology, fertiliser regimes, and sowing date. Information on temperate coriander crops is considerably scarcer. Available general growing advice for coriander (commercial and garden-scale) varies considerably, but almost universally includes good drainage and avoidance of waterlogging as vital aspects for crop performance.

Published research on UK coriander crops exists in the form of two AHDB reports, one detailing the phenomenon of bolting (early flowering) (Wiltshire, 2009) and the other examining the effects of variety and growth conditions for pot production and optimised shelf-life (Flowers and Bashtanova, 2008). Research has not been conducted to determine optimum growing conditions or cultivation techniques for outdoor coriander in the UK. Consequently, practices which may help to reduce or alleviate CYD have not been formally assessed.

1.7.8 Previous work on coriander yield decline

In 2014 the AHDB funded a study to investigate the potential causes of CYD (CP-117, completed by K. Fraser in 2017). This work laid the foundation for the present MRes study. Through glasshouse pot trials, Fraser (2017) established that coriander yield decline is a real phenomenon which can be replicated under controlled experimental conditions. Yield decline was replicated in experiments using a variety of different soil types (both field soils and standard composts) and coriander varieties, indicating that the cause of this particular yield decline is linked to the plant, and not the soil. Fraser's (2017) study compared soil rhizosphere microbial communities in healthy and yield decline plants, showing a significant difference between the two groups. The work ultimately concluded that current practices for growing coriander result in plant compounds and crop debris which may attract a deleterious microbial community that persists and leads to reduced coriander growth in subsequent crops. The exact nature of this problem was not clarified in the study. However, extensive microbial community data provided a baseline for further studies. Most importantly, the work provided further possibilities of agronomic practices to potentially alleviate coriander yield decline. Following on from Fraser (2017), the present study therefore furthered investigations in soil desiccation and tillage experiments, and also microbial studies.

1.8 Focus of current study

1.8.1 Overall aims

The problem of yield decline in coriander has serious implications for UK growers. Therefore, the overall aims of this MRes dissertation are to help establish soil and crop management strategies which will reduce or prevent CYD, and provide further

understanding into the potential causes of CYD. It is hoped that this work will provide valuable knowledge to coriander growers and the UK horticulture industry. On a wider scale, this study may contribute towards a greater understanding of the yield decline phenomenon—an area severely lacking in comprehensive studies of specific cropping systems (Bennett, *et al*., 2012).

1.8.2 Individual objectives

Crop management practices are fundamental to the 'composition, diversity, and function of the soil microbial community', and therefore may significantly alter soil processes, soil structures, and plant growth (Gonzalez-Chavez, *et al*., 2010). This project focuses on several soil management strategies known to assist with soil management and influence soil microbial communities. Such practices, along with associated microbial studies, have been evaluated in terms of their potential to reduce or alleviate CYD. The individual objectives of this project are outlined below.

- To assess tillage at different depths as a means of influencing soil compaction and also the position and concentration of coriander root residues and associated microorganisms (Chapter 2).

- To evaluate seed planting density as a factor potentially influencing the occurrence and severity of CYD (Chapter 2).

- To determine whether coriander can be grown for second cycle without yield decline, if 'optimum' conditions for growth are met (Chapter 2).

- To examine desiccation/drying of crop soils after harvest as a means to potentially alter microbial communities and soil structure; indirectly assessed through resulting crop yields (Chapter 3).

- To assess the effectiveness of soil sterilisation in preventing or reducing CYD (Chapter 3).

- To compare soil microbial communities associated with healthy vs. yield decline coriander plants (Chapter 3).

Chapter 2: Glasshouse pot trials to assess crop and soil management strategies for reducing Coriander Yield Decline

2.1 Introduction

2.1.1 Modern crop production vs. traditional practices

Growing populations and mounting economic pressures have driven changes in the way crops are grown, and also in the crop environment itself. (Reeves, 2017; Knox, *et al*., 2011). Operations have become increasingly specialised and production has intensified. However, the products and practices that facilitate modern cropping systems are being increasingly linked to the degradation of the wider environment and also yield declines in crops (Knox, *et al*., 2011; Tilman, *et al.*, 2002, Reeves, 2017).

Yield declines are particularly associated with crops grown in monocultures, or restricted arable rotations (Knox*, et al*., 2011; Bennett, *et al.*, 2012). These methods are being increasingly used in place of traditional crop rotations. The reasons for this tendency in the UK stem from shortages in arable land and general economic pressures, but also affordable access to fertilisers and pesticides, which fulfill functions traditionally achieved by crop rotations (Knox, *et al*., 2011; Bullock, 1992; Karlen, *et al*.; 1994; Ball, *et al*., 2005). These changes in crop production do not just characterise major agricultural crops; they are also relevant to the horticultural sector.

The UK's most economically important herb, coriander, suffers from a severe form of yield decline which can reduce yields by 50% (Fraser, 2017). The problem is apparently compounded by limited land availability. This factor, combined with the high value of the crop influences planting decisions for growers (Robert Gibbs, pers. comm., 2017). This situation precludes longer rotations that may help to alleviate or prevent the problem of coriander yield decline (CYD) (Fraser, 2017). CYD has serious negative impacts on the UK horticultural industry, and further insight into management options is needed. In addressing this issue, the importance of traditional crop rotations will be outlined. Following this, the specific agronomic

practices of growing coriander in the UK will be defined, before presenting the experimental work which aims to provide new knowledge for growers.

Traditional crop rotations: a declining practice

Along with tillage, crop rotation is a fundamental influence on soil quality, crop performance, and sustainable cropping systems (Munkholm, *et al*., 2013). Crop rotation can be defined as the sequential production of diverse crop species on the same land (Karlen, *et al*., 1994). The practice can be traced back to the Han dynasty of China more than 3,000 years ago (MacRae and Mehuys, 1985). Early agriculturalists were aware of a link between low yields and continuous cropping, with rotational cropping known to offer better productivity. Nonetheless, early farmers did not understand why this was the case (Karlen, *et al*., 1994). It is now general knowledge that crop rotations help to maintain soil structure, fertility, and diverse populations of microbial communities. They also help to manage levels of weeds, pests and diseases (Bullock, 1992; Karlen, *et al*.; 1994; Ball, *et al*., 2005; Knox, *et al*., 2011).

The Norfolk four-course rotation system of the 17th Century was a defining feature of British agriculture, and implemented a systematic approach to combined farming (Robinson and Sutherland, 2002). This type of crop rotation was widely practiced until the agricultural advances of the 1940s. It used a four-crop rotation: a root crop, two cereals, and a grass ley (Robinson and Sutherland, 2002). However, from the 1940s onwards, agriculture in the UK has favoured shorter and less diverse rotations. Longer rotations are often considered impractical or not viable, largely due to land availability constraints (Bennett, *et al*., 2012). As mentioned previously, this fact influences many cropping systems, including those of coriander. Regardless of the overall decline in traditional rotations, these systems persist virtually unchanged in many organic systems, particularly in Scotland (Knox, *et al*., 2011).

2.1.2 UK coriander growing methods

UK coriander is grown under cover in pots, and as a field crop (the subject of this study), which is generally sold as packaged leaves. It is grown in many parts of the country, and as such is subject to a range of environmental conditions. This fact,

combined with its relative newness as a crop, has resulted in cultivation and planting methods in the UK being highly variable. Beyond the tendency to grow two successive crops in a season (early spring and early summer) before a (probably) shortened crop rotation, there is no standard protocol for: planting density, crop debris management, tillage, crop rotation, fertilisation, *etc.* (Robert Gibbs, pers. comm., 2017; also reviewed in the grower questionnaire conducted by Fraser, 2017).

As a field crop, coriander is considered particularly 'problematic' by many UK growers (Flowers and Bashtanova, 2008; Philip Dodd, pers. comm, 2017; Robert Gibbs, pers. comm., 2017). It is prone to bolting, and is also susceptible to sudden yellowing of the leaves (Bashtanova and Flowers, 2011). It is affected by a phenomenon known as 'June blues', causing patches of bluish discolouration, which render the leaves unsalable (Phillip Dodd, pers. comm., 2017). Coriander is prone to a number of specific and general pests and pathogens including fungal/oomycete diseases caused by: *Fusarium* spp., *Pythium* spp., and *Erisyphe polygoni*; and it is also affected by bacterial blights (Diederichsen, 1996). These are in addition to the issue of yield decline in coriander—a serious, but ill-defined problem facing UK growers.

2.1.3 Chapter aims and objectives

Overall aims

This chapter focuses on the glasshouse pot trials that were conducted to assess crop and soil management strategies with potential to reduce CYD. In light of the highly diverse agronomic methods used by UK coriander growers and lack of research into optimum growth methods, the aim of these experiments was to provide insight into the impact of specific cultural practices on the occurrence of CYD. This, in turn, would inform a set of optimised conditions for coriander growth in a second crop cycle; thereby providing useful information for growers and informing further investigations into the potential causes of CYD.

Individual objectives:

- To assess the effectiveness of deep ploughing as a means of altering crop soils; changing the position and concentration of coriander root residues, and potentially reducing the occurrence of CYD in a subsequent crop.

- To assess whether the superficial disturbance provided by harrowing is sufficient to reduce CYD; an experimental factor which may have contributed to the high levels of decline observed of a previous AHDB (Agriculture and Horticulture Board) funded study (Fraser, 2017).

- To evaluate the potential effects of three different planting densities on the occurrence and severity of CYD.
- To investigate the impact of fertiliser regime on coriander yield and CYD in the glasshouse.

- To assess the occurrence of CYD in an experiment where optimum conditions for coriander growth are provided for a crop grown in the same soils for a second cycle.

2.2 Materials and methods

2.2.1 Glasshouse conditions: general method for growing coriander in the glasshouse

General methods for growing coriander followed those used by Fraser (2017) which established yield decline in the glasshouse in two successive crops cycles. Growth experiments were carried out at the glasshouse facilities of SASA (Science and Advice for Scottish Agriculture, 55.9237° N, 3.3429° W) in Edinburgh. These were maintained at 20ºC in the daytime (reaching a maximum of 25ºC), and 18ºC in the evenings. Glasshouse lighting (high pressure sodium *Papillon 270*—600 watt lamps, Papillon Luminaires) was set to achieve a photoperiod of sixteen hours, mimicking UK summer conditions. Plants were watered daily and given a single application of Chempak 3 balanced (20-20-20 N, P, K) fertiliser at a rate of 7.5 mg/cm^2 at four weeks' growth (this was later modified in some experiments). Standardised potting compost (John Innes 2) and Coriander var. Santos (sub-variety 'Rani') seeds were

chosen for these trials, as yield decline was previously established irrespective of soil type and coriander variety (Fraser, 2017). Furthermore, Santos is the most common variety used by UK growers (Robert Gibbs, pers. comm., 2017). Planting density initially followed Fraser, 2017 (.27 seeds/cm^2), and later became an experimental factor in its own right.

2.2.2 Experimental set-up and design

Experiments were organised in randomised complete block designs using GenStat (VSN, 2011). Blocks comprised the fixed factor in these set-ups, accounting for the gradient of watering expected due to hose length and bench positions in the glasshouse. Treatments were randomly assigned within each block. Each crop cycle required approximately eight weeks (or as few as six, depending on season) to reach the optimum height (15-19 cm) and leaf number (five to seven basal leaves), as suggested by coriander growers and previous work (Robert Gibbs, pers. comm., 2017; Fraser, 2017). To facilitate the growth of a second crop cycle, first cycle crops were harvested such that the entire plant biomass was removed from the soil surface, leaving minimal traces of plant residue above ground. After harvest, seeds were re-sown to simulate the growth of a second crop in the same soils (see Figure 2.1 below for method overview).

To compare treatments affecting coriander growth in a second crop cycle (thus assessing the occurrence/severity of yield decline) coriander was grown for a first cycle in 'virgin' or control soils, those without a prior coriander cropping history. Pots were then subjected to various treatments (described in detail for each experiment in Sections 2.2.5-2.2.8), before being sown again for a second crop cycle (using soils with one prior coriander crop) or occasionally a third crop cycle (using soils with two prior coriander crops) (Figure 2.1).

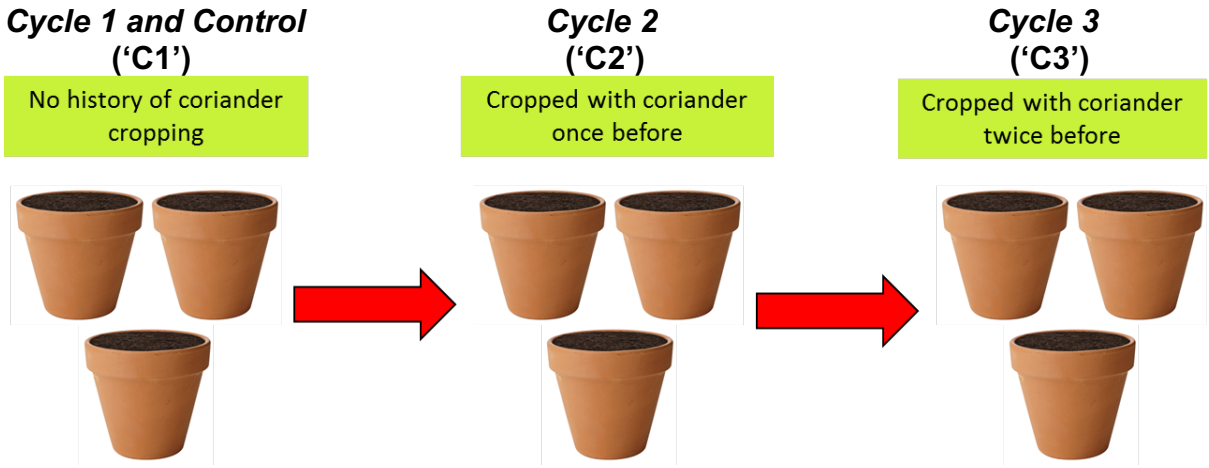

Figure 2.1: Basic method for growing coriander for two or three crop cycles

2.2.3 Data collection

C2 (and sometimes C3) growth data were collected for 'individual plant biomass' and 'total yield per pot'. The former was determined by randomly sampling whole plants from each pot and measuring both fresh and dry weights of roots, shoots, and leaves. A calculation was then made for 'above ground weight', consisting of the combined weight of shoots and leaves; and 'below ground weight', consisting of the weight of the root only. Replications of plants sampled depended on the number of pots and size of plants (limited by the size of the drying oven) produced in the experiments. Plants were oven dried at 60°C for approximately 24 hours (according to Fraser, 2017). The root to shoot ratio of plants was calculated by using the following formula: $\frac{Dried\ root\ g}{Dried\ shoot\ g}$

Total yield per pot was also determined as a means of providing a more relevant form of data for growers. This was done by calculating the (fresh) weight of all plants in a given pot, cut at approximately 3 cm above the soil surface to mimic the technique used by growers in the field (Robert Gibbs, pers. comm., 2017). All crop data were recorded and organised in Microsoft Excel workbooks, which were also used to produce charts and tables. Standard error (SE) was calculated for all obtained means for plant metrics. This was done by dividing the standard deviation (SD) (output from R) by the square root of the number of samples/observations (n), as in the following formula: $SE = \frac{SD}{\sqrt{n}}$. These calculations were then added as error bars to Excel graphs.

2.2.4 Statistical analyses

Statistical analyses were performed using R (R Core Team, 2013). Normality of distribution of measured characteristics was tested using a Shapiro-Wilk test ($p \geq 0.05$). Data sets with non-normal distributions were transformed using the natural logarithm or square root. Analysis of variance (ANOVA) or a Welch two sample t-test (in the case of comparisons of two treatment levels) were performed to detect significant differences in means between treatments (*e.g.*, desiccation, ploughing, harrowing, different densities) and controls for each crop. Where normal distributions were not obtained, non-parametric tests were used (Kruskal-Wallis rank sum test or Mann-Whitney U test). Post-hoc analyses were carried out to determine individual differences between treatments (Tukey test 95% confidence interval (CI) groupings, or a Nemenyi Test for non-parametric analyses). Significant differences in treatment vs. control plant biomass and yield per pot were used to inform yield decline data. To obtain sufficient replication for each experimental treatment, multiple plants were taken from each sample pot, ranging from two to ten replicate pots per experimental treatment. Therefore, replicate sample units (plants) were not statistically independent. To ensure that a 'pot effect' did not contribute to the difference between treatments, 'pot' was tested as a random factor in mixed model analyses of variance where possible, to ensure that significant differences in plant metrics (*e.g.* dry or fresh weights) were not a result of the experimental artefact of a particular pot.

2.2.5 Assessing the impact of harrowing on a second cycle coriander crop

Coriander seeds were sown at the standard density (36 seeds) in 13 cm (1 litre) pots in order to examine the effect of harrowing on coriander crop soils (in contrast to more compacted, non-harrowed soils). Four replicate pots were re-sown using the same parameters for the second crop with each of two treatments: 1) harrowing: disturbing only the upper soil layers using a fork to a depth of 5 to 7 cm, before re-sowing or 2) simply re-sowing by inserting seeds with minimal soil disturbance. Four control pots were also sown and plants were harvested at approximately eight weeks' growth. Individual plant biomass data (for fresh and dry above and below ground weights) were collected for 30 replicate plants, and also total yield per pot (four replicates).

2.2.6 Comparing the impacts of deep ploughing vs. harrowing on the occurrence of CYD

Coriander seeds were sown at the standard density (166 seeds) in 28 cm (15 litre) pots in order to assess the effect of a simulated deep plough on a second cycle coriander crop (in contrast to a more superficial harrowing). Four replicate pots were re-sown for a second cycle with the same general parameters for each of two treatments: 1) simulated ploughing to a depth of approximately 20 cm, or 2) harrowing, as described above (2.2.5). To simulate ploughing, 20 cm of crop soils were removed from pots, and inverted, so that the upper soil layers were roughly situated at the bottom of the pots. Harrowing was simulated by disturbing only the upper soil layers using a fork, before re-sowing. Four control pots were also sown. The second crop cycle was harvested after approximately eight weeks. A third cycle was then sown in the same manner, and harvested at eight weeks. Individual plant biomass (fresh and dry above and below ground weights) data were collected for the second cycle crop (30 replicate plants), and total yield per pot (four replicates) were determined for both second and third cycles.

2.2.7 Examining the effects of different seed planting densities on the occurrence and severity of CYD

Coriander seeds were sown in 13 cm (1 litre) pots using three different planting densities: a high density=36 seeds (.27 seeds/cm^2), a medium density=20 seeds (.15 seeds/ cm^2), and a low density=3 seeds (.025 seeds/cm^2). A second crop cycle was then sown with the same parameters, with two replicate pots sown for each of the three planting densities. Two control pots for each of these densities were also sown, totaling 12 pots for the experiment. Plants were harvested after approximately eight weeks to determine biomass for individual plants (fresh and dry above and below ground weights) for ten replicate plants and also total yield per pot.

