Potential for biofumigation against soilborne diseases of potato caused by *Rhizoctonia solani,* and for effects on soil microbial communities

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy (Plant Pathology)

at

Lincoln University

by

Le Phuoc Thanh

Lincoln University

2018

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The soilborne diseases of potato (*Solanum tuberosum*) caused by *Rhizoctonia solani* (stem canker and tuber black scurf) are important factors limiting yields and quality of intensively managed potato crops in New Zealand. International studies have shown that biofumigation using *Brassica* plants is a promising component of integrated disease management with potential to reduce agrichemical usage for disease control. To date, there is limited evidence on the efficacy of biofumigation for controlling soil-borne diseases of potato in New Zealand. The goal of this research was to investigate the biofumigation potential of selected plants for suppression of *R. solani* AG3-PT and AG2-1, which cause potato diseases.

In vitro studies showed that of the 10 plant types tested, three, including 'Caliente' mustard, brown mustard and 'Nemat' arugula, gave the greatest inhibition of the mycelium growth of *R. solani* AG3-PT and AG2-1 isolates, at 5-10 g of macerated plant tissues per Petri dish, or 5-10% (w:w) when incorporated into soil. Shoot or shoot plus root tissues of selected biofumigants, harvested at the mid-flowering growth stage, were the most effective for reducing mycelium growth of *R. solani* isolates. Increased conversion of *R. solani* mycelium into sclerotia was observed at 1% or 5% (w:w) soil incorporation for 'Caliente' mustard and 1% (w:w) for brown mustard or 'Nemat' arugula. The subsequent germination of retrieved *R. solani* sclerotia was reduced at 10% (w:w) soil incorporation of 'Nemat' arugula. The subsequent mycelium growth from retrieved *R. solani* colonised barley grains was completely inhibited at 10% (w:w) soil incorporation of 'Caliente' mustard, brown mustard or 'Nemat' arugula.

Biofumigation potential against *R. solani* AG3-PT was assessed in different soil edaphic conditions, using qPCR and measurements of soil microbial activity (dehydrogenase activity, DHA). Biofumigant treatments at soil pH of 6.6, 20°C and 40% water holding capacity (WHC), or at 15°C and 40% WHC, gave the greatest reductions of *R. solani* AG3-PT inoculum levels in soil. In addition, biofumigant treatments at pH 6.6, 15°C, and 40 or 70% WHC, or in the combination of 15°C and 40% WHC, resulted in the greatest DHA levels. The soils amended with macerated 'Caliente' mustard tissue (5% w:w) and after incubation at 15°C, reduced the stem canker severity on potato 'Jersey Benne' plants, and increased plant height and dry biomass compared with the pathogen inoculated controls.

The effects of the selected biofumigant treatments for reducing *R. solani* AG3-PT inoculum, and suppressing infection of 'Russet Burbank' potato plants, were assessed in a shadehouse experiment, using qPCR and DHA assessments. The results showed that the biofumigant treatments reduced and maintained *R. solani* AG3-PT inoculum (and potentially other AGs) at low levels during the experiment, in soil, and stems, stolons and tubers of potato plants. However, there was no differential effect of biofumigant treatments on soil microbial activity (DHA). The biofumigant treatments also reduced severity of tuber powdery scab caused by *Spongospora subterranea*, and incidence of dead stems caused by *Colletotrichum coccodes*. Potato plant yield parameters were increased by the biofumigant treatments.

Effects of cover crops, including 'Caliente' mustard, oat or 'Graza' radish, on soil microbial communities were assessed by DHA measurements, soil carbon utilisation profiles (CUP), and PCR-DGGE in soil samples taken from three potato field trials near Timaru (2015/16, 2016/17) and Ashburton (2015/16), Canterbury. DHA increased after cover crop treatments. The events of crop incorporation and plant growth over the sampling period and the oat treatment affected the CUPs of the soil microbial communities. Results from PCR-DGGE showed that different cover/potato crops or growth stages strongly affected the structure (species composition), richness, and diversity indices for communities of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. In addition, cover crop treatments affected the soil microbial communities. 'Caliente' mustard altered the community structures of total fungi in the three field trials, and reduced the richness and diversity indices for total fungi communities in the two Timaru field trials. 'Caliente' mustard also affected the Proteobacteria community structures in soil samples from the two Timaru field trials, but increased richness and reduced the diversity of Betaproteobacteria in soil from the Timaru trial (2015/16). This biofumigant treatment also reduced the diversity of Alphaproteobacteria in soil samples from the Ashburton field trial (2015/16). 'Graza' radish treatment only affected AMF community structure in samples from one field trial (Timaru, 2016/17), but had no effects on the community structures, richness or diversity of AMF or the other microbial groups in samples from the other fields. Oat treatment reduced the richness of the total fungi and Gammaproteobacteria communities in soil from the Timaru field trial (2016/17).