2.2.8 Investigating the impact of fertiliser regime on coriander growth and the occurrence of CYD

Coriander seeds were sown in 13 cm (1 litre) pots at the standard density (36 seeds). A second cycle was then sown using the same parameters, for two levels of treatment: 1) ‘fertilised’ with a base-dressing of 0.5g Chempak 3 balanced fertiliser;

or 2) ‘non-fertilised’. Three replicate pots were sown for each treatment, alongside three controls for each treatment, for a total of 12 pots. Plants were grown for approximately eight weeks, and all were given the standard application of fertiliser at four weeks’ growth (as described in Section 2.2.1). Data were collected for individual plant biomass (fresh and dry above and below ground weights) from 21 replicate plants per treatment and total yields per pot.

2.2.9 Assessing ‘optimum’ growth conditions for impact on CYD

Based on results obtained in the above experiments, an ‘optimised’ coriander growth trial was carried out to determine if CYD still occurred, even if coriander was grown under what could be optimum conditions. Coriander seeds were sown in 23 cm (5 litre) pots at a density of .088 seeds/cm^2 (37 seeds) for ten replicate pots. The crop was grown for approximately six weeks, whereby plants were harvested and crop soils were allowed to dry out in their pots for two weeks. Pots were then harrowed, watered, base-dressed with fertiliser (1.5 g Chempak 3), and then re-sown with coriander (at the same density), alongside ten control pots. Cycle 2 was grown for approximately six weeks; only total yields per pot were calculated.

2.3 Results

2.3.1 Assessing the impact of harrowing on coriander yield

Harrowing coriander crop soils before sowing a subsequent crop in the same soils had an observable impact on the size of individual plants, and also the overall yields per pot produced. Interestingly, this effect was not evident in the root size of individual plants (fresh or dry) ($p>0.05$), or the root to shoot ratios ($p>0.05$). In contrast, coriander plants grown in control (C1) soils had significantly greater fresh above ground weights (shoots and leaves) than C2 harrowed plants (CI 95% $p=0.006$), and C2 non-harrowed plants (CI 95% $p=0.003$) (Figure 2.2a). Likewise, the dry above ground weights of plants showed the same effect, with control (C1) plants larger than both the C2 harrowed plants (CI 95% $p=0.035$), and C2 non-harrowed plants (CI 95% $p=0.038$) (Figure 5). Control (C1) soils also produced the largest total mean yield per pot, which was significantly greater than that of the C2 non-harrowed pots (95%CI $p=0.040$), but not statistically different from the C2 harrowed pots ($p>0.05$). Figure 2.2b highlights this effect: C2 non-harrowed pots

declined by 34% relative to the control (C1), while C2 harrowed pots declined by just 20%. Overall, results show that harrowing helps to reduce CYD in a second coriander crop.

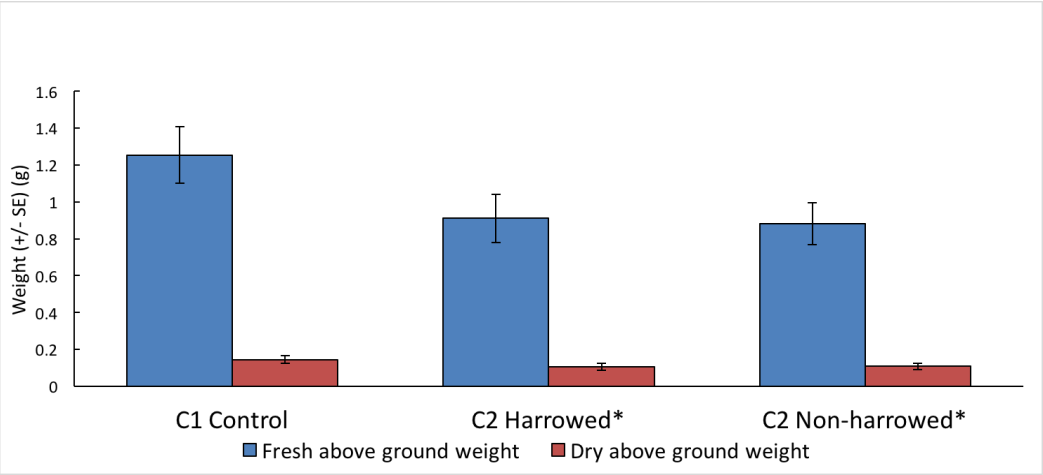

Figure 2.2a: The effect of harrowing on the above ground weights (+/-SE) of coriander Coriander was initially grown and harvested. After harvest, soil was either harrowed or left compacted; coriander was then re-sown for another crop cycle (C2). Figure shows mean above ground weights (fresh and dry) for C1 Control, and C2 Harrowed and C2 Non-harrowed treatments with significant differences (*) between both treatments vs. Control (C1) (fresh and dry weights). (Means calculated from 30 replicate plants/treatment).

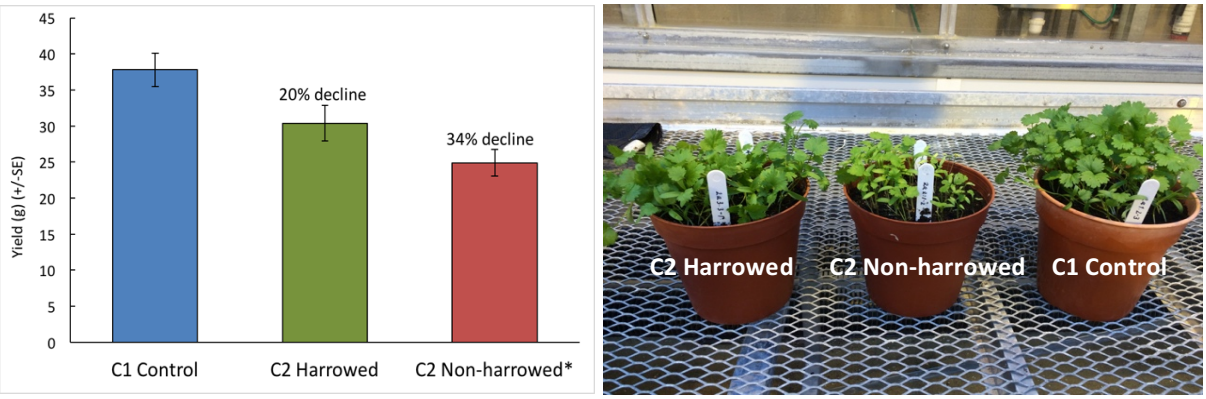

Figure 2.2b: The effect of harrowing on coriander total yield per pot (+/-SE) Coriander was initially grown and harvested. After harvest, soil was either harrowed or left compacted; coriander was then re-sown for another crop cycle (C2). Figure (left) shows mean total yields per pot for C1 Control, and C2 Harrowed and C2 Non-harrowed treatments with significant difference (*) only between Control (C1) and C2 Non-harrowed pots; relative declines from the Control are also shown; also shown (right) an example of coriander for each treatment at four weeks' growth. (Means calculated from four replicate pots per treatment).

2.3.2 Comparison of ploughing and harrowing on total coriander yield

Coriander grown in fresh control (C1) soils produced greater overall yields per pot than coriander grown in soils that had had one crop before (C2) or two crops before (C3), irrespective of the tillage treatment applied. The control (C1) pots produced

significantly greater yields than both the C2 ploughed pots (CI 95% p=0.031), and the C2 harrowed pots (CI 95% p=0.042). To illustrate this, C2 ploughed pots experienced a decline of 27% relative to the control (C1), compared to a decline of 15% in C2 harrowed pots (Figure 2.3). This situation was reversed for Cycle 3, where C3 harrowed pots declined by 32% relative to the control, compared to a 26% decline in yields of C3 ploughed pots (Figure 2.3). In measuring individual plant biomass for Cycle 2 pots (only total yields per pot were calculated for Cycle 3), no statistical differences (p>0.05) were found for any of the individual plant metrics; therefore, no data is shown.

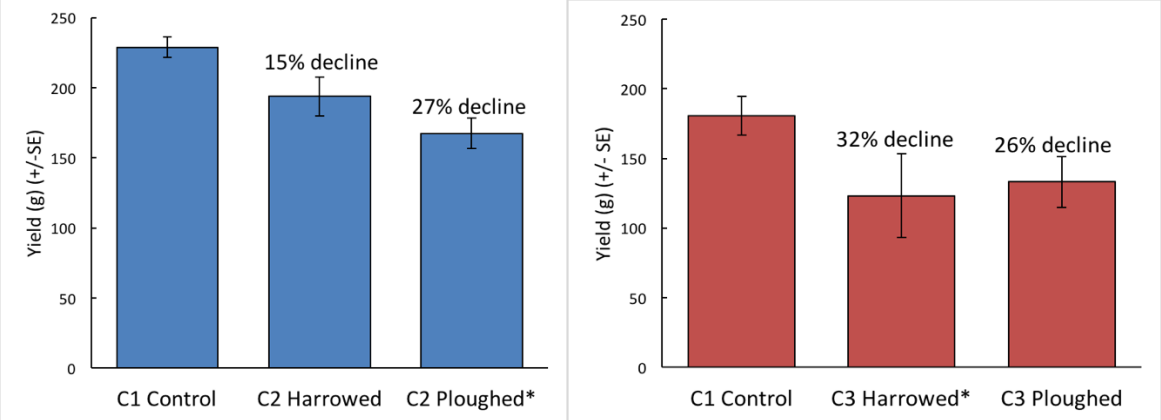

Figure 2.3: The effect of harrowing and ploughing on coriander total yields per pot (+/- SE)
Coriander was initially grown and harvested (C1). After harvest, soil was either harrowed or ploughed and re-sown with coriander for two more growth cycles (C2 and C3). Figures show mean yields per pot (C2 on left, and C3 on right), with two treatments and a control (C1); also showing relative percent decline from the C1 Controls ('*' indicates statistically significant difference from control). C2 ploughed pots yielded significantly less than the control, while in C3, the same was true for harrowed pots. (Means calculated from four replicate pots per treatment).

2.3.3 Examining the effects of different seed planting densities on the occurrence and severity of CYD

Coriander grown in fresh control (C1) soils and coriander grown for a second cycle in the same soils (C2) both indicated that planting density affects coriander growth. This was seen in the individual plant biomass and total yields per pot. Coriander plants grown at the low density of just three seeds per pot (both in C1 soils and C2 soils) produced the largest above and below ground weights, although variability was highest within these plants (Figure 2.4a). This difference in biomass was found to be

statistically significant for: fresh above ground weights (p= 3.12^{e-14}), dry above ground weights (p=1.34^{e-12}), fresh below ground weights (p=3.97^{e-10}), and dry below ground weights (1.53^{e-07}). Although a defined difference in total yields per pot can be seen between the three different densities in Figure 2.4b, no variance was detected in an ANOVA (p>0.05). This may reflect the high levels of variability seen in the C2 low density and C1 medium density pots. Relative to C1 control pots, the C2 high density pots experienced the highest rate of decline at 41%, with the C2 low density pots at 34%, and the C2 medium density pots showing the least decline at 13% (Figure 2.4b).

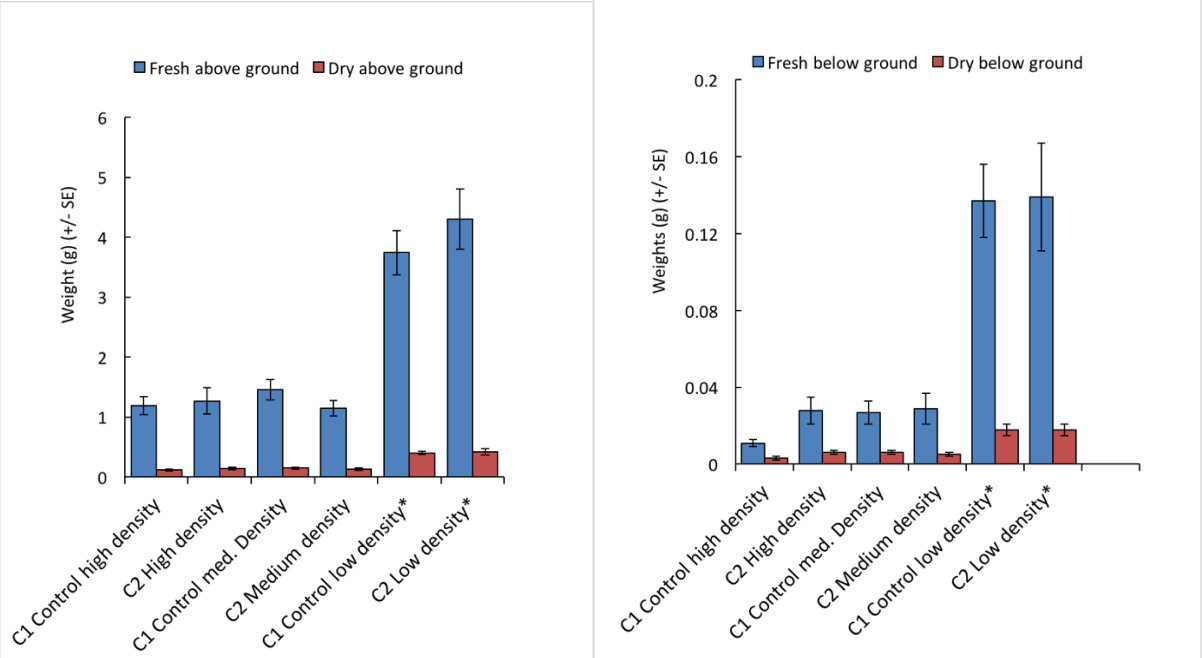

Figure 2.4a: The effect of planting density on the above and below ground weights (+/- SE) of coriander
Coriander sown at three different planting densities was grown and harvested (C1). Coriander was re-sown at the same planting densities (C2) alongside controls (C1). Figure shows mean above ground weights (left) and mean below ground weights (right) for controls (C1) and C2 for the three densities; low density pots produced significantly larger plants (above and below ground). '*'Denotes significant difference in low density plant weights (C1 and C2 above and below ground) in comparisons with all other treatments. (Means calculated from ten replicate plants per treatment).

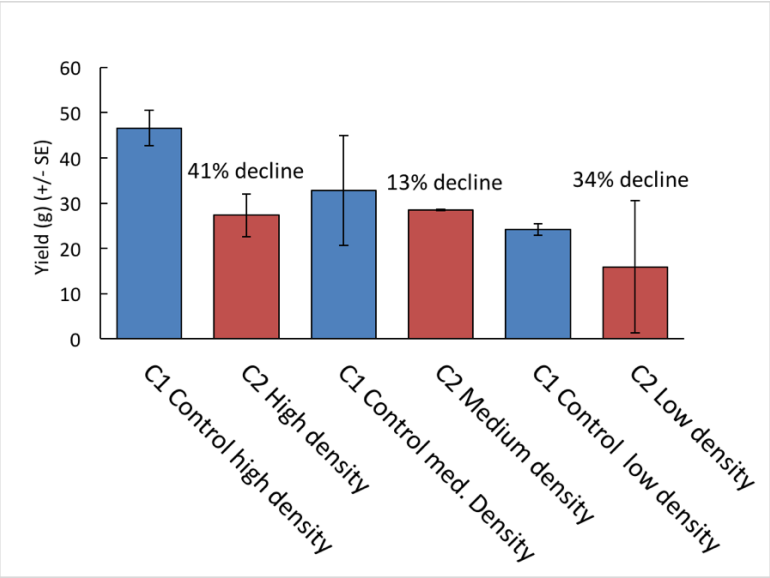

Figure 2.4b: The impact of planting density on coriander total yield per pot (+/-SE) Coriander sown at three different planting densities was grown and harvested (C1). Coriander was re-sown at the same planting densities (C2) alongside fresh controls (C1). Figure shows total mean yield per pot with percent C2 declines from relative C1 controls for each density. (Means calculated from two replicate pots per treatment).

2.3.4 Investigating the impact of fertiliser regime on coriander growth and the occurrence of CYD

Fertiliser regime (base-dressing pots with fertiliser vs. not base-dressing with fertiliser before sowing a second coriander crop) was a further influential factor on the size of individual plants, but not on the overall yields per pot. Significant differences were found for each of the plant metrics, with the exception of dry above ground weights ($p>0.05$). The fresh above ground weights of plants were significantly different between treatments ($p= 0.022$); fertilised plants (grown in both fresh control (C1) soils and in C2 soils) had greater fresh above ground weights (Figure 2.5a). Although post-hoc analyses failed to reveal specific pairwise differences (95% CI $p>0.05$), fertilised pots produced fresh above ground weights approximately 20-30% greater than those of non-fertilised pots. The fresh weights of plant roots differed significantly between treatments ($p= 0.003$), but with a contrasting result. Figure 2.5a shows that the largest fresh root (below ground) weights were found in C2 non-fertilised plants (which failed to show pairwise significance in post-hoc analyses, $p>0.05$, likely due to high variability). Dry below ground weights were likewise significantly different for coriander grown in the C1 control fertilised soils vs. the C2 non-fertilised soils (CI 95% $p=0.024$) (Figure 2.5a).

Total yields per pot were significantly different (p=0.002). Coriander yields per pot for fresh control soils (C1) were greater than yields per pot for coriander grown for a second cycle (C2) (Figure 2.5b), with pairwise differences (CI 95%) shown below:

C2 Non-fertilised vs. C1 Control non-fertilised	p= 0.005
C2 Fertilised vs. C1 Control fertilised	p= 0.041
C2 Non-fertilised vs. C1 Control fertilised	p= 0.003

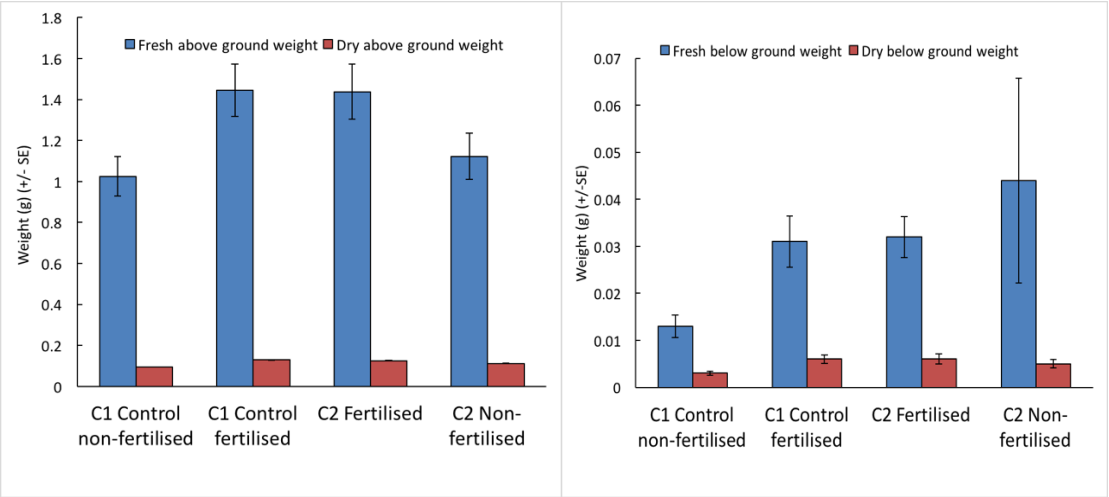

Figure 2.5a: The impact of fertiliser regime on above and below ground weights of coriander (+/-SE)
Coriander was grown in fresh compost and harvested (C1). Coriander was then sown in the same soils (C2) alongside controls (C1) which were either base-dressed with fertiliser ('fertilised') or not base-dressed with fertiliser ('non-fertilised'). Figure shows above ground weights (left), and below ground weights (right). (Means calculated from 21 replicate plants per treatment).

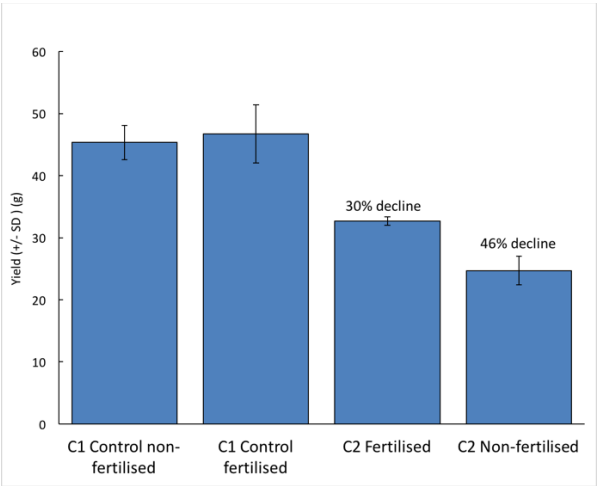

Figure 2.5b: The impact of fertiliser regime on coriander total yield per pot (+/-SE)
Coriander was grown in fresh compost and harvested (C1). Coriander was then sown in the same soils (C2) alongside controls (C1) which were either base-dressed with fertiliser ('fertilised') or not base-dressed with fertiliser ('non-fertilised') Figure shows total yields per pot, with percent C2 declines from relative C1 control pots. (Means calculated from three replicate pots per treatment).

2.3.5 Assessing a set of 'optimum' conditions for their impact on CYD

For the optimum growth conditions experiment, coriander was grown and harvested. Before sowing a second cycle (C2), crop soils were left to dry out for two weeks, harrowed, base-dressed with fertiliser, and then sown at a medium-low planting density alongside C1 control pots. Even after growing coriander under these seemingly optimum conditions, C2 yields still declined significantly (p=0.005). This difference is illustrated by the 28% decline experienced in C2 pots, relative to C1 control pots (Figure 2.6).

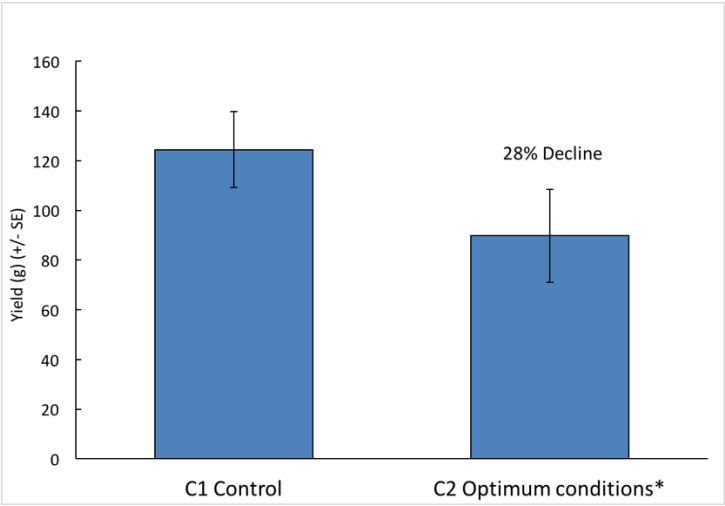

Figure 2.6: The impact of optimum growth conditions on total coriander yield per pot (+/-SE)
Coriander was sown at a medium/low planting density in fresh soils and harvested (C1). Crop soils were left to dry out for two weeks, then harrowed, and base-dressed before re-sowing. Figure shows significantly greater mean total yields for C1 Controls (*) vs. C2 optimum conditions coriander (grown for a second cycle using optimum conditions). (Means calculated from ten replicate pots per treatment).

2.4 Discussion

2.4.1 Limitations and benefits of glasshouse experiments

Glasshouse pot trials are the standard means by which the beginning stages of agricultural/horticultural research are typically carried out. In the present study, these experiments were vital to understanding how different crop management practices influenced the occurrence and severity of CYD. In the case of this project, and crop research in general, glasshouse studies are fundamental to informing larger scale field trials, and the subsequent investments behind them. These experiments enable the answering of scientific questions through maintaining control

over a wide range of experimental variables. They also allow the easy application of treatments/environmental limitations and subsequent interpretation of results and also replicability for future research (Passioura, 2006).

However, glasshouse based experiments incur considerable limitations. Growing plants in pots has several downsides which make results difficult to interpret and to apply to field conditions. Factors such as atypical water relations, temperatures, and soil structures all influence the roots and rhizosphere interactions, and thus plant growth and experimental results (Passioura, 2006). Experimental artefacts can be inadvertently introduced into these results. These include often overlooked factors such as pot size and water and nutrient supply, all of which greatly influence plant size (Passioura, 2006; Poorter, *et al.,* 2012a; Poorter, *et al*.,2012b; Nesmith and Duval, 1998). Furthermore, rhizosphere interactions may be very different in a field environment. Even if field soils are used in experiments, there is generally a loss of the structural features that influence these interactions in the field (Pankhurst, *et al*., 2002; Passioura 2006). For this reason, experimental artefacts have been considered wherever possible when interpreting the results of this study.

2.4.2 The effect of different depths of tillage

Along with crop rotation, tillage practices are fundamental to crop performance; affecting the chemical, physical, and biological properties of soils (Munkholm, *et al.*, 2013; Miura, *et al*., 2008). Ploughing after a coriander crop was proposed as a means to potentially reduce CYD in a subsequent crop (Fraser, 2017). In fact, this practice is used by some growers who do not get CYD (Fraser, 2017). While full tillage or ‘deep ploughing’ can negatively impact soil structure, it is also known to potentially alleviate some of the effects of deleterious microorganisms (Bennett, *et al*., 2012). Furthermore, it was thought that this technique may change the concentration and position of coriander crop residues, thereby influencing the occurrence of CYD (Fraser, 2017).

Overall, the results of the ploughing experiment did not provide conclusive evidence towards the influence of deep ploughing on the occurrence of CYD, at least in a glasshouse scenario. In the second crop cycle, pots which were ploughed declined

by 27%, compared to a smaller decline of 15% in harrowed pots. The third crop cycle presented a contrasting result with ploughed pots showing slightly less decline (26%) than harrowed pots (32%). This experiment required 28cm pots with a soil volume of 15 litres (15 times the volume of the 13cm pots used in most other experiments in this study). These pots produced very large plants (and no stunting of growth in second and third cycles), and also maintained obviously different water relations to experiments carried out in smaller pots. Crop soils remained dry and friable, even in the second and third cycles. For this reason, a complete inversion of soils was challenging, and perhaps not completed in a manner representative of deep ploughing. Besides the potential experimental influence of large pots, it must be noted that in these early stages of experimental work, pots were not base-dressed with fertiliser before sowing a subsequent crop. Although plants had access to a large volume of nutrient rich compost, a nutritional disadvantage was still likely in the subsequent crop cycles. This may have contributed to the greater overall decline in the third cycle. While a general level of decline was observed in these experiments, the influence of nutrient availability and inconsistent tillage methods confounds interpretation in terms of the effect on CYD.

Harrowing was also proposed as an experimental treatment for this study, as a lack of harrowing before sowing the second cycle of coriander may have influenced the level of CYD achieved in previous glasshouse studies (Fraser, 2017; Kim Parker, pers. comm., 2017). Re-sowing in compacted soils could have contributed to the defined yield decline effect achieved, which was typically 50% (Fraser, 2017). In terms of soil preparation for crops, ploughing is considered a kind of primary tillage practice. Harrowing, is then a final step in the soil preparation process whereby seedbeds are finished with a shallow cultivation. This technique has important benefits for crops and soils, including: creating a finer textured soil, protecting the soil surface from rapid drying, improving air and water penetrability, influencing microbiological process, and facilitating better nutrient availability (Folnovic, 2017). Nevertheless, it is unclear whether UK coriander growers always harrow before sowing a crop.

In the glasshouse, results of the harrowing experiment showed that overall yields for harrowed pots were 14% greater than yields for non-harrowed pots. However, a considerable level of decline was still seen in both of the treatments, with

significantly smaller plants (above ground weights) being produced in both treatments for the second cycle crop. Like the ploughing experiment, nutrient availability must be noted as a potential contributing factor (pots were not base-dressed before re-sowing). Notwithstanding, results showed that harrowing had a significant positive impact on coriander yield in a second cycle. It is likely that the numerous biotic and abiotic influences of harrowing (or a lack of it) contributed to the significant difference between harrowed and non-harrowed pots compared to the control. For this reason, harrowing was included as a factor in the set of 'optimum' conditions for coriander growth in subsequent experiments.

2.4.3 The effect of planting density

Besides crop rotation and tillage, a number of other cultural practices influence the performance of crops—potentially relevant to CYD. Plant spacing and density of crops are known to have significant impacts on numerous factors associated with plant growth. As an example, maize (*Zea mays L.*) has been highly studied in this regard, and stand density has been found to strongly influence plant architecture, growth and developmental patterns, and grain yields. (Sangoi, 2001; Widdicombe and Thelen, 2002; Abuzar, *et al*., 2011). Additionally, plant spacing has been shown to have important effects on levels of weed surpression (Weiner, *et al.*, 2001) and also disease. Research on *Sclerotinia sclerotiorum* (white mold) has shown that row widths wider than the conventional drill width spacings can reduce the disease in soybean (Widdicombe and Thelen, 2002).

A previous AHDB report found the growth of pot-grown coriander to be highly influenced by different planting densities in the glasshouse (Flowers and Bashtanova, 2008). This suggests that planting density may have influenced the level of yield decline observed in the previous study (Fraser, 2017), and also represents an important factor in understanding conditions that may contribute to CYD. In carrying out the present research, it was discovered that field coriander growers use a wide range of planting densities. The planting density experiment therefore reflected this variation, whilst considering the potential impact on CYD. The 'high' density used in this experiment, (.27 seeds/cm^2) 36 seeds for a 13 cm (1 litre) pot, was used throughout the previous study, and is typical of that used for pot

grown coriander. The 'low' density used in this experiment reflected a planting density used by an outdoor grower who does not generally get CYD (Robert Gibbs., pers. comm., 2017). At just 3 seeds (.025 seeds/cm^2) per 13 cm (1 litre) pot, it represented a ten-fold difference from the high density. An intermediate figure between the two densities (0.15 seeds/cm^2=20 seeds) was also used.

In considering the limitations of this experiment, it must be noted that coriander in the field is 'drilled' in rows. In this way, long, narrow clumps of plants are produced with a very high density in one dimension (within the row), and a very low density in the other dimension (between the rows) (Bleasdale, 1984; cited in Weiner, *et al*., 2001). While the dimensions of planting density achieved in the field were not replicated in this study, considerations for the potential impact of overall planting density on growth and yields were deemed to be an important aspect which may influence the occurrence of CYD.

The results of the density experiment showed a marked difference in the size of plants, with the low density pots producing much larger above and below ground plant parts. This is not surprising, given the comparative increase in availability of light, nutrients, and rooting volume (Poorter, *et al*., 2012a). Nonetheless, variability amongst low density plant weights was very high, perhaps resulting from a growing environment with inconsistent levels of moisture, rooting volume and nutrients. Interestingly, the results of total yields per pot presented a different scenario. Although replication was low, the medium density showed limited decline (13%), compared to the low density (34%) and the high density (41%), with considerably more decline. Results indicate the potential for planting density as an important factor when determining 'optimum' conditions for coriander, and also the limitation of CYD. Observations supporting this were not quantified. However, it appeared that particularly with the lower density plants, the second crop cycle exhibited a pronounced loss of soil structure, potentially associated with greater exposure to water and potential waterlogging (also potential leaching of nutrients). The higher density pots appeared to maintain better water relations, but may have incurred greater decline in response to the lack of light and nutrients, associated with increased competition (Berendse and Möller, 2009). Overall, results suggest that planting density could be an important influence on CYD. This factor should be

considered, not just in terms of maximising yields, but in terms of limiting the occurrence of CYD.

2.4.4 The influence of fertiliser regime on experimental results

In addition to planting density, crop nutrition management is a vital influence on plant yield. As mentioned previously, fertiliser regime is also an important consideration that can become an experimental artefact in pot trials. Initial experiments in this study used a single application of fertiliser, as described in the general methods (2.2.1). However, this method inherently puts second cycle crops at a nutritional disadvantage, compared to control pots where coriander is sown in fresh, nutrient-rich compost. The high levels of yield decline achieved in the previous CYD study (approximately 50%, Fraser, 2017) (and also in some experiments in the present study) were likely influenced by this fact.

The results of the fertiliser experiment showed a significant difference in plant size depending on treatment. This coincides with the CYD observed in the previous study, and also the 'stunting of growth' reported by growers in reference to CYD in the field (Fraser, 2017). Above ground plant weights were significantly greater for fertilised pots (both control and second cycle). Below ground weights were greatest for non-fertilised cycle two pots. This could reflect the tendency of plants to change their biomass allocation patterns in response to environmental conditions/stress. It must be noted that variability was high in this instance, perhaps reflecting the heterogeneity of soil conditions and nutrient availability after a previous crop. It has been suggested that plants in low-nutrient environments may allocate more resources to their roots in order to increase their capacity to uptake limited soil resources (Müller, *et al*., 2000; Berendse and Möller, 2009; Poorter, *et al*., 2012c). Likewise, the small roots observed in non-fertilised control pots may indicate a contrasting response to the abundance of nutrients in fresh compost.

In terms of overall yields for the fertiliser experiment, second cycle crops showed a significant overall decline from controls. Fertilised second cycle pots declined by 30%, compared to 46% in non-fertilised pots. It is likely that factors noted in previous experiments also contributed to this general result (*e.g.,* the high planting density

and lack of harrowing), but may not account for all of this CYD effect. It is standard practice for growers to 'base-dress' a growing area with fertiliser before sowing a crop (Robert Gibbs, pers. comm., 2017; also reviewed in the grower questionnaire in Fraser, 2017). In the case of coriander, a fast-growing and leafy crop, availability of nitrogen is particularly important for successful yields (Robert Gibbs, pers. comm., 2017). In terms of CYD, a lack of nutrients is an unlikely causal agent in the field. Most crops grown commercially in shortened rotations are provided with ample fertiliser and depletion of major nutrients is unlikely (Bennett, *et al*., 2012). However, this does not discount the involvement of other mechanisms, such as deleterious rhizosphere microorganisms, which may impede the actual delivery/uptake of plant growth substances or nutrients to plants (Bennett, *et al*., 2012).

2.4.5 Performance of coriander under a set of 'optimum' conditions for a second crop cycle

Crop yields are influenced by a huge range of factors. These include a plant's genetic character, but also governing conditions for growth: *e.g.* climate/weather, soil fertility, pest and disease control, soil water stress, nutrient availability; and the physical, chemical, and biological properties of soils (Bennett, *et al*., 2012; Reeves, 2017). It has been suggested that the current practices for growing coriander in the UK may be creating a crop environment that is detrimental to yields (Fraser, 2017). Through examination of a range of factors affecting plant growth in the glasshouse (depth of tillage, planting density, and fertiliser regime), a concluding experiment was designed to assess a set of potentially optimum conditions for coriander growth in a subsequent cycle.

The optimum conditions experiment used a combination of factors to facilitate an ideal growing environment for a second cycle of coriander. Harrowing was used as it was shown to have positive effects on yields in a previous experiment. A moderate-low planting density was selected, based on previous planting density insight and grower advice (Robert Gibbs, pers. comm., 2018). Crop soils were also dried/desiccated in accordance with the positive results for the practice shown in previous work (Fraser, 2017), and in a further experiment discussed in Chapter 3. Larger pots were chosen (23 cm (5 litre), rather than the standard 13 cm (1 litre) as

they provide a more stable growing environment with less water stress (Nesmith and Duval, 1998; Poorter, *et al*., 2012a). However, even after facilitating these optimised conditions, the second crop cycle still showed a statistically significant decline in yield of 28%. It is possible that a longer period of desiccation would have improved growth. Pots were relatively large, but were only allowed to dry for a period of two weeks; as opposed to the period of four weeks used in the desiccation experiment (conducted in13cm pots) (Chapter 3). Additionally, it is possible that the density chosen was not optimum for a second crop cycle, in combination with the other experimental factors. In any case, results suggest that conditions may have fallen short of 'optimum', and also that a further mechanism may have contributed to the occurrence of CYD in this case—likely a microbiological influence.

2.5 Chapter 2 Conclusions

This experimental work suggests that coriander yield and the occurrence of CYD are influenced by a combination of interacting factors, at least in the glasshouse. Overall results have been characterised by high variability within experiments, both in terms of individual plant size and total yields. Although the effect of ploughing on CYD was not conclusive, an insightful result was shown in the harrowing experiment. This indicated that harrowing facilitated better yields in second cycle crops. Planting density was also shown to be a potentially important consideration in addressing CYD limitation. This factor strongly influenced the size of individual plants, and also affected the levels of decline in yields of a second crop. Fertiliser had a significant effect in this study, and likely contributed to the result of small, stunted plants in second crop cycles of the previous study. The optimum conditions experiment did not prevent CYD, perhaps due to the shortened time of desiccation, or a density which was not optimum for the size of the pot. However, even under attempts at growth optimisation, the level of decline was pronounced in second cycle yields. This may reflect some of the many experimental artefacts inherent in re-growing in the same pot and soils in the glasshouse. However, these results also make the case for a further causal agent in CYD.

Overall, results of the pot trials provided important insight into some of the conditions associated with the occurrence and severity of CYD. They also showed potential for

a further mechanism, beyond soil physical properties, which must be considered. Chapter 3 will therefore examine the potential microbial involvement in CYD, which may be indirectly linked to the abiotic factors and cultural practices examined in this experimental chapter.