This study has provided supportive information that biofumigant plants and crops could be incorporated as a management strategy to control economically important diseases of potato.

Keywords: *Rhizoctonia solani*, anastomosis group (AG), biofumigation, dehydrogenase, qPCR, MicroResp™, PCR-DGGE, *Spongospora subterannea*, *Colletotrichum coccodes*, richness, diversity, fungi, AMF, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria.

Acknowledgements

I am grateful to my supervisory group, Assoc. Prof. Eirian Jones, Assoc. Prof. Hayley Ridgway and Prof. Richard Falloon, for their expertise advice, support, encourage and inspiration. Special thanks to my main supervisor, Assoc. Prof. Eirian Jones, for her patience, trust and positive attitude to my ability to finish the thesis, and her constructive comments and guidance during my writing. I appreciate to my associate supervisors, Assoc. Prof. Hayley Ridgway and Prof. Richard Falloon for their patience, thorough guidance and critical comments during my lab and field work and writing.

I acknowledge to New Zealand Aid Program in Viet Nam for giving me the scholarship; Lincoln University and Foundation for Arable Research (FAR, New Zealand) for funding my research; the committee of ICPP 2003 Inc. Plant Pathology Travel Grants for funding me to attend the conferences. I would like to extend my special thanks to my scholarship advisors, Sue Bowie, Jayne Borrill and Mandy Buller, for their advice and numerous supports not only my studying but also my life in New Zealand.

I would like to thank all Lincoln University staff supporting during my studying: Candice Hume for well-organised labs and orders; Sandy Hammond for teaching the MicroResp technique and organised labs; Sandy Hammond, Dr Celine Blonde and Dr Jana Monk for teaching me DGGE technique; Roger Creswell for allowing me to access the labs to analyse the soil samples; Brian Kwan (Bioprotection Centre) for allowing me to use the qPCR machine; Leanne Hassal for helping in orderings; Brent Richards for supporting me in setting up the greenhouse/shade house experiments; Emma Gambles, Marianne Duncan and Robyn Wilson for supporting me in the administration stuff.

I would like to thank my close friends Nitesh Chand, Pus Weisis and Merriam Toalak, and I am glad to know all of you. I would like to thank my friends, especially Anish Shah, Jing Liu and Dr Neeraj Purushtham Balraj, in the Plant Microbiology/Pathology research group for lab instructions and chatting. I would like to thank all the demonstrators for showing me the measurements of dehydrogenase activity and soil organic matter (Dr Youngnam Kim), soil texture (Mina Lee) and soil water holding capacity (David Rex).

I would like to thank Jen Linton (FAR) for organising the field trips to collect soil samples. Special thanks to Dr Farhat Shah (Plant & Food Research) for supporting me in *Rhizoctonia solani* isolation; Farhat Shah, Sarah Sinton and Steve Dellow (Plant & Food Research) for supporting me in field soil samplings and giving me information of the field trials.

My gratitude and deepest appreciation to my lovely wife, Tran Thi Kim Hanh, for her sacrifice, patience, enthusiasms and support me. Special thanks to my little daughter, Le Tran Quynh Anh (or Kiwi), as a wonderful gift for my wife and I during our journey to New Zealand, and also as my motivation to finish the thesis.

Special thanks to my parents for your love, support and sacrifice in my life; and to my brothers and sisters for your love and support.