Chapter 3: Microbiological studies of coriander yield decline

3.1 Introduction and chapter overview

Chapter 2 results indicated that selected growing conditions resulted in varying levels of CYD when coriander crops were grown successively in the same soils. Specifically, evidence showed that harrowing, using different sowing densities, and fertiliser regime, contributed to reduced levels of decline in the glasshouse, both in terms of the individual size of plants and total yields per pot. However, even after facilitating a set of 'optimum' growth conditions for a second crop cycle in the glasshouse, decline still occurred. This suggests the possibility of microbial community involvement in the problem, which was also hypothesised in the previous CYD study (Fraser, 2017). There is increasing evidence to support changes in agronomic practice as means to improve both the productivity of crops, and also longer-term sustainability of cropping systems though influencing (amongst other things) the biology of soils (Watt, *et al.*, 2006). Chapter 3 focuses on this concept: the fact that the soil microbiome changes in response to particular soil and crop management practices. Here, glasshouse experiments and microbial studies on a grower's soil which showed signs of CYD aimed to provide further insight into the microbial communities associated with CYD.

3.1.1 The crop soil microbiome and the rhizosphere

A variety of abiotic and biotic factors shape soils and consequently influence plant growth. Amongst these, the activities of microbial communities are intimately linked to many aspects of the physiology and development of plants (Mendes, *et al*., 2013). Likewise, agricultural productivity is highly dependent on the presence of a foundation of these microbial communities, many of which exist in soils (Bakker, *et al*., 2012). Within the agricultural environment, it has been found that different crop

species, soil types, and temporal changes determine microbial community compositions (Wieland, *et al*., 2001; Berg and Smalla, 2009; Bakker, *et al.*, 2012; Smalla, *et al*., 2001). The complex microbiome of crop soils is a 'hot topic' in agricultural research, and becoming an increasingly important area of study in addressing the problem of yield decline in crops (reviewed in Bennett, *et al*., 2012). Of particular relevance to crop production are the plant-driven interactions of microbial communities that are specifically associated with plant roots.
The rhizosphere can be defined as the soil adjacent to and influenced by plant roots, through the release of plant substances which affect microbial activity (Sørensen, 1997; Nihorimbere, *et al*., 2011; Hartmann, *et al*., 2009). Microorganisms of the rhizosphere directly and indirectly influence the composition and also the productivity of plant communities (Schnitzer *et al*., 2011; Mendes, *et al*., 2013). Soil and rhizosphere microorganisms are considered 'sensitive' to small changes in abiotic conditions, including factors associated with environmental stress and soil disturbance (Chaparro, *et al.*, 2012). They are therefore considered bioindicators of soil quality (Mendes, *et al*., 2013). Plants exert selective forces on the soil microbiome, largely through root exudates; a phenomenon which has been known since the hypotheses of Lorentz Hiltner (Hiltner, 1904, reviewed in Hartmann, *et al*., 2009; Schroth and Hildebrand, 1964). In doing this, plants shape the rhizosphere and a spectrum of plant-microbe interactions affecting plant growth and crop yields (Bakker, *et al.*, 2012; Berg and Smalla, 2009; Hartmann, *et al*., 2009).

Rhizosphere microorganisms are thought to play an important role in the fitness and health of their plant hosts; contributing to the acquisition of plant growth substances/nutrients, imparting levels of resistance to plant pathogens and disease, and supporting plant growth during abiotic stress (Nehorimbere, *et al*., 2010; Mendes, *et al*., 2013). Likewise, less desirable rhizosphere interactions also occur. Plant pathogens may colonise the rhizosphere (Mendes, *et al*., 2013), or plants may 'select' for deleterious rhizosphere microorganisms (DRMOs) (mentioned in Chapter 1), or also form negative mycorrhizal associations (NMOs) (Bennett, *et al*., 2012). Both of these scenarios have been implicated as causal agents of crop yield decline, and are examined in further detail in the discussion section of this chapter (3).

3.1.2 Methods of studying the soil microbiome and rhizosphere communities

Traditional studies of the plant microbiome and the rhizosphere were characterised by methods of isolation and culturing using different growth media and environmental conditions (Laksmanan, *et al*., 2014). Such techniques present a large degree of selective bias, and it has been estimated that less than one percent of environmental microorganisms are culturable by standard techniques (Amann, *et al*., 1995; Hugenholtz, *et al.*, 1998; Davis, *et al.*, 2005). However, advances in cultivation-based approaches have managed to isolate and study some rarely culturable microorganisms (Davis, *et al*., 2005; Stewart, 2012). Recent advances are non-culture-based techniques that have revolutionised understanding of soil microbial diversity with next-generation DNA sequencing (NGS) technologies. These range from PCR amplicon sequencing of genetic markers, to 'metagenomics' which provide analysis of sequence data generated from the total pool of extracted environmental DNA (Jacquiod, *et al*., 2016). A variety of techniques for environmental DNA sampling have been able to detect cryptic organisms, facilitate large-scale studies, and generate great volumes of data for molecular systematics (Bass, *et al.* 2015).

3.1.3 Previous microbial studies of CYD

The AHDB study by Fraser (2017) provided the first microbial studies of CYD in the UK. This work established a potential link between microbial communities and yield decline through comparing the rhizosphere and bulk soils of healthy and yield decline coriander. Using Illumina NGS techniques, the main findings were that a rhizosphere effect was observed after continuous coriander cropping, with rhizosphere soil microbial communities markedly different to the corresponding bulk soil microbial communities in healthy coriander. Further findings showed that microbial communities of healthy vs. yield decline plants differed considerably, though separate instances of decline showed different patterns of relative abundance of fungi and bacteria. A hypothesised fungal cause in the CYD problem was suggested, based on the dominance of the fungal phylum Mucoromycota in yield decline soils, which were only half as abundant in healthy soils.

3.1.4 Chapter aims and individual objectives

Overall aims

This chapter aimed to further explore crop and soil management options to reduce CYD, with a focus on the biological properties of coriander crop soils. Specifically, microbiological community changes were explored, including those associated with: a) manipulating the soil environment and b) those occurring in a grower's field soil experiencing CYD. Glasshouse studies provide further evidence for practices to improve conditions for growth and reduce CYD, but also support the hypothesis of microbial community involvement in the problem. To further elucidate a potential microbial cause associated with CYD, microbial community studies provide insight into differences in the relative abundance of taxa associated with healthy and yield decline coriander plants.

Individual objectives

- To assess desiccation/drying of crop soils as a means to potentially reduce CYD through changing soil structure, whilst altering associated microbial community composition.

- To investigate the effect of soil sterilisation on CYD. This work uses a grower's field soil showing evidence of CYD and assessed coriander growth in sterilised (autoclaved) soil compared to non-sterile soil.

- To elucidate changes in the microbial community composition of bulk and rhizosphere soils through identifying fungal and bacterial taxa associated with healthy and decline soils, from both a grower's field soils and from the above desiccation experiment soils.

- To determine specific microorganisms which may associated with CYD; thereby providing a base for future study.

3.2 Materials and methods

General methods for growing coriander in the glasshouse, as well as data collection and statistical analyses are outlined in Chapter 2 (2.2.1). In addition to the standard application of fertiliser at four weeks' growth, both of the pot experiments in Chapter

3 were base-dressed with fertiliser before sowing a second crop cycle. As outlined in Chapter 2, 'C1' or 'control' indicates fresh soils with no history of coriander cropping; 'C2' refers to coriander grown in the same soils for a second crop cycle.

3.2.1 Investigating the impact of drying out crop soils before sowing a second coriander crop

Coriander seeds were sown in 13 cm (1 litre) pots at the standard density (36 seeds) and harvested after approximately eight weeks. Three replicate pots with their crop soils intact were either: 1) left to dry out for a period of four weeks ('desiccated'), or 2) watered daily for four weeks ('watered'). A second crop was then sown in the same soils at the initial planting density, alongside C1 controls. Pots were harvested after approximately eight weeks, whereby total yields per pot (three replicates), and individual plant biomass (30 replicate plants) were collected.

3.2.2 Assessing the effect of soil sterilisation on CYD and coriander growth in an affected field soil

Two field soils were obtained from a coriander grower who experiences yield decline. Yields data were not available for the fields sampled. One soil sample was taken from a field with a healthy crop, and a second soil sample was collected from an adjacent field which had produced a poor crop with stunted growth symptomatic of CYD (Figure 3.1). These crops were sown just one week apart. Both of the field soils were used to grow crops in the glasshouse, which produced similar, severely stunted plants in each case. Plants and soil from the same fields were later used for the microbial studies outlined in section 3.2.3. A glasshouse experiment was set up to test sterilisation as a possible means to alleviate CYD in a crop grown in the 'healthy' field soil (which had contained a previous crop in the field, and was confirmed to be CYD affected). To facilitate this, half of the soil was sterilised by running it through an autoclave for two cycles (high-pressure saturated steam reaching 121°C). Ten 12 cm (700 milliliter) pots were sown at the standard density (30 seeds) consisting of: five pots with sterilised soil, and five pots with non-sterilised soil. Plants were grown for approximately six weeks, whereby the individual plant biomass (20 replicate plants), and total yields per pot (five replicates) were collected for the two treatments.

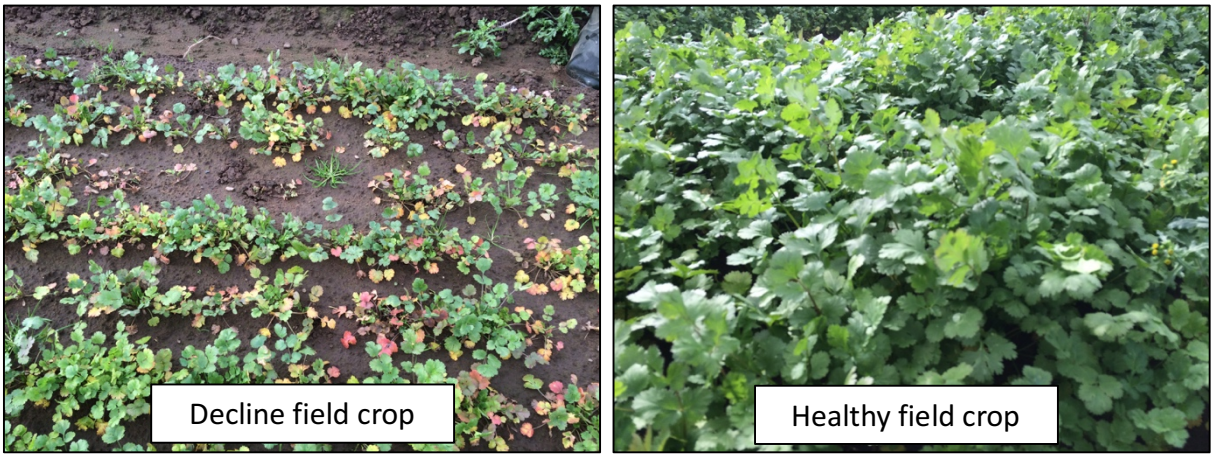

Figure 3.1: Field soils used in the sterilisation experiment and microbial studies
Figure shows the two field soils used: (left) from a field with a yield decline crop, (right) from a field with a healthy crop; both field soils were used to grow coriander in the glasshouse—each producing poor, CYD symptomatic plants.

3.2.3 Microbial studies of coriander field soils and desiccation experiment soils

Soil DNA extractions

Plant and soil samples were collected from the grower's field soils described in 3.2.2 and pictured in Figure 3.1. These represented a 'healthy' coriander crop and a yield decline coriander crop with two levels of decline ('moderate CYD' and 'severe CYD') (pictured in Figure 3.2). Plants and soils were also collected from the desiccation experiment (3.2.1). DNA extracts were prepared for twelve samples in order to examine the associated microbial communities. Rhizosphere and bulk soils were collected, with 'rhizosphere' constituting soils clinging tightly to roots (Hilton, *et al*., 2013), and 'bulk' soils as those not adhering to roots. Rhizosphere and bulk soils were taken from the following six soils (for a total of 12 samples): 1) Healthy field soil; 2) Severe CYD field soil; 3) Moderate CYD field soil; 4) Desiccation experimental control (C1); 5) Desiccation experiment 'desiccated' treatment (C2); and 6) Desiccation experiment 'watered' treatment (C2). DNA extractions were performed using a PowerSoil® DNA isolation kit (MO BIO laboratories, Inc.). The manufacturer's protocol was followed with 0.25 g of soil processed for each sample. Rhizosphere samples required four to five plants to provide sufficient soil for extraction. Purified DNA was stored in a freezer at -20°C until further use.

Figure 3.2: Examples of plant samples taken from a grower's field crops
Figure shows a plant from the healthy field crop (left) and two plants taken from the poor field crop (middle and right), which show two levels of CYD.

Polymerase Chain Reaction (PCR) and amplification

PCR and amplification were carried out for fungi and bacteria. The internal transcribed spacer (ITS) ribosomal DNA region was chosen for PCR amplification due to its universality in the molecular systematics of fungi (Schoch, *et al*., 2012). PCR primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used. For bacteria, the 16S (ribosomal RNA (rRNA) gene was also chosen as it is the preferred genetic technique for performing taxonomic studies of bacterial species (Clarridege, 2004). PCR primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1027R (5'-GCACACAGGCCACAAGGG -3') were used. The PCR reaction recipe aliquot per sample was as follows: 6.4 µl H_20, 0.8 µl forward primer, 0.8 reverse primer, 10.0 µl. Type-it Multiplex PCR mastermix (QIAGEN©), 2.0 µl purified DNA. PCR reactions were performed in a Veriti ^{TM} Thermal Cycler (Applied Biosystems) for each gene region. A cycle was run for ITS with a temperature profile of 5 min at 95°C; followed by 35 cycles of: 30 seconds at 95°C, 60 seconds at 55°C and 60 seconds at 72°C; followed by 6 min at

72°C, and then a holding temperature of 15°C. The cycle for 16S was run with a temperature profile of 90 seconds at 98°C; followed by 30 cycles of: 20 seconds at 98°C, 20 seconds at 60°C, and 60 seconds at 72°C. These 30 cycles were followed by five minutes at 72°C, and then a holding temperature of 15°C.

MinION nanopore sequencing (Oxford Nanopore Technologies)

The metagenomic sequencing workflow used generally follows that of Edwards, *et al*. (2016). PCR product quality/concentration was quantified using Qubit 4 Fluorometer (ThermoFisher Scientific) to determine DNA concentrations (ng/µl) for ITS and 16S for each of the twelve samples. Concentrations of samples ranged from 34.4 to 160.4 ng/µl. The required volume to provide 200 ng genomic DNA for each sample was determined. Volumes of input DNA were then adjusted with nuclease-free water to make a total volume of 7.5 µl for each of the twelve barcoded samples. A Rapid Barcoding Sequencing kit (SQK-RBK004) (Oxford Nanopore Technologies (ONT)) was used according to the manufacturer's protocol (Appendix), with the input mass of 400ng total genomic DNA (200 ng ITS and 200 ng 16S) required for 1D nanopore sequencing. The addition of the optional AMPure XP bead (ONT) purification step was also carried out to increase throughput through the cleaning up and concentrating of pooled material. The sequence library was prepared using 5 µl barcoded DNA. This was instead of the protocol's recommended 10 µl, as DNA quality and concentrations were deemed sufficient (Vince Mulholland, pers. comm. 2018) to use a smaller quantity of barcoded DNA and potentially enable a second sequencing run in the event of failure. Barcoded DNA was loaded into a FLO-MIN106 (ONT) flow cell. DNA sequencing was carried out with MinION Mk1B DNA Sequencer (ONT) using the sequencing software MinKNOW v.1.7.3 (ONT) with a 48-hour sequencing workflow (which was terminated at approximately 12 hours due to computer shutdown). 1D basecalling was performed remotely via the ONT Metrichor platform, where default settings and live basecalling were enabled. Metrichor includes scripts for the cloud-based taxonomic profiling of metagenomic

samples (Edwards, *et al*., 2016). During basecalling, reads were categorised as either pass or fail by the Epi2Me software, pass reads including those having passed a quality score threshold of 9. The EPI2ME platform WIMP (What's In My Pot) (ONT) was enabled to facilitate quantitative and qualitative sequence data analysis, as well as broad taxonomic classification. Albacore v.1.2.1(ONT) was used to convert FASTA5 files to FASTQ files and also to demultiplex barcoded results. The Kaiju program (Version 1.6.2 webserver http://kaiju.binf.ku.dk) was then used to provide further taxonomic classification (Menzel, *et al*., 2016). Kaiju analyses were conducted using the NCBI nr+euk database containing protein sequences from microbes, viruses and eukaryotes with low complexity filtering and in *greedy* mode (minimum match length:11; minimum match score: 75; allowed mismatches: 5). Outputs were visualised as Krona plots. Relative abundance percentages were calculated for bacterial phyla, fungal phyla, classes within Ascomycota, and for the most abundant ascomycete taxa (genera or species). Index Fungorum (2018) was used for currently accepted names of fungi.

3.3 Results

3.3.1 The effect of soil desiccation on CYD in a second crop cycle

The desiccation experiment showed a pronounced difference in coriander growth depending on the two treatments applied before sowing the second crop ('C2 watered' vs. 'C2 desiccated'). This was evident in the size of individual plants, and also for total yields per pot. Soils which had been watered daily before sowing a second coriander crop produced the largest individual plants (above and below ground), but the lowest total yields per pot (*i.e*. there were fewer plants in the watered pots, but those that grew were larger than those in the desiccated pots). The fresh above ground weights of C2 watered plants were significantly greater than those of C2 desiccated plants ($p=0.002$), and C1 control plants ($p=2.00^{e-04}$) (Figure 3.3a). However, dry above ground weights were not statistically different ($p> 0.05$). Figure 3.3a also illustrates the significantly larger fresh root weights obtained for C2 watered plants, compared to C1 control plants ($p=5.90^{e-06}$), and C2 desiccated plants

($p=2.46^{e-05}$). Dry below ground weights showed a similar effect, with C2 watered treatment plants again significantly larger than C1 controls (95% CI p=0.005) and C2 desiccated treatment plants (95% CI p=0.011). Root to shoot ratios followed the same pattern: C2 watered ratios were significantly larger than those of C1 controls (p=0.001) and C2 desiccated ($p=3.80^{e-04}$). The total yield for C2 watered pots (16.3 g +/- 13.4) was highly variable and not significantly lower (p>0.05) that C1 control pots (41.2 g +/- 8.3) or C2 desiccated pots (39.6 g +/- 9.0) (Figures 3.3b and 3.3c).