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Chapter 1

Introduction

1.1 Potato

Potato (*Solanum tuberosum* L.), an annual tuber-producing plant in the Solanaceae, is predominantly grown in temperate climates (latitudes 45-57°N) as summer food crops, and in subtropical zones (latitudes 23-34°N) as winter/spring/autumn crops (Graves, 2001; Hijmans, 2001; Stevenson *et al.*, 2001; Lutaladio and Castaldi, 2009). In addition, 25% of the worldwide potato cropping area is in the cool highlands (above 1,000 m above sea level) of the tropics (Hijmans, 2001). In 2013, world potato production was approx. 376.5 million tonnes (with 19.3 million hectares of cultivation, giving an average yield of 19.5 t/ha), and ranked fourth for production volume, only exceeded by rice, wheat and maize (FAOSTAT, 2015). Potato is the world's most important non-grain food crop (Stevenson *et al.*, 2001; Lutaladio and Castaldi, 2009).

Potato cultivation is affected by more than 40 harmful pests, including insects, pathogens (fungi, oomycetes, bacteria, nematodes, viruses) and weeds (Stevenson *et al.*, 2001; Oerke, 2006; Fiers *et al.*, 2012). The estimated annual yield losses caused by pests is between 24-59% (averaging 40%), of which 15% (7-24%) was caused by pathogens (Oerke, 2006). The magnitude of losses depends on the level of applied disease control methods (Oerke, 2006). Appropriate management of potato diseases has been estimated to reduce the yield loss by at least 26.4 million tonnes per annum (based on data for world potato production in 2013).

Potato is one of the most economically important crop plants in New Zealand, and is the fourth most important export crop commodity behind wine, kiwifruit and apples (Aitken and Hewett, 2014). The annual contribution of the potato industry to the New Zealand economy is more than NZ $500 million (Olsen, 2015). In 2014 there were 174 growers cultivating 10,329 ha of potato crops, and producing 511,875 tonnes (Aitken and Hewett, 2014), equating to an average yield of 50 t/ha. This is one of the greatest average potato yields worldwide (Olsen, 2015). However, potential yields in the Canterbury region of New Zealand could reach 90 t/ha, based on computer modelling (Sinton *et al.*, 2013). Field surveys and experiments conducted during 2012-2013 by Sinton *et al.* (2013) and Michel *et al.* (2013) illustrated that diseases caused by *Rhizoctonia solani* Kühn (Ogoshi, 1996) and *Spongospora subterranea* f.sp. *subterranea* (Falloon, 2008), and soil compaction are major yield-limiting factors for potato crops in Canterbury. These factors have been estimated to result in a "yield gap" of 30-40 t/ha. In certain circumstances, *R. solani* disease of potato could account for

approx. NZ $75 million annual economic losses in New Zealand (Potatoes New Zealand, unpublished data; cited in Das *et al.*, 2014). Therefore, suitable management strategies are essential to reduce these losses, particularly those caused by *R. solani* and *S. subterranea*, and to maintain economic returns from potato production. Disease caused by *R. solani* on potato in New Zealand was the focus of the present study.

1.2 *Rhizoctonia solani*

1.2.1 The pathogenic fungus

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* [A. B. Frank] Donk) is a soilborne, necrotrophic Basidiomycete pathogen which has a wide host range (Ogoshi, 1996; Agrios, 2005; Tsror, 2010; Ferrucho *et al.*, 2012; Wharton and Wood, 2013; Crop Protection Compendium, 2015).

Rhizoctonia solani hyphae are multinucleate, initially hyaline but becoming brown or grey, due to the accumulation of melanin in the cell walls at they mature. The characteristic hyphal branches are 45 or 90^{o} to the main hypha (Figures 1.1 and 1.2B). Each newly-branched hypha has a constriction at its base and a septum near the branching point (Roberts, 1999; García *et al.*, 2006; Crop Protection Compendium, 2015). The hyphae are classified into three types, including runner (aerial) hyphae, lobate hyphae, and monilioid cells (Crop Protection Compendium, 2015). The runner hyphae (Figures 1.1 and 1.2B) grow over host plant surfaces or on *in vitro* culture media. The runner hyphae form lobate hyphae or appressoria which are responsible for infection of host tissues. The monilioid cells (Figure 1.2C) are involved in formation of sclerotia (Crop Protection Compendium, 2015).

The fungus can produce sclerotia, which are in a quiescent viable state. The sclerotia have no rind and medulla structures, and are formed from compact bodies of loosely aggregated melanised hyphae (Figure 1.2A) (Coley-Smith and Cooke, 1971; Sumner, 1996; Ritchie *et al.*, 2013; Crop Protection Compendium, 2015). The black sclerotia that form on potato tuber surfaces result in black scurf, an important quality-limiting disease (Strand, 2006; Atkinson *et al.*, 2010). The melanin provides protection from unfavourable environmental conditions, agrichemicals, and antagonism from other soil microorganisms (Tavantzis and Bandy, 1988; Tavantzis *et al.*, 1989; Keijer, 1996; Sumner, 1996; Henson *et al.*, 1999; García *et al.*, 2006; Anees *et al.*, 2010; Ritchie *et al.*, 2013).

Figure 1.1 Hypha of *Rhizoctonia solani* (Sneh *et al.*, 1996).

Figure 1.2 Some structures of *Rhizoctonia solani*: (A) Sclerotium cells; (B) Aerial hyphae; (C) Monilioid cells (Crop Protection Compendium, 2015).

1.2.2 Anastomosis groups of *Rhizoctonia solani* on potato

Rhizoctonia solani isolates can be distinguished into groups, and subgroups, which are varieties/special forms (*formae specialis*) or races/strains (Agrios, 2005), based on hyphal anastomosis interactions, pathogenicity, colony morphology, biochemical and/or genetic characteristics (Carling, 1996; Carling *et al.*, 2002; García *et al.*, 2006; Tsror, 2010). Based on light microscope observations of hyphal fusion reactions with known tester isolates, *R. solani* strains have been categorised into anastomosis groups (AGs) (Carling *et al.*, 1988, 2002; Carling, 1996; Roberts, 1999; García *et al.*, 2006; Sharon *et al.*, 2006; Brierley *et al.*, 2013). Polymerase chain reaction (PCR) and analysis of ribosomal deoxyribonucleic acid-internal transcribed spacer (rDNA-ITS) sequences have also been used for identification of *R. solani* AGs (Carling *et al.*, 2002; García *et al.*, 2006; Sharon *et al.*, 2006; Brierley *et al.*, 2013; González, 2013; Arakawa and Inagaki, 2014).

A total of 13 AGs (assigned from AG1 to AG13) are currently recognised for *R. solani* and identified as causing diseases in numerous host plants (Carling *et al.*, 1988, 2002; Carling, 1996; Roberts, 1999; García *et al.*, 2006; Tsror, 2010). The different AGs have different plant hosts and geographic distributions (Brierley *et al.*, 2013). Anastomosis Group 3-PT (a subgroup from AG3) is the dominant group isolated from potato cropping systems worldwide, particularly from diseased tubers (Banville *et al.*, 1996; Tsror, 2010; Ferrucho *et al.*, 2012; Brierley *et al.*, 2013; Ritchie *et al.*, 2013; Wharton and Wood, 2013; Woodhall *et al.*, 2013; Djébali *et al.*, 2014; Özer and Bayraktar, 2015). In addition, other *R. solani* AGs, including AG2-1, AG2-2, AG4, AG5, AG7, AG8 and AG9, and some binucleate *Rhizoctonia* (BNR) isolates, can cause diseases in potato (Tsror, 2010; Woodhall *et al.*, 2011, 2012; Yang and Wu, 2012, 2013; Brierley *et al.*, 2013; Özer and Bayraktar, 2015). In New Zealand, Das *et al.* (2014) reported that AG3-PT was the most commonly isolated *R. solani* AG from black scurf symptomatic potato tubers,

accounting for 85% (110/129) of isolates obtained, followed by AG2-1 (14%) and AG5 (1%). In Peru, AG4 was the main group in warm potato growing regions (Anguiz and Martin, 1989), and AG3-PT was the predominant group in cool environments (Anguiz and Martin, 1989; Virgen-Calleros *et al.*, 2000). This indicated that climate influences the distribution of the *R. solani* AGs infecting potato.