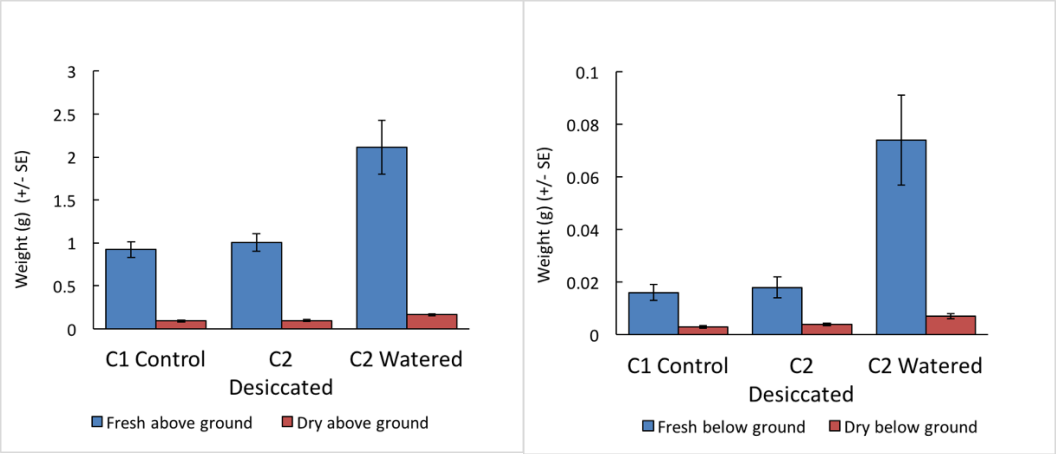

Figure 3.3a: The impact of desiccation on above and below ground weights of coriander (+/-SE)
Coriander was grown in fresh soils (C1) and harvested. Crop soils were either left to dry out for four weeks 'desiccated', or watered daily for four weeks 'watered', then re-sown (C2) alongside controls (C1). Figure shows largest above ground weights for C2 Watered plants (left) and also largest below ground weights for C2 Watered plants (right). (Means calculated from 30 replicate plants per treatment).

Figure 3.3b: The impact of desiccation on growth of a second coriander crop
Coriander was grown in fresh soils (C1) and harvested. Crop soils were either left to dry out for four weeks 'desiccated', or watered daily for four weeks 'watered', then re-sown (C2) alongside controls (C1). Figures shows plant growth at harvest: C1 Control pots and C2 Desiccated pots had similar yields; C2 Watered pots produced poor yields.

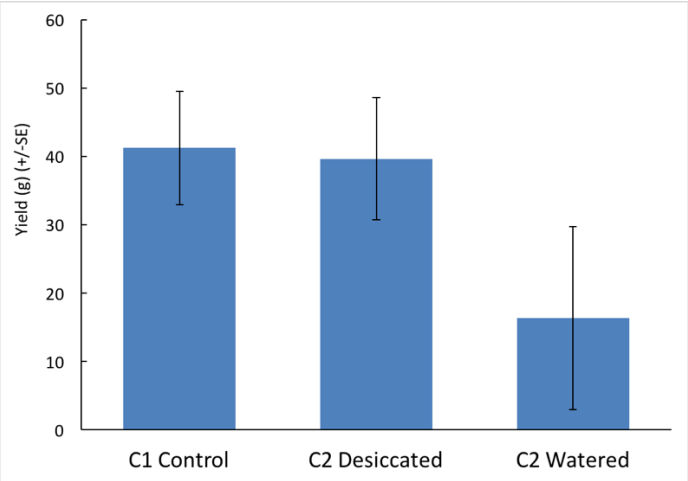

Figure 3.3c: The impact of desiccation on coriander total yields per pot (+/-SE)
Coriander was grown in fresh soils (C1) and harvested. Crop soils were either left to dry out for four weeks 'desiccated', or watered daily for four weeks 'watered', then re-sown (C2) alongside controls (C1). Figure shows: C1 Control pots and C2 Desiccated pots had similar yields, C2 Watered pots yielded comparatively low (but high variability). (Means calculated from three replicate pots per treatment).

3.3.2 The effect of soil sterilsation on coriander growth in a grower's field soil showing CYD

A grower's field soil (which been used to grow one previous coriander crop) produced larger plants and greater total yields per pot after sterilisation. A Welch two sample t-test (95% CI) confirmed significant differences for all measured characteristics of sterilised vs. non-sterilised soils, with the exception of root to shoot ratio (see Table 3.1). Figure 3.4a illustrates the dramatic difference in above and below ground biomass for treatments: sterilised soils produced approximately 70% larger plants (combined above and below ground weights). Total yields per pot were approximately 50% greater in the sterilised soils, compared to non-sterilised soils (Figure 3.4b).

Sterilised vs. Non-sterilised	***Welch two sample t-test results***		
Fresh above ground weights	t=10.86	df=38	$p=3.28^{e-13}$
Dry above ground weights	t=7.16	df=38	$p=2.43^{e-07}$
Fresh below ground weights	t=4.52	df=38	$p=1.10^{e-04}$
Dry below ground weights	t=4.61	df=38	$p=8.60^{e-05}$
Root to shoot ratio	t=2.03	df=38	*p=0.050*
Total yield per pot	t=3.90	df=8	*p=0.012*

Table 3.1: Welch two sample t-test output for sterilisation experiment
Figure shows that sterilisation significantly impacted the production of individual plant biomass, and total yields per pot.

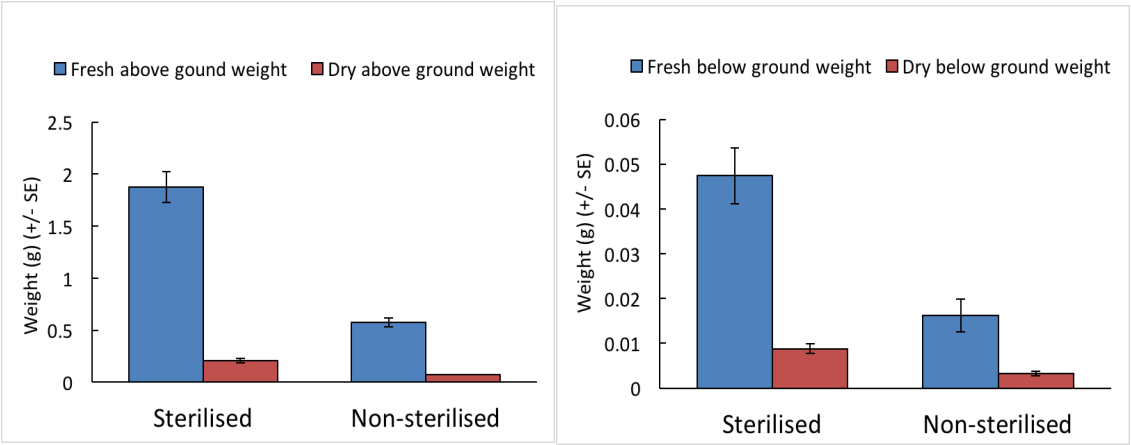

Figure 3.4a: The effect of soil sterilisation on above and below ground weights of coriander (+/-SE)
Field soils that had contained one previous crop of coriander were either 'sterilised' or left 'non-sterilised' before sowing a subsequent crop in the glasshouse. Figure shows the dramatic difference between treatments for above ground weights (left) and below ground weights (right), with sterilised soils producing much larger plants. (Means calculated from 20 replicate plants per treatment).

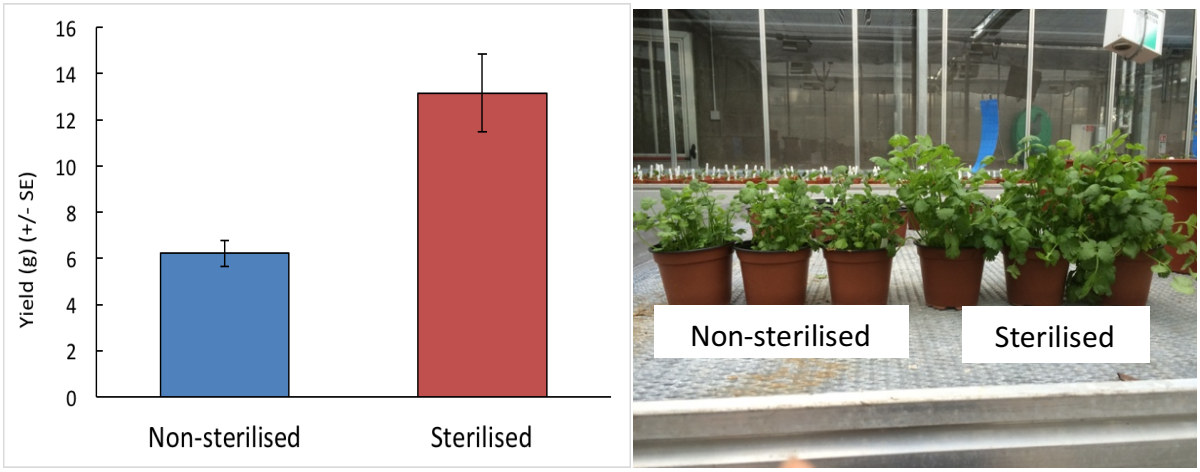

Figure 3.4b: The effect of sterilisation on coriander total yield per pot (+/-SE)
Field soils that had contained one previous crop of coriander were either 'sterilised' or left 'non-sterilised' before sowing a subsequent crop in the glasshouse. Figure shows total yield per pot for two soil treatments (left) with corresponding photo of growth at harvest time (right). (Means calculated from five replicate pots per treatment).

3.3.3 Metagenomic study of bulk and rhizosphere soils using MinION

MinION sequencing (ONT) produced 1,032,110 reads that passed the quality filter, with a total yield of 65.2 million bases. Average sequence length was 635 base pairs, with an average quality score of 9.95 (out of 12) (EPI2ME WIMP, ONT). The

broad taxonomic breakdown of sequences was: <1% Archaea, <1% Viruses, 19% Eukaryota, and 81% Bacteria (EPI2ME WIMP, ONT). Higher taxonomic resolution was provided through Kaiju web server (Menzel, *et al*., 2016), with the number of reads classified for each sample shown in Table 3.2. This shows that an overall rhizosphere effect (Berendesen, *et al*., 2012), particularly evident for the field soil samples, with more than three times the number of reads for rhizosphere compared to corresponding bulk soil for samples. This effect was also seen in the C1 control of the desiccation experiment (with twice the number of classifications for rhizosphere vs. bulk soil), but not for the other two soil treatment samples.

Sample	*Reads Classified*	*Total reads*
1) Healthy rhizosphere	45,029	58,740
2) Healthy bulk soil	13,249	17,726
3) Severe CYD rhizosphere	65,921	89,547
4) Severe CYD bulk soil	19,767	28,248
5) Moderate CYD rhizosphere	79,898	108,554
6) Moderate CYD bulk soil	23,748	33,219
7) C1 Control rhizosphere	63,535	97,939
8) C1 Control bulk soil	33,965	45,487
9) C2 Desiccated rhizosphere	68,748	102,431
10) C2 Desiccated bulk soil	73,172	103,996
11) C2 Watered rhizosphere	64,347	94,746
12) C2 Watered bulk soil	62,127	92,162

Table 3.2: Kaiju web server classification results
Table shows the total number of quality reads produced in MinION and number of reads classified in Kaiju web server. Samples 1-6 represent field soil samples from two coriander crops (healthy and CYD with two levels of decline); samples 7-12 were taken from the desiccation experiment and represent the two levels of C2 treatment and a C1 control.

Phylum level analysis

Identification at phylum level was carried out for bacteria and fungi to give a broad overview of potential microbial community changes within the field soil samples and desiccation experiment soil samples. Taxonomic assignments within the six most abundant bacterial phyla/super phyla are shown in Figure 3.5. Bacterial taxa classified for the twelve samples did not show clearly defined changes within either of the soil sample groups. Proteobacteria and Firmicutes were interchangeably dominant in both the field soils and in the desiccation experiment soils, throughout the rhizosphere and bulk soils. Distribution of fungal phyla across samples showed

more pronounced shifts between soils, as illustrated in Figure 3.6. This was particularly evident in the field soil samples, where relative abundances of ascomycetes in the rhizosphere of the CYD soils (both for severe CYD 81% and moderate CYD 82%) were nearly double that of the healthy field soil rhizosphere (44%). Within the desiccation experiment soils, the most notable difference between samples is the dominance of basidiomycetes classified in the bulk soil of the C2 desiccated sample, at 48% relative abundance (Figure 3.6). This is in contrast to basidiomycetes classified for bulk soils of C1 control (24%), and C2 watered soils (15%).

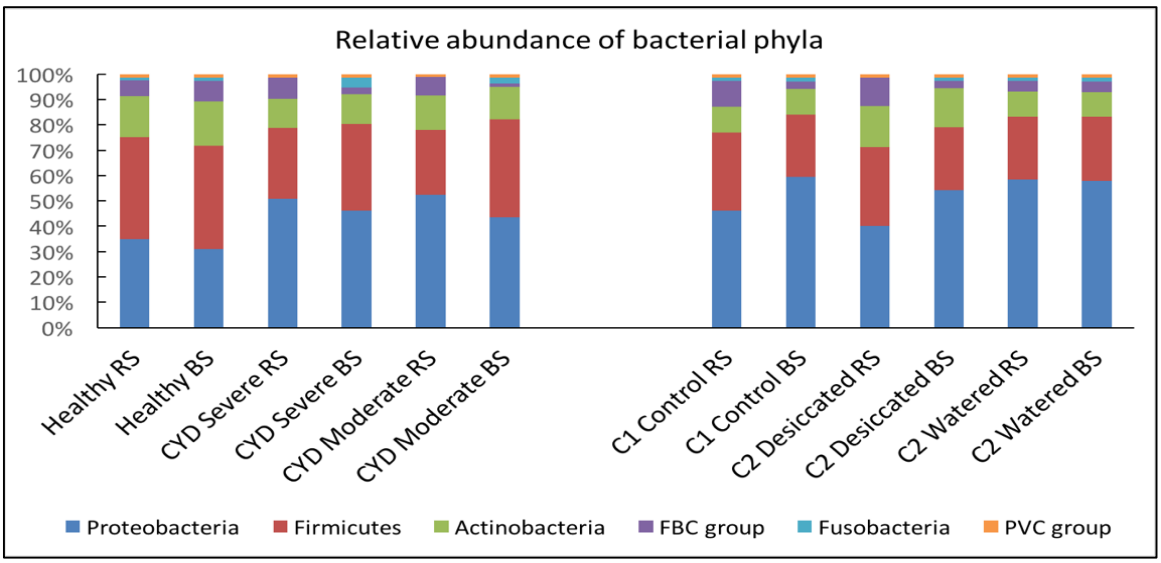

Figure 3.5: Relative abundances of bacteria for twelve soils samples
Figure shows the relative abundances of the main bacterial phyla classified for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil; 'FBC group'=Bacterioidetes/Chlorobi group; 'PVC group'= Planctomycetes, Verrucomicrobia, Chlamydiae group; (relative abundance % for total bacteria classified for each sample).

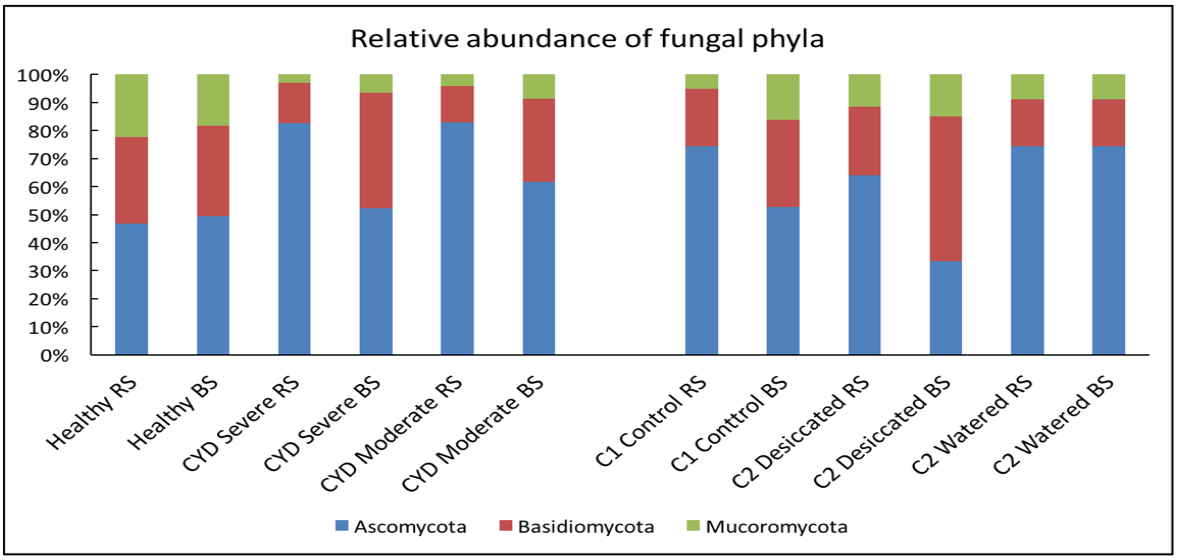

Figure 3.6: Relative abundances of fungal phyla for twelve soils samples
Figure shows the relative abundances of three fungal phyla classified for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil; (relative abundance % for total fungi classified for each sample).

Higher taxonomic resolution of fungi

Considering the overall dominance of ascomycete fungi classified, further taxonomic resolution within Ascomycota was carried out to provide additional insight into potential microbial community change. Figure 3.7 shows the relative abundance of six ascomycete classes. The most defined difference in comparing the six field soil samples is the shift from a dominance of Sordariomycetes in the healthy field soils (rhizosphere and bulk soil), to a dominance of Dothideomycetes in the CYD soils (rhizosphere and bulk soil for both levels of CYD). A type of rhizosphere effect can also be seen within the CYD samples, with a decrease in the abundance of Eurotiomycetes in the rhizosphere, compared to the bulk soil CYD samples. The desiccation experiment soils present a very different pattern of relative ascomycete abundance. The C1 control and the C2 desiccated samples show a similar distribution of rhizosphere fungi with a dominant relative abundance of Sordariomycetes, at 91% and 87%, respectively. However, the C2 watered soils show no clear distinction between rhizosphere and bulk soil fungi. C2 desiccated bulk soils have a very different distribution of ascomycetes compared to the other samples: 15% Taphrinomycetes (compared to <3% in the other two bulk soil samples), and 8% Saccharomycetes (compared to <2% in the other two bulk soil samples).