1.2.3 The disease cycle of *Rhizoctonia solani* and symptoms on potato

Rhizoctonia solani infects potato plants from inoculum sources such as infected seed tubers and resting propagules in the soil (Figure 1.3). At the beginning of the crop growing season, the pathogen propagules germinate and infect the developing sprouts, stolons, roots, and stems of potato plants. If the pathogen severely damages the primordia of sprouts and roots, this can result in the death of the tips of the sprouts and roots resulting in the appearance of "sprout tipping" and "root nipping" symptoms. Early severe disease can result in delayed or reduced plant emergence, reduced numbers of stems and stolons, decreased seedling and plant vigour, and lead to weakened plants and reduced crop stands. In addition, secondary or tertiary sprouts may be produced to compensate for damage to the primary sprouts. In some cases, severely infected seed tubers only produce stolons without stems, which are called "no top" symptoms (Banville, 1989; Hide *et al.*, 1989a, 1989b; Banville *et al.*, 1996; Fox, 2006; Strand, 2006; Wharton and Wood, 2013).

Rhizoctonia solani infections can cause brown, necrotic and sunken lesions (cankers) on host stems, stolons and roots (Figures 1.4, 1.5 and 1.6). Severe cankers can girdle stems blocking the movement of water, nutrients and carbohydrates in plants, leading to stem death. Stolon cankers may also reduce tuber formation, or cause formation of small and distorted tubers (Hide *et al.*, 1989a, 1989b; Banville *et al.*, 1996; Jeger *et al.*, 1996; Strand, 2006; Brierley *et al.*, 2013; Wharton and Wood, 2013).

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Figure 1.3 The disease cycle of potato stem canker and tuber black scurf, caused by *Rhizoctonia solani* (Wharton and Wood, 2013).

Potato diseases caused by *R. solani* primarily occur on underground plant parts, but some aboveground symptoms may also result from damage to roots, stolons and stems (Banville *et al.*, 1996; Brierley *et al.*, 2013). These include leaf rolling, purple pigmentation or chlorosis (Figure 1.7) on the leaves, and stunting, rosetting and production of aerial tubers on plant stems (Figure 1.8). When potato canopies overlap or under yet-to-be identified favourable conditions, hymenia (the sexual stage) of *R. solani* can form on aboveground potato stems near the soil surface (Figure 1.9) (Banville *et al.*, 1996; Strand, 2006; Brierley *et al.*, 2013; Wharton and Wood, 2013).

Later in the crop growing season, *R. solani* will infect daughter potato tubers. The fungal hyphae initially grow over the surfaces of progeny tubers, and then form sclerotia, which are brown to black, irregular in shape, and firmly adhered to the tuber skins (Figure 1.10). The sclerotium symptom on tubers is called black scurf (Hide *et al.*, 1989a, 1989b; Banville *et al.*, 1996; Strand, 2006; Tsror, 2010; Brierley *et al.*, 2013; Wharton and Wood, 2013). *Rhizoctonia solani* infections of tubers often stimulate formation of cork cell layers within tubers (Figure 1.16), away from the infection points. This process blocks further invasion of the pathogen into tuber tissues, reduces translocation of its toxic substances into adjacent healthy tuber tissues, and prevents the flow of nutrients and water from the healthy to diseased tissues (Agrios, 2005). This may explain why black scurf symptoms are only seen on the outside tuber skins, and do not cause severe physical damage within the tubers. Other disease symptoms on potato tubers caused by *R. solani* include dry core (Figure 1.11),

deformation (Figures 1.12 and 1.13), greening, and “giraffe neck” or “elephant hide” skin (Figure 1.14) (Brierley *et al.*, 2013; Crop Protection Compendium, 2015).

Damage on the underground parts of potato plants caused by *R. solani* can be seen from 3 to 4 weeks after seed tuber planting, as bare patches in crops where young plants have failed to emerge (Figure 1.15) (Anees *et al.*, 2010; Crop Protection Compendium, 2015). Sometimes, severe stem canker can kill whole plants at more advanced stages of growth, and these are characterised by general collapse resulting from severe rotting of the stem bases (Brierley *et al.*, 2013). Observations and evaluations of tuber disease caused by *R. solani* and other pathogens have generally been conducted at potato harvest (Banville *et al.*, 1996). Disease symptoms on potato incited by *R. solani* can be confused with physiological disorders as well as other soilborne diseases. The pathogen generally only infects the sprouts, stolons, roots and tuber surfaces, and does not decay seed tubers. The lesions caused by *R. solani* are always dry and usually sunken (Wharton and Wood, 2013).