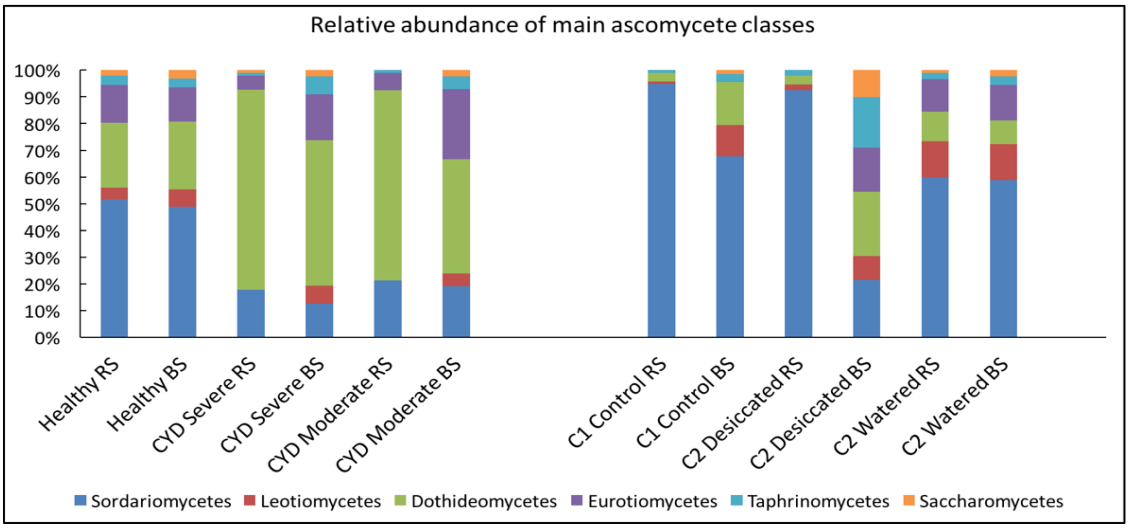

Figure 3.7: Relative abundances of ascomycete classes for twelve soil samples
Figure shows the relative abundances of six ascomycete classes for field soils (left) and desiccation experiment soils (right) with 'RS'=rhizosphere and 'BS'=bulk soil; (relative abundance % for total ascomycetes classified for each sample).

Further taxonomic resolution was obtained for field samples and desiccation experiment samples, to potentially identify fungi associated with yield decline (or healthy coriander). Figure 3.8 shows the relative abundance of the most common ascomycete taxa classified (to genus or species level). *Valsa mali* is particularly dominant in the healthy field soils. *Bipolaris sorokiniana, Leptosphaeria maculans,* and *Cenococcum geophilum* account for the dominance of Dothideomycetes in the CYD rhizosphere (both levels). These fungi also characterise the defined difference in rhizosphere vs. bulk soil fungi, which is not apparent pattern in the healthy field soils. Figure 3.9 illustrates the most common ascomycete taxa classified in the desiccation experiment. Like the field soil samples, *Valsa mali* is again highly prevalent, and dominates both of the C1 control samples, and the C2 desiccated rhizosphere soil. This is in contrast to the C2 desiccated bulk soil, which is characterised by a dominant relative abundance of *Trichoderma auroviride* (Sordariomycetes), and only 1% *V. mali.* *Neurospora tetrasperma* (Sordariomycetes) is the most abundant taxon in bulk and rhizosphere soils of the C2 watered samples, which show no distinction between rhizosphere and corresponding bulk soil.

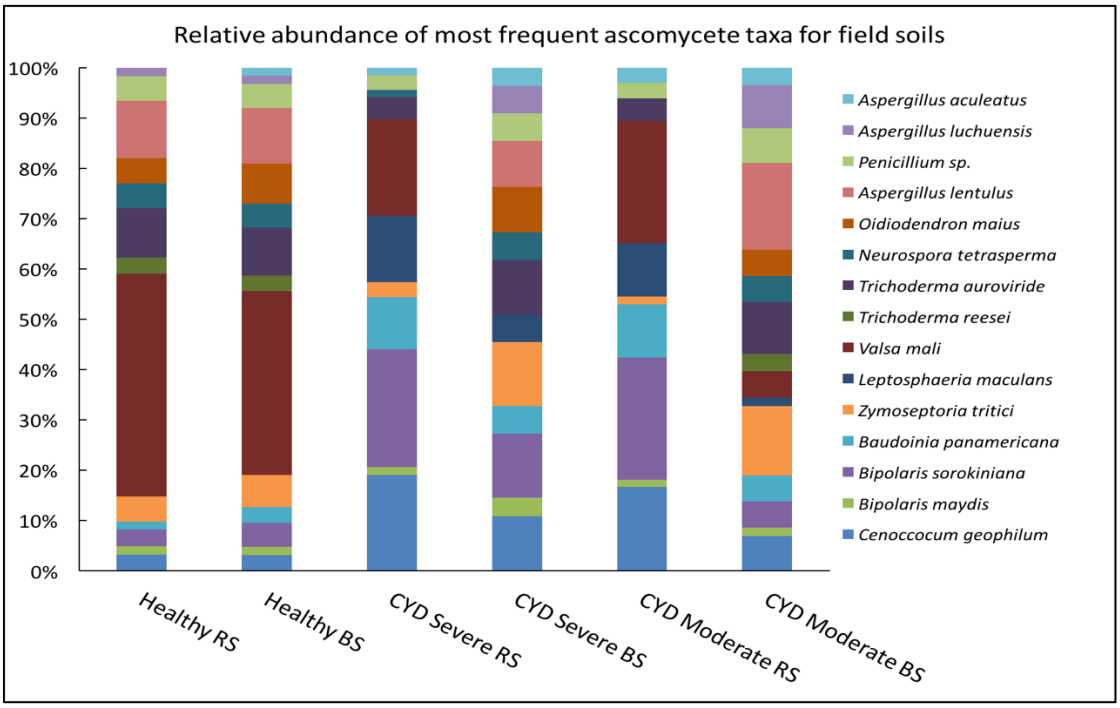

Figure 3.*8: Relative abundance of ascomycete genera and species for field soils*
Figure shows the relative abundance of the most frequently classified ascomycete taxa for the six field soils (to genus or species level); with a defined rhizosphere effect in the CYD samples; (relative abundance % for total ascomycetes classified for each sample).

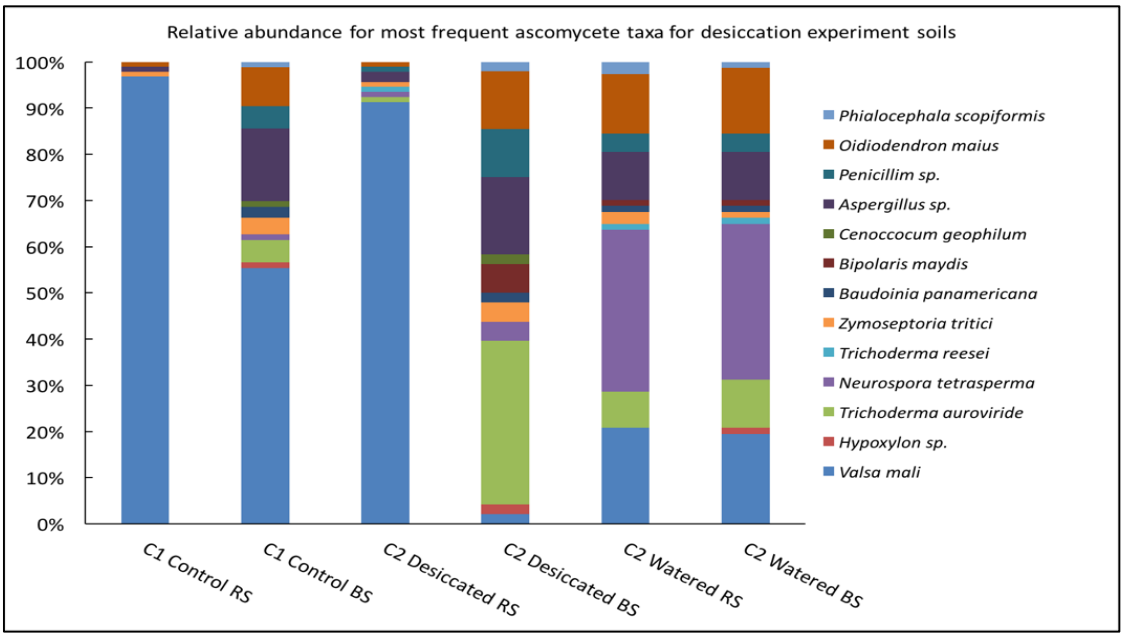

Figure 3.9 *Relative abundance of ascomycete genera and species for desiccation experiment*
Figure shows the relative abundance of the most frequently classified ascomycete taxa for the six field soils (to genus or species level); with a defined rhizosphere effect seen in the C1 control and C2 desiccated samples.

3.4 Discussion

3.4.1 The effects of desiccation on coriander growth in a second crop

The air drying of soils

In the desiccation experiment, coriander grown in soils that were dried out for four weeks before sowing a second crop, did not experience yield decline. The difference in mean total yields for C2 desiccated pots and C1 control pots was negligible; and the mean above ground fresh weight of C2 desiccated plants was actually slightly higher than that of C1 controls. Importantly, the fact that C2 desiccated pots did not experience yield decline, differentiates soil drying as a treatment apart from those used in Chapter 2 experiments (tillage, fertiliser regime, and sowing density). It is likely that the effect of drying out the crop soils after growing coriander helped to maintain the soil physical structure. Air-drying soils has been shown to facilitate the stability of soil aggregates, which helps to store soil organic matter and maintain overall soil structure and plant productivity (Kaiser, *et al*., 2015; Six, *et al*., 2004). But equally important, it is also likely that the microbial community composition of these soils was altered in the drying process. In this case, it appeared that drying had a beneficial effect on soil microbiota; specifically, resulting in very different fungal communities to those of the poor-performing C2 watered treatment pots. Observations of the microbial communities found in the desiccation experiment soils will be discussed in further detail in Section 3.4.4.

In contrast to the C2 desiccated pots, coriander grown in soils that were watered for four weeks before sowing a second crop performed very poorly. C2 watered pots produced much larger plants than the other two treatments, but very low comparative yields per pot. A plausible explanation for this increase in plant size, may be an indirect consequence of the decline in soil structure observed in the C2 watered pots. Poor soil structure led to waterlogging and the emergence and survival of few plants. This created an overall lowered plant density, which may have facilitated the growth of larger plants. This effect was similarly observed in the planting density experiment in Chapter 2 (2.3.3). In the density experiment, the lowest planting density resulted in much larger plants, but a clear degradation of soil structure in the second crop, due to excess water exposure. This consequently led to significant decline in yields for a second crop sown in the same soils at low planting density.

The soil microbiome of crops is highly influenced by agricultural management practices (Chaparro, *et al*., 2012). One such practice is solarisation, a technique for partial sterilisation which was first described as a method for controlling pathogenic fungi (Katan and DeVay, 1991). This technique is carried out by placing plastic sheets on soils during a hot and dry season for four to six weeks to facilitate 45°-55°C in the upper soil layers. Solarisation can kill many soil pests, pathogens, and weed seeds (Katan, 1981; Stapleton and Vejay, 1986; McGovern and McSorley, 1987). The practice is used throughout many parts of India, in place of chemical crop treatments and fumigation (Gill, *et al*., 2017), and has been used in Australia to reduce yield decline and improve growth of monoculture sugarcane (Magarey, 1996). Interestingly, it is also a practice used in southern Spain by coriander growers who do not get CYD (Victoria Langdale, pers. comm., 2017). Early studies by Katan, *et al*. (1976), showed that pathogenic fungi were greatly reduced when soils were heated to 45°-50°C, but that even milder treatments, including air-drying soils had similar effects (Fukunushi, 1980; Kaiser, *et al*., 2015). Potentially supporting the effect observed in the desiccation experiment in this study, is the fact that soil drying can perform some of the same functions as solarisation. Kaiser, *et al*., (2015) found that one of the major effects of air-drying soil (at ambient temperature), was the death of a large proportion of the soil-inhabiting microorganisms. Although the magnitude of this effect depends on the drought resistance and adaptation of the microbiota of the particular soil, the impact may still be significant. Besides affecting soil biotic properties, air-drying soils can also profoundly change the physical and chemical characteristics of a soil (Kaiser, *et al*., 2015).

In explaining the effects observed in the 'watered' plants in this experiment, it is well known that waterlogging is a major abiotic stress for plants. It quickly changes the soil environment and results in a suite of factors which can negatively affect plant growth and development: *e.g*. soil compaction, hypoxia/anoxia, toxic bi-products, and physiological and morphological changes (Parent, *et al*., 2008). As soil water content has direct and indirect effects on soil oxygen and nutrient availability, it is a significant influence on soil microbial composition (Ya-Juan, *et al*. 2012). Overall, it is likely that the 'decline' effect produced in the C2 watered treatment pots (and the C2 low density pots in Chapter 2) was symptomatic of waterlogging in pots. While waterlogging and lack of drainage affect plants in agricultural systems, these

experimental results may not reflect the same mechanisms of yield decline experienced by growers in the field, which have primarily been characterised by small, stunted plants.

3.4.2 The impact of sterilisation of a grower's field soil showing evidence of yield decline

The level of sterilisation achieved in the present study was not determined prior to sowing coriander. However, the contrast between the sterilised and non-sterilised soils confirmed an effect from the sterilisation. This difference was pronounced in the individual plant size, and the total yield per pot. Consistent with similar studies, no difference in the growth of coriander between the two soils was apparent during the first weeks of plant growth (Zhang, *et al*., 2016). It is likely that many of the beneficial microbiota were eliminated during the autoclaving process, inhibiting early plant growth and establishment (Zhang, *et al*., 2016). However, after this time, improved growth of the sterilised crops became apparent. The overall results suggest that the sterilisation process had eliminated a deleterious microbial element from the soils. This gives further weight to a microbial cause in CYD, at least in the case of the affected field soil. Importantly, it also confirms the pretense to this study: that a deleterious effect may occur in healthy coriander crop soil, when a subsequent crop is introduced into the same soil. This will be further explored in the microbial studies discussion (3.4.4). Another consideration in this experiment, is the fact that soil sterilisation has also been shown to facilitate increased availability and acquisition of nutrients, which may have further contributed to the effect of improved growth (Troelstra, *et al*., 2001; Costa, *et al*., 2006).

Along with solarisation and air-drying of soils, other non-chemical means have been used to control soil pathogens and also address yield decline associated with microbial causes. Heat 'sterilisation' includes a variety of techniques to raise soil temperatures above the levels achieved in solarisarisation. Soils are generally heated beyond 60°C, which is thought to be sufficient to eliminate most pests and pathogens (Baker, 1962). Some of the many modes of heat sterilisation include: steam sterilisation, hot water treatments, microwaving, and hot air treatments. Methods of application vary considerably depending on the scale of operation, indoor

or outdoor use, the soil type, and the level of soil infestation (Runia, and Molendijk, 2009). Steam sterilisation was used to improve growth of *Angelica sinensis* (Apiaceae), which suffers from yield decline in continuous cropping systems (Zhang, *et al*., 2016). Sterilisation has also been used to achieve similar results in studies of yield decline in other affected crops: *e.g*. cucumber (Ruan, *et al*., 2001), apple (Leinfelder and Merrin, 2006), pepper (Hou, *et al.*, 2006) and soybean (Zhang, *et al.*, 2007). Interestingly, this method is also used by a UK coriander grower in Guernsey to limit CYD. In this particular case, coriander is grown under glass in the soil (not in pots) (Simon Harty, pers. comm., 2018). Soils are steam-sterilised in between growing two to three crops of coriander before a soil rotation. In this case, yields from soils that are sterilised before sowing the second crop of coriander are approximately 30% larger than yields from unsterilised soils. Furthermore, these sterilised soils enable a 'second cut' of coriander. However, soils that are not sterilised do not facilitate this second cut, as they go to seed after harvest.

3.4.3 Limitations of microbial and metagenomics studies of soils

While culture-independent methods, including metagenomics, have enabled great advancements in understanding the microbial world, they are not without limitations and biases. Firstly, the quality of DNA used determines the end result, and the potential for a meaningful characterisation of the microbial communities of a given environment (Verma, *et al*., 2017). Obtaining high quality DNA from soils is made difficult by the fact that soil is a highly heterogeneous mixture. It consequently contains many substances that can contaminate DNA and also inhibit the PCR process (Verma *et al*., 2017). There is no standard protocol for conducting microbial studies of soils, and a number of biases can be introduced at various stages. Type of DNA extraction kit, primer design, and PCR parameters are amongst a few of the choices that will have an effect on the community structure observed in microbial sequencing analyses (Albertsen, *et al*., 2015; Jacquiod, *et al*., 2016). Additional bias may accompany the ever-widening choice of bioinformatics tools for sequence analyses (Verma, *et al*., 2017). Furthermore, important qualitative and quantitative effects occur with sequence database choice, and also the annotation procedure used (Jacquiod, *et al*., 2016). Finally, the present study is limited by a small sample size. Results presented in this chapter are therefore observations of microbial

communities and must be interpreted as such; more extensive studies are required to provide greater depth and more conclusive insight.

3.4.4 Examining the microbial communities of coriander field soils and desiccation experiment soils

The build-up of specific pests or pathogens in soils is considered a major contributing factor in the yield declines that affect crops grown in monoculture or shortened rotations (Bennett, *et al.*, 2012). A classic example of this is *Gaeumannomyces graminis* var*. tritici*, the fungal pathogen of wheat and barley known as 'take-all' which severely reduces yields of crops grown continuously in the same soils (Cook, 2003). Another well-defined case is that of peas (*Pisum sativum*), which suffer yield losses due to the build-up of legume foot and root rots (including *Pythium* spp. and *Fusarium* spp.) when crops are grown in the same soils without sufficient crop rotation breaks (Oyarzun, Gerlagh and Hoogland, 1993; Bødker, Leroul and Smedegaard-Petersen, 1993). Hilton *et al.* (2013) also determined that two fungal pathogens, *Olpidium brassicae* and *Pyrenochaeta lycopersici,* were implicated in yield decline of repeatedly cropped oil seed rape.

However, confirming the involvement of particular pests and pathogens in incidences of yield decline is not easy (Bennett, *et al*., 2012). There is increasing support for the involvement of communities of microorganisms which are not specific pathogens. DRMOs (deleterious rhizosphere microorganisms) have been linked to numerous examples of yield decline in crops grown in monoculture and shortened rotations (reviewed in Bennett, *et al*., 2012). These organisms operate in a different manner to pathogens, in that they do not penetrate vascular tissue, or cause major disease symptoms (Schippers, *et al*., 1987). Early studies by Schippers, *et al*. (1987) suggested that DRMOs restrict plant growth without obvious signs of disease. Some of the deleterious activities of DRMOs include altering a plant's uptake of water, ions, and plant growth substances; through limiting root growth and function (Schippers, *et al*., 1996; Schippers, *et al*., 1987). Even with the availability of adequate soil nutrients, the implication is that soil microbial communities may be partly responsible for yield reduction in crops grown in monoculture and shortened rotations. Soil sterilisation experiments have provided evidence to support the existence of DRMOs

in yield decline soils, and transfer studies have shown that such organisms may also be harboured in crop debris (Bennett, *et al.*, 2012; Nehl, *et al*., 1997).

The microbial communities of healthy and yield decline field soils

In examining the field soils sampled in this study, changes in bacterial community structure between samples were not obviously linked to yield decline. However, a pronounced difference in fungal taxa between the healthy and yield decline samples was observed. There was a clear dominance of ascomycetes in the CYD samples, particularly in the rhizosphere. Interestingly, the relative abundance of Mucoromycota was significantly lower in the CYD samples (bulk and rhizosphere), compared to the healthy field soils. This is in contrast to the pattern observed in the previous CYD study, where mucoromycetes (synonym zygomycetes) were much more abundant in yield decline soils, and thought to contribute to the CYD effect observed (Fraser, 2017).

Higher taxonomic resolution illustrated a clear increase in relative abundance of Dothideomycetes (and a corresponding reduction in Sordariomycetes) in the CYD samples, particularly in the rhizosphere. Dothideomycetes is the largest class of Ascomycota (Kirk, *et al*. 2008). This very diverse group includes many saprobes, but also many plant pathogens of great economic impact (Ohm, *et al*., 2012; Hyde, *et al.,* 2013). The healthy coriander field soils were dominated by Sordariomycetes, the second largest class of ascomytaceous fungi. Like Dothideomycetes, the Sordariomycetes class contains fungi with highly diverse ecologies, including numerous plant pathogens (Kirk, *et al*., 2008). Disease is considered an exception in plant-microbe interactions. Fungi are particularly known to vary their ecology, depending on host and environment; with interactions ranging from parasitism to mutualism and everything in between (Kogel, *et al*., 2006). This diversity means that functional modes, *e.g*. plant growth effects for the fungi classified in this study, could not be ascertained and attempting to assign a particular function to a fungal community would be a challenging undertaking and require further extensive study.

An increase in Dothideomycetes was clearly observed in the CYD samples in this study. But due to the many limitations present, results for taxonomic resolution beyond class level should be interpreted as points of interest for further study, rather

than definitive microbial community patterns. Nonetheless, some specific taxa were found to account for the increased contribution to relative abundance of Dothideomycetes in the CYD rhizosphere. These, along with *V. mali,* which accounted for the dominance of Sordariomycetes in the healthy soils, are described below.

Bipolaris sorokiniana

Bipolaris sorokiniana Shoemaker (Pleosporaceae, Dothideomycetes) is ubiquitous where wheat and barley are grown. It causes common root rot, leaf spot disease, seedling blight, head blight, and black point. This fungus is considered a global concern, and causes significant yield losses (Kumar, *et al*., 2002). While it is mainly known as a pathogen of Poaceae crops, it has also been observed on numerous other diverse hosts (Farr and Rossman, 2018). Importantly, this fungus accounted for just 2% of ascomycetes in the healthy rhizosphere soils, but 16% in both of the CYD severe and moderate decline rhizospheres. This indicates a notable population shift, and that *B. sorokiniana* may warrant further investigation as a potential DRMO associated with CYD.

Cenoccocum geophilum

Cenoccocum geophilum Fr. (Gloniaceae, Dothideomycetes) is one of the most common soil fungi forming ectomycorrhizal (EM) associations, and is well-known for its wide host and habitat range (Trappe, 1964; Lobuglio, 1999; Peter *et al*., 2016). It is one of the most ubiquitous EM fungi with woody plant roots, but has also been known to form associations with numerous herbaceous species (Obase, *et al*., 2017). It is the only mycorrhizal species in Dothideomycetes, a class particularly known for its plant pathogens (Spatafora, *et al*., 2012). In genomic studies, Peter, *et al*. (2016) showed that whilst genes characteristic of ectomycorrhizal basidiomycetes are present, this species still holds a significant set of genes known to be involved in pathogenesis. Intriguingly, *C. geophilum* contributed to the defined difference in fungal community structure between healthy and CYD rhizospheres. The relative abundance (total ascomycetes) of this fungus was 2% in the rhizosphere of healthy field soil, in contrast to 11% and 13% in the moderate and severe CYD rhizospheres, respectively. The ecology of *C. geophilum* in this particular case is unknown. However, it is interesting to note that in certain instances, mycorrhizal fungi have

been hypothesised to contribute to yield decline in monocultured crops (Bennett, *et al*., 2012). Negative mycorrhizal associations (NMAs) have been observed in connection with yield decline in tobacco, and corn, amongst other crops (Bennett, *et al*., 2012); though no evidence has been found specifically for *C. geophilum*. In studies by Johnson, *et al*. (1997), a proliferation of mycorrhizal fungi was found to be negatively correlated with corn yields. The hypothesis for this result, was that the build-up of specific mycorrhizal fungi functioned in diverting plant resources, consequently lowering yields. In this way, some mycorrhizal associations may incur more costs than benefits, resulting in a kind of parasitism/negative association rather than the usual beneficial behaviour (Johnson, *et al*., 1997).

Leptosphaeria maculans

Leptosphaeria maculans Ces. and De Not (Leptosphaeriaceae, Dothideomycetes) is a cosmopolitan pathogen of *Brassica* spp., particularly oilseed rape, but is documented to affect numerous other herbaceous and woody plants (Farr and Rossman, 2018). It is known to cause black leg, canker, dry rot, and leaf spot in its hosts. *L. maculans* also contributed to the difference in rhizosphere fungi between healthy and CYD field soil samples. It was not detected in the healthy soils, but was found to comprise 7% and 9% of the relative abundance of ascomycetes for the moderate and severe CYD rhizosphere samples, respectively. This indicates another potentially deleterious association which would benefit from further investigation.

Valsa mali

Valsa mali Miyabe and G. Yamada (Valsaceae, Sordariomycetes) is an extensively studied necrotrophic fungal pathogen of apple. It is widely known throughout Eastern Asia, but its global distribution is unclear. Records of the fungus are scarce in the UK, besides a description of the species on apple by Grove (1935). The abundance of this fungus in the microbial sequence data was unexpected (particularly the desiccation experiment samples) and may indicate sequence misidentification or overrepresentation in the reference database used. In any case, *V. mali* contributed to the dominance of Sordariomycetes in the healthy field soil samples accounting for approximately one quarter of the ascomycetes classified for these samples (rhizosphere 27% and bulk 23%). The relative abundance of *V. mali* was lower in the rhizosphere of the CYD samples (severe CYD 13% and moderate 16%), and very

low in CYD bulk soils (severe CYD 0% and moderate CYD 3%). The role of this fungus cannot be hypothesised, but it is interesting that its abundance decreased in the CYD samples compared to the healthy field soil samples. Although a very small difference, it is also slightly less abundant in the severe CYD, compared to the moderate CYD. This observation indicates a kind of beneficial association between coriander and *V. mali*, particularly given the unusually high abundance of the fungus in the desiccation experiment C1 control and C2 desiccated rhizosphere soil samples. However, this result is interpreted with caution, given the incongruity of this fungus in coriander soils.

The microbial communities of the desiccation experiment samples

The desiccation experiment soils presented distinct microbial communities to the field soils. This is not surprising, given the very different physical and biological properties of the compost, compared to field soil; and the nature of growing plants in pots in a controlled environment. This also reflects findings of the previous CYD study: that incidences of yield decline may not have a singular microbial cause (Fraser, 2017). Like the field soil samples, the desiccation experiment soils did not present obvious changes in bacterial community structure. These samples showed a pronounced difference in fungal taxa between the healthy and yield decline samples. At phylum level, the most notable difference between samples was the relative abundance of basidiomycetes in the C2 desiccated bulk soil at 48%, compared to 29% in C1 control bulk soil, and 15% in C2 watered bulk soil. This indicates that the process of drying may have changed the fungal composition of the soil. It is possible that the drying process initially eliminated a large proportion of the fungi present (as shown by Kaiser, *et al*., 2015), before soils were then re-colonised by a greater abundance of novel fungi, not originally present in the compost. The rhizospheres communities at phylum level, were very similar for the three soil types.

In examining the relative abundance of classes of Ascomycota, the rhizospheres of the C1 control and C2 desiccated sample are very similar; characterised by the dominant abundance of Sordariomycetes at 91% and 87%, respectively. This may reflect the similarity in growth and yield between these two crops, as the C2 desiccated plants showed no CYD. The C2 watered samples had very little distinction between the rhizosphere and bulk soils, and fewer Sordariomycetes. The

C2 desiccated bulk soils showed the most even distribution of fungal classes, perhaps reflecting the effect of drying on the soils. Along with the caveat of study limitations mentioned previously, specific taxa which account for some of the overall fungal community patterns observed in the desiccation experiment soils are described below.

Valsa mali

As mentioned previously, *Valsa mali* may be overrepresented in reference databases. In this study, the taxon appears to be associated with a healthy coriander rhizosphere. *V. mali* represented 89% and 84% of the ascomycetes in the C1 control rhizosphere and C2 desiccated rhizosphere, respectively. It was less prevalent in the rhizosphere of the poorly performing C2 watered treatment sample (16%). It would be interesting to ascertain whether this fungus is present in other coriander cropping systems, as it did not feature in the previous CYD study (Fraser, 2017).

Trichoderma auroviride

Trichoderma auroviride Rifai (Rifai, 1969—name not verified in Index Fungorum) (Hypocreaceae, Sordariomycetes) was detected in both the field soils and the desiccation experiment soils. *Trichoderma* spp. are well-studied for their potential to control plant pathogens, and also for their growth-promotion effect on plants (Brotman, *et al*., 2013; Marín-Guirao, *et al*., 2016). Some strains of the genus, including *T. auroviride*, are considered economically beneficial for their potential to reduce plant disease in agricultural environments (Harman and Kubicek, 2014). It is intriguing that this fungus comprised 17% of the ascomycete taxa for the C2 desiccated bulk soil, compared to 4% in C1 control bulk soil, and 8% in the C2 watered bulk soil. This pattern was not replicated in the bulk soils of the field samples (they all had 6% relative abundance). However, the abundance of this fungus in the C2 desiccated bulk soils could indicate that *T. auroviride* contributed to the successful growth of coriander without CYD in the desiccation experiment.

Neurospora tetrasperma

Neurospora tetrasperma Shear and B.O. Dodge (Sordariaceae, Sordariomycetes) is a species in the genus commonly called 'red bread mold', which 'has long been known as a bakery pest and has caused much loss to bakers as well as to housewives' (Shear and Dodge, 1927). The biology of *Neurospora* spp. have been extensively studied in the laboratory, and *N. tetrasperma* has become a 'model organism'. But ironically, the natural habitat and distribution of the genus is not well understood (Koritala and Lee, K., 2017). As is the case in Genbank, it is likely that this organism is overrepresented in other DNA reference databases. Nevertheless, *N. tetrasperma* accounted for approximately one quarter of the ascomycetes in the C2 watered bulk and rhizosphere soils, compared to <2% in the other desiccation experiment samples. Amongst other things, this may indicate that the fungus had a deleterious effect on C2 watered treatment coriander growth. Likewise, the environmental conditions created in the C2 watered pots were obviously very different to those of the other treatments, and could have favoured the growth of *N. tetrasperma.*

3.5 Conclusions

The pot trials outlined in Chapter 3 showed that management practices which directly alter the microbiota of soils can reduce/eliminate CYD, at least in a glasshouse environment. Both soil drying and soil sterilisation treatments improved coriander growth in a second crop cycle, compared to crops grown in untreated soils. Although the nature of yield decline in the field soils was obviously very different to that of the desiccation experiment, it appears that in both cases, a deleterious microbial component was eliminated from the soils in glasshouse experiments. It is also possible that both of these techniques improved the soil physical properties, including affecting nutrient availability.

The metagenomic study presented in this chapter provided a snapshot of the soil microbial communities associated with two diverse coriander cropping systems. These two sets of samples showed different fungal community patterns; likely the product of different plant genotypes, very different growing conditions, and a host of other factors influencing the biological properties of soils (*e.g*. nutrients and fertiliser regime, planting density, cropping history). The field soil samples showed a distinct

difference in fungal communities between the healthy and CYD rhizospheres. This provides further evidence for the hypothesis of the previous study (Fraser, 2017): that CYD may have a fungal causal element, which presents as changes in rhizosphere fungal communities from healthy to yield decline coriander (at least in some cases in the field). The desiccation experiment samples showed very different fungal communities. Interestingly, the rhizosphere fungi of the high-yielding coriander treatments (C1 control and C2 desiccated) were very similar. The bulk soil of the C2 desiccated treatment indicated that drying the soil before sowing the second crop, had a profound effect on the fungi present. This was reflected in the successful growth of the crop, and apparent elimination of CYD. While these studies are not conclusive, they provide useful observations to help direct future study and inform the field trials required to assess potential management options, which will be discussed in Chapter 4.

Chapter 4: General discussion, overall conclusions, and management options

4.1 Introduction and chapter overview

Modern agriculture has greatly increased capabilities of food production. It is important to maintain these benefits, but also address the corresponding drawbacks (Watt, *et al*., 2006). There are growing concerns that agricultural intensification is placing enormous pressure on the health and functions of soils. Consequently, the long-term productivity of these soils is in jeopardy, with crop yield decline being one of the negative outcomes of intensification (Tilman *et al*., 2001; Tilman, *et al.,* 2002; Trivedi, *et al*., 2016). Numerous instances of yield decline have been identified within current cropping systems, where the functions of traditional crop rotations have largely been replaced by synthetic fertilisers and pesticides (Bullock, *et al*., 1992; Tilman, *et al*.; 2002, Ball, *et al*., 2005; Bennett, *et al.,* 2012). It is becoming increasingly important to address the problem of yield decline, by understanding the causes, and by adopting management practices to limit its effects. This presents a considerable challenge in the case of CYD. UK coriander is grown in diverse cropping systems, and thus far, the precise causes of CYD have not been elucidated. This reflects the complexity of plant-soil-microbe interactions, and that numerous factors are likely interacting in CYD.

Chapter 4 summarises current understanding of the phenomenon of CYD in the UK, and reviews the overall results of experimental work conducted in the present study. It also provides insight into potential management options based on evidence gained from glasshouse pot trials and microbial community observations. Avenues of future study will be mentioned, including field trials essential to fully evaluate potential management strategies.

4.2 The coriander yield decline phenomenon

In this study, coriander yield decline has been observed in a grower's field, and also in the glasshouse, as a real phenomenon. Plants showing 'decline' did not exhibit obvious symptoms of disease. CYD was observed in the form of lower yields due to lack of emergence, smaller plants, or both. Nonetheless, this problem is still not well-defined. As discussed in Chapter 2, the very diverse practices used to grow

coriander commercially make it difficult to pin-point the exact conditions under which the problem occurs. That this problem is said to persist in soils for up to eight years is not widely confirmed (Tom Davies pers. comm. cited in Fraser, 2017). Growers with access to large areas of land tend to avoid CYD by using four to five-year crop rotations (Robert Gibbs., pers. comm., 2018). However, this is not possible for many growers. Given the combination of land constraints and the high value of coriander, growers tend to use shorter, and less diverse rotations than the traditional crop rotations referred to in Chapter 2. However, it is not known exactly how long these rotations tend to be, and whether they are consistent between different growers. It is possible that some growers inadvertently crop another Apiaceae species within their rotation 'break' period (*e.g*. parsley, carrot, parsnip), which may perpetuate decline in the subsequent coriander crop. Furthermore, it is unclear whether part of the CYD effect stems from the fact that growers tend to sow two consecutive crops, before a rotation break. This may be an important consideration, since it is not just the number of years between crops, but also the frequency of crops which is associated with the build-up of pathogens (and potential DRMOs) in crop soils (Bennett, *et al*., 2012). Clearly, it is important to provide optimum growing conditions for coriander through practices that limit the occurrence of CYD. However, given that the use of shortened rotations in coriander cropping is unlikely to change due to the lack of available land, the most realistic and effective management options for CYD may be those that directly alter the biology of soils; potentially eliminating deleterious microbial communities implicated in CYD.

4.3 Summary of experimental findings

The glasshouse pot trials conducted during this study showed that coriander yield and the occurrence of CYD are likely influenced by a combination of interacting factors. Harrowing/relieving compaction in the superficial soil layers was shown to produce better yields in second cycle crops, compared to leaving soils un-harrowed. Planting density was also shown to be a potentially important consideration in addressing CYD limitation in the glasshouse, as this factor affected the levels of decline in yields of a second crop. Fertiliser levels had a significant effect in pot trials, and likely the previous CYD study (Fraser, 2017). Lack of sufficient fertiliser produced stunted plants and smaller yields (not unlike CYD symptoms in the field).

Attempts at providing optimum growth conditions to prevent CYD were not successful. It is possible that these conditions fell short of 'optimum' in this case. While the overall results of pot trials showed that the above factors influenced the severity of CYD, it appeared that a deleterious soil microbial element was still significantly contributing to CYD. To illustrate this, even when coriander growth conditions were optimised based on previous pot trial data, average yield per pot for a second coriander crop was still only 62% of that obtained in the control pots.

The pot trials which employed techniques to directly alter the microbial properties of soils were much more effective in reducing CYD in the glasshouse. Both drying out crop soils and soil sterilisation appeared to eliminate CYD. These experiments also provided further evidence for the existence of a deleterious microbial element in the soils.

Microbial community studies were not replicated (due to financial resource restrictions), so must be considered superficial observations. However, these still provided insight into differences in the fungal communities detected in the rhizosphere of healthy coriander compared to yield decline coriander collected from a grower's field soils. The desiccation experiment soils also showed pronounced changes in fungal communities. This indicated that drying out the soils before re-sowing coriander had a profound effect, and perhaps 'reset' the rhizosphere fungal community to a population similar to that of the healthy control soils.

4.4 Potential management options for CYD

Results of this study showed that the implementation of some cultural practices may provide improved conditions for coriander growth, and potentially reduce the occurrence of CYD. Likewise, certain cultural practices currently being used may cause a build-up of deleterious microbial communities in soils, which affect a subsequent coriander crop. In any case, it must be noted that CYD is likely a complex problem, involving many interrelated factors. It has been said that advancements in productivity in cropping systems rarely result from the interactions of a single factor; rather they arise from synergistic interactions amongst many things working together (Watt, *et al.*, 2006). Accordingly, implementation of potential soil management options for CYD first require assessment on a larger scale and also in

field trials. Furthermore, future treatments for CYD must be considered in the context of long-term productivity and sustainable cropping systems.

4.4.1 Determining the parameters of an effective crop rotation to limit CYD

Greater understanding of the cultural practices used by UK coriander growers is essential to further defining CYD, and best implementing treatments. In addressing management options, it must be noted that the most successful method for increasing or maintaining crop yields is to extend the length of the crop rotation used (Bennett, *et al.*, 2012; Bullock, 1992; Karlen *et al.*, 1994;). Whilst crop rotations are inextricable from addressing the issue of CYD, the parameters of an effective rotation to avoid CYD have not been determined. Long rotations are impractical for most coriander growers. Additionally, the effectiveness of rotation breaks is not just determined by length, but also by the crop species grown (Bennett, *et al.*, 2012). Some of the crops used by coriander growers during rotation breaks may not be effective at disrupting the yield decline seen in subsequent coriander crops. It could be that more diverse crop species are needed between coriander crops. Finally, it may be that cropping frequency is another contributing factor to CYD—perhaps 'double-cropping' is not an advisable practice for all coriander cropping systems. A longer-term study into effective rotations and cropping frequency of coriander would therefore be highly beneficial.

4.4.2 Providing optimum growing conditions to limit CYD

All cultural practices directly or indirectly affect the biological properties of soil, which in turn, significantly contributes to the overall health and productivity of soil (Abawi and Widmer, 2000). Soil borne disease is the most severe when soil conditions are poor: *e.g.* inadequate drainage, poor structure, low organic matter, low fertility, and soil compaction (Abawi and Widmer, 2000). It is likely that poor soil conditions exacerbate the occurrence and severity of CYD in the field. Therefore, attempts to provide optimum soil conditions for coriander should be part of mitigating CYD. Pot experiments in this study showed that some management techniques may help to reduce decline (or likewise exacerbate CYD, if not considered).

Whether compaction is an issue contributing to CYD in the field is unknown, *e.g.*

growers may drill into the same crop for a second time, or use minimal tillage. Nonetheless, pot trial evidence showed that compacted soils resulted in significantly greater levels of CYD than harrowed soils. It therefore appears that alleviating compaction could be part of a combination of factors to limit CYD. However, facilitating this in a field scenario may present difficulties within the scope of most UK coriander cropping systems. Reflecting UK land shortages, many growers rent fields on a seasonal basis (Robert Gibbs, pers. comm., 2018). Therefore, levels of compaction may be dependent of previous cropping (by other growers), in addition to weather conditions. Changing the tendency to sow two consecutive crops in a season (potentially causing compaction by sowing into the same drills) would be difficult to implement against the lure of the potential financial gains implied in harvesting two coriander crops, instead of one.

Discussions with several coriander growers revealed the use of a wide variety of planting densities. Pot trials in this study suggested that perhaps greater efforts should be made to assess optimum densities for a particular field cropping environment. Doing so may help to limit conditions which favour CYD. Determining an optimum planting density would depend on many factors: *e.g.* the soil type and growing environment, coriander variety, and rate of emergence of plants; but may be an important consideration in providing conditions to limit CYD. Owing to the complexity involved in determining an optimum planting density, this task could present a challenging undertaking for growers (or scientists), particularly as the growing environment would likely change considerably with rotations. As coriander seed is relatively cheap, there is a tendency towards high density planting (Robert Gibbs, pers. comm., 2018). However, as exemplified by a grower who does not experience CYD (Robert Gibbs, pers. comm., 2018), a lowered general planting density may help to curb the occurrence or severity of CYD

Finally, lack of plant nutrition may contribute to the severity of CYD, as shown in the glasshouse. This is not to say that ample fertiliser is not applied to crops, but that limited nutrient uptake may be occurring in CYD field soils. Whilst maintaining adequate crop nutrients may seem obvious, this factor should be included within the combined factors to manage CYD. More adequate soil testing could address a potential lack of nutrition (which may exacerbate CYD) by determining the status of

plant available nutrients and also informing better fertiliser application. The obvious limitations here would come from the short-term costs of regular implementation of such testing, as well as more targeted, and potentially more expensive fertiliser application.

4.4.3 Drying out crop soils as a means to reduce the occurrence of CYD

Desiccation of crop soils was found to effectively limit or eliminate CYD in the glasshouse. But how effective or feasible is drying out crop soils in the field? Allowing soils to dry out before ploughing is a common practice to limit soil structure damage. An effort to dry out coriander crop soils is also implemented by a grower who does not get CYD—in this case, waiting two to three weeks to carry out post-harvest ploughing, weather permitting (Robert Gibbs, pers. comm. 2017). In a field situation, the reality of drying out soils as a post-harvest treatment for CYD would be highly weather dependent, and potentially challenging. As part of a combination of factors for optimum growing conditions, it may be beneficial to dry out soils for as long as possible to limit CYD in a subsequent coriander crop. However, the level of dryness required for the treatment of CYD is unknown. Effective drying was achieved in this study under 'ambient' conditions in a glasshouse. Two weeks of drying appeared to be insufficient in the case of the 'optimum conditions' experiment; but four weeks of drying produced an effective treatment in the desiccation experiment (which used smaller pots). Temperature and soil type would largely dictate the rate of drying and the level of dryness achieved in the field. Nonetheless, efforts to dry soils after a coriander crop could certainly contribute to overall improved conditions for growth and limitation of CYD in combination with the management practices described above (limiting compaction, optimum planting density, and adequate plant nutrition). To confidently advise growers on soil-drying, field trials would be required.

4.4.4 Soil sterilisation as a management option for CYD

Soil sterilisation eliminated CYD in the affected field soil used in glasshouse pot trials in this study. This reflects the efficacy of soil sterilisation, which has long been used to treat incidences of yield decline (Russell and Peteherbridge, 1912; Russell and Hutchinson, 1913). The practice is also used by a grower to limit CYD in a covered coriander cropping system (Simon Harty, pers. comm., 2018). Besides limiting yield decline, sterilisation has also been shown to improve the ability of plants to adapt to

continuous cropping (Zhang *et al*., 2007), as well as releasing nutrients previously unavailable to the crop (Raffle, 2005).

Soil sterilisation may be the most effective treatment for CYD (within the current understanding of the problem), but the drawbacks of this practice must be considered. Soil microorganisms are integral to virtually all soil processes. As such, microbial community composition and diversity largely determine the sustainable productivity of agricultural soils (Barrios, *et al*., 2007; van der Heijden, *et al*., 2008). Therefore, suppressing or eradicating problematic soil microorganisms should be just one aspect of many practices to promote soil health (Katan, *et al*., 2017). Because of the inherent toxicity and increasingly restricted use of chemical sterilisation methods, steam sterilisation has been promoted as a more environmentally friendly means of treating infected soils. Consequently, non-chemical sterilisation technology is continually advancing. But the process is still considered expensive, slow, and labour-intensive; particularly for field-scale operations (Simon Harty, pers. comm., 2018). Furthermore, it is often difficult to treat soils to sufficient depth to achieve the desired results. As a management option for CYD, soil sterilisation needs further investigation, and also cost-benefit analysis.

4.5 Future work

Future work on CYD in the UK should further assess the management strategies examined in this study, with more extensive replication and within the complexity of a field environment. This must be done before advising growers on changes to current coriander cropping practice.

Unlike major crop species, coriander has been the subject of limited research. Further study is needed to facilitate yield optimisation in the crop, and also a better understanding of the factors which contribute to CYD. In directing future work, greater investment should be made in commercial breeding programs and the development of more robust coriander strains for UK conditions. Additional research is also needed to better define the parameters of an effective coriander crop rotation.

Together with the previous CYD study (Fraser, 2017), the present study helped provide a baseline for future microbial studies of CYD. Studies across more coriander cropping systems could further elucidate the involvement of specific microorganisms. Continued research is needed to develop microbial community profiling for CYD, which could better explain this type of yield decline and also drive more targeted management approaches. This could potentially create CYD detection strategies to inform planting decisions and treatments. As costs of NGS techniques decrease (and knowledge of coriander crops and associated soil types increases), this may become a more routine practice for detecting and dealing with yield decline.

As stated above, understanding the potential role of microorganisms in CYD may be key to developing effective means of managing the problem. Further studies are needed, not only for the elucidation of specific taxa, but to understand functional modes of rhizosphere fungi implicated in CYD. Of particular interest are Dothideomycetes, which appeared to increase in abundance in the yield decline coriander in this study, compared to the healthy crop sampled. This class is particularly known for pathogenicity, but also has a great diversity of life strategies. A better understanding the ecology and potential negative associations of these fungi with coriander could be a promising avenue of further study.

Biofumigation is a potential avenue of treatment to eliminate DRMOs. This was recommended as a management option in CP117 (Fraser, 2017), and could be a worthwhile study avenue. Just as microorganisms have been implicated in CYD and many other incidences of yield decline, they could also be used as a means of treatment. The use of biocontrol agents to eliminate fungal DRMOs may provide another valuable study area. Of particular relevance to this study, is the possibility of using *Trichoderma* spp., or other microorganisms which are known to be antagonistic to fungal plant pathogens.

4.6 Conclusions

Given the limited scope of this study, CYD was not resolved. However, this work succeeded in expanding current understanding of the problem; adding evidence to

support its existence as a real phenomenon. Glasshouse studies confirmed that certain crop and soil management practices impact the occurrence and severity of CYD. These require further investigation to better inform growers of their efficacy and practicality in the field. Additional support for at least a partial microbiological cause in CYD was established, which appeared to be fungal in the case of the field soils examined. This provides a baseline for further studies, which will help to design more targeted management options. Overall, the present study revealed new insight about CYD and directions for beneficial future study. Finally, this study also provided valuable insight into the yield decline of a specific cropping system, which is increasingly important research for modern agriculture, and the future of UK horticulture.

Apendix: ONT Rapid Barcoding Sequencing Kit Protocol

Rapid Barcoding Sequencing (SQK-RBK004)
Version: RBK_9054_v2_revB_23Jan2018 Last update: 10/05/2018

Before starting checklist

Materials
Rapid Barcoding Sequencing Kit (SQK- RBK004)
Flow Cell Priming Kit (EXP-FLP001)
Consumables
1.5 ml Eppendorf DNA LoBind tubes
0.2 ml thin-walled PCR tubes
Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
Agencourt AMPure XP beads (optional)
Freshly-prepared 70% ethanol in nuclease- free water (optional)
10 mM Tris-HCl pH 8.0 with 50 mM NaCl (optional)
Equipment
Ice bucket with ice
Microfuge Timer
Thermal cycler at 30° C and 80° C
Pipettes and pipette tips P2, P20, P100, P200, P1000

Preparing input DNA

Prepare the DNA in Nuclease-free water.
Transfer ~400 ng genomic DNA into a DNA LoBind tube Adjust the volume to 7.5 µl with Nuclease-free water Mix by flicking the tube to avoid unwanted shearing
Spin down briefly in a microfuge
Record the quality, quantity and size of the DNA.

IMPORTANT

Criteria for input DNA

Purity as measured using Nanodrop - OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2
Average fragment size, as measured by pulse-field, or low percentage agarose gel analysis >30 kb
Input mass, as measured by Qubit - ~400 ng
No detergents or surfactants in the buffer

Check your flow cell

Set up the MinION, flow cell and host computer
Once successfully plugged in, you will see a light and hear the fan.
Open the MinKNOW GUI from the desktop icon and establish a local or remote connection. If running a MinION on the same host computer, plug the MinION into the computer.
If running a MinION on a remote computer, first enter the name or IP address of the remote host under Connect to a remote computer (if running from the Connection page), or Connections (if running from the homepage) and click Connect.
Choose the flow cell type from the selector box. Then mark the flow cell as "Selected"

Click "Check flow cells" at the bottom of the screen. R9.4.1 FLO-MIN106
R9.5.1 FLO-MIN107
Click "Start test".
Check the number of active pores available for the experiment, reported in the System History panel when the check is complete.
Flow cell check complete.

Library preparation

Thaw kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:
Fragmentation Mix RB01-12: not frozen, briefly spin down, mix well by pipetting
Rapid Adapter (RAP): not frozen, briefly spin down, mix well by pipetting
Sequencing Buffer (SQB): thaw at RT, briefly spin down, mix well by pipetting*
Loading Beads (LB): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use Flush Buffer (FLB) - 1 tube: thaw at RT, briefly spin down, mix well by pipetting*
Flush Tether (FLT): thaw at RT, briefly spin down, mix well by pipetting
In a 0.2 ml thin-walled PCR tube, mix the following:
7.5 µl 400 ng template DNA
2.5 µl Fragmentation Mix RB01-12 (one for each sample)
Mix gently by flicking the tube, and spin down.
Incubate the tube at 30° C for 1 minute and then at 80° C for 1 minute. Briefly put the tube on ice to cool it down.
Pool all barcoded samples in your desired ratio, noting the total volume.
IMPORTANT
If barcoding four or more samples, increased throughput can be achieved through cleaning up and concentrating the pooled material using AMPure XP beads as outlined in Steps 6-15. Otherwise, for a more rapid sample preparation, transfer 10 µl of pooled sample from Step 5 into a clean 1.5 ml Eppendorf DNA LoBind tube, and proceed directly to Step 16.
Prepare the AMPure XP beads for use; resuspend by vortexing.
To the entire pooled barcoded sample from Step 5, add an equal volume of resuspended AMPure XP beads, and mix by flicking the tube.
Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.
Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.

Keep on magnet, wash beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the 70% ethanol using a pipette and discard.
Repeat the previous step.
Spin down and place the tube back on the magnet. Pipette off any residual 70% ethanol. Briefly allow to dry.
Remove the tube from the magnetic rack and resuspend pellet in 10 µl of 10 mM Tris-HCl pH 7.5-8.0 with 50 mM NaCl. Incubate for 2 minutes at RT.
Pellet the beads on a magnet until the eluate is clear and colourless.
Remove and retain 10 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
Remove and retain the eluate which contains the DNA in a clean 1.5 ml Eppendorf DNA LoBind tube
Dispose of the pelleted beads
End of optional steps.

Add 1 µl of RAP to 10 µl barcoded DNA.
Mix gently by flicking the tube, and spin down. Incubate the reaction for 5 minutes at RT.
The prepared library is used for loading into the MinION flow cell. Store the library on ice until ready to load.

Priming and loading the SpotON Flow Cell

IMPORTANT
Please note that the Sequencing Tether (SQT) tube will NOT be used in this protocol. It is provided in the kit for potential future product compatibility.
Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FLB) at RT before placing the tubes on ice as soon as thawing is complete.
Mix the Sequencing Buffer (SQB) and Flush Buffer (FLB) tubes by vortexing, spin down and return to ice. Spin down the Flush Tether (FLT) tube, mix by pipetting, and return to ice.
Open the lid of the nanopore sequencing device and slide the flow cell's priming port cover clockwise so that the priming port is visible.

IMPORTANT
Care must be taken when drawing back buffer from the flow cell. The array of pores must be covered

by buffer at all times. Removing more than 20-30 µl risks damaging the pores in the array.
After opening the priming port, check for small bubble under the cover. Draw back a small volume to remove any bubble (a few µls):
Set a P1000 pipette to 200 µl Insert the tip into the priming port
Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip

Prepare the flow cell priming mix: add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FLB), and mix by pipetting up and down.
Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes.
Thoroughly mix the contents of the SQB and LB tubes by pipetting.
In a new tube, prepare the library for loading as follows:
34 µl Sequencing Buffer (SQB)
25.5 µl Loading Beads (LB), mixed immediately before use 4.5 µl Nuclease-free water
11 µl DNA library
IMPORTANT
The Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.
Complete the flow cell priming:
Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.
Mix the prepared library gently by pipetting up and down just prior to loading.
Add 75 µl of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.
Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION lid.

Starting a sequencing run

Double–click the MinKNOW icon located on the desktop to open the MinKNOW GUI. If your MinION was disconnected from the computer, plug it back in.
Choose the flow cell type from the selector box. Then mark the flow cell as "Selected". Click the "New Experiment" button at the bottom left of the GUI.
On the New experiment popup screen, select the running parameters for your experiment from the individual tabs.
Output settings - FASTQ: The number of basecalls that MinKNOW will write in a single file. By default this is set to 4000
Output settings - FAST5: The number of files that MinKNOW will write to a single folder. By default this is set to 4000
Click "Begin Experiment"
Allow the script to run to completion.
The MinKNOW Experiment page will indicate the progression of the script; this can be accessed through the "Experiment" tab that will appear at the top right of the screen
Monitor messages in the Message panel in the MinKNOW GUI
The basecalled read files are stored in :\data\reads
Progression of MinKNOW protocol script
The running experiment screen
Experiment summary information
Check the number of active pores reported in the MUX scan are similar (within 10-15%) to those reported at the end of the Flow Cell Check
If there is a significant reduction in the numbers, restart MinKNOW.
If the numbers are still significantly different, close down the host computer and reboot.
When the numbers are similar to those reported at the end of the Flow Cell Check, restart the experiment on the Connection page. There is no need to load any additional library after restart.
Stopping the experiment is achieved by clicking "Stop experiment" button at the top of the screen.
Check the temperature is approximately 34° C.
Check pore occupancy in the channel panel at the top of the experimental view.
A good library will be indicated by a higher proportion of light green channels in Sequencing than are

in Pore. The combination of Sequencing and Pore indicates the number of active pores at any point in time. A low proportion of Sequencing channels will reduce the throughput of the run.
Recovering indicates channels that may become available for sequencing again. A high proportion of this may indicate additional clean up steps are required during your library preparation.
Inactive indicates channels that are no longer available for sequencing. A high proportion of these as soon as the run begins may indicate an osmotic imbalance.
Unclassified are channels that have not yet been assigned one of the above classifications
Monitor the pore occupancy Duty time plots
Monitor the development of the read length histogram. Trace viewer
Onward analysis of MinKNOW basecalled data
Open the Desktop Agent using the desktop shortcut.
Click on the New Workflow tab in the Desktop Agent and select the FASTQ barcoding workflow.

Select the workflow parameters.

Select the quality score cut-off (this defaults to 7 unless changed)
Select "Yes" in answer to "Detect barcode?"
If you are working with human data, please tick "Yes" in answer to "Is the data you are about to upload a whole or partial human genome?", and confirm that you have consent from the subject to upload the data.
Check the correct settings are selected in the Desktop Agent.
Click "Start Run" to start data analysis.
Follow the progression of upload and download of read files in the Desktop Agent.
Click on VIEW REPORT.
Click on VIEW REPORT to navigate to the Metrichor website, this can be done at any point during data exchange
Return to the Desktop Agent to see progression of the exchange

Close down MinKNOW and the Desktop Agent
Quit Desktop Agent using the close x.
Quit MinKNOW by closing down the web GUI. Disconnect the MinION.

Prepare the flow cell for re-use or return to Oxford Nanopore.

If you would like to reuse the flow cell, follow the Wash Kit instructions and store the washed flow cell at 2- 8 °C, OR
Follow the returns procedure by washing out the MinION Flow Cell ready to send back to Oxford Nanopore.

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