Diseases caused by *R. solani* result in quantitative and qualitative losses in potato production (Banville, 1989; Banville *et al.*, 1996; Tsror (Lahkim) and Peretz-Alon, 2005; Atkinson *et al.*, 2010, 2011; Brierley *et al.*, 2013). Potato yield losses of 10-15% are commonly caused by the pathogen, but can be up to 30% in severe cases (Banville *et al.*, 1996; Crop Protection Compendium, 2015). Black scurf on potato tubers generally does not greatly affect final crop yields (Strand, 2006; Atkinson *et al.*, 2010). However, these symptoms on tubers significantly reduce their marketable value, and also downgrade seed tubers (Tsror, 2010; Atkinson *et al.*, 2010, 2011). The economic losses due to reduced tuber quality have frequently been greater than those due to reductions in yield (Crop Protection Compendium, 2015). A study in the USA showed that *R. solani* caused yield losses of approx. 19%, but lowered the proportion of US-1 grade tubers by 35% (Carling *et al.*, 1989; Crop Protection Compendium, 2015). A study in Canada reported that potato diseases incited by *R. solani* reduced total yields by 9-16%, but the marketable yields and the proportions of size A grade tubers decreased by 21-31% (Banville, 1989; Crop Protection Compendium, 2015).

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Figure 1.4 Stem canker (Das, 2013).

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Figure 1.5 Root canker (Das, 2013).

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Figure 1.6 Stolon lesion ("nipping") (Das, 2013).

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Figure 1.7 Leaf chlorosis (Brierley *et al.*, 2013).

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Figure 1.8 Aerial tubers (Das, 2013).

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Figure 1.9 *Thanatephorus cucmeris* hymenia (Das, 2013).

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Figure 1.10 Black scurf on a tuber (Brierley *et al.*, 2013)

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Figure 1.11 Dry core on a tuber (Brierley *et al.*, 2013)

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Figure 1.12 Tuber deformations (Das, 2013)

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Figure 1.13 Tuber deformation (Brierley *et al.*, 2013).

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Figure 1.14 Elephant hide on a tuber (Brierley *et al.*, 2013).

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Figure 1.15 Reduced plant emergence (Brierley *et al.*, 2013).

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Figure 1.16 Cork layer of cells at the surface of a potato tuber lesion after *Rhizoctonia solani* infection (Ramsey, 1917; cited in Agrios, 2005).

1.2.4 Pathogen survival

Under dry conditions, *R. solani* sclerotia can survive for up to 6 years (Gadd and Bertus, 1928; cited in Coley-Smith and Cooke, 1971). The pathogen can survive between crops as propagules in soil, in or on plant debris, in volunteer potato plants, in stored tubers (Agrios, 2005; García *et al.*, 2006; Tsror, 2010; Fiers *et al.*, 2012; Ritchie *et al.*, 2013; Wharton and Wood, 2013), and, sometimes, in compost amendments (Tsror, 2010; Noble, 2011).

Persistence of *R. solani* propagules in soil may vary for different AGs depending on edaphic conditions, including soil depth, moisture, temperature, aeration, pH, organic matter content, parasites and predators (Coley-Smith and Cooke, 1971; Anees *et al.*, 2010). *Rhizoctonia solani* isolates from AG1, AG2-1, AG2-2, AG4 and BNRs, cultured in cornmeal-sand as inoculum sources, survived in fallow soil for 283 days, while two isolates of AG3 were not recovered from soil after 86 days (Bell and Sumner, 1987). Baird *et al.* (1993) reported that AG4 in colonised peanut (*Arachis hypogaea*) shell residues remained viable for up to 2 years, but recovery was only 1%. In addition, recovery was greatest for colonised peanut shells on the soil surface compared with those buried at depths of 8 cm or 25 cm. Recovery of beneficial *Trichoderma* spp. was greater from peanut shells colonised with *R. solani* AG4, and these antagonists were suggested to be responsible for reducing the viability of *R. solani* AG4. Yulianti *et al.* (2007) reported that, under laboratory conditions, *R. solani* AG2-1 survived as a saprophyte for up to 6 months in a soil containing 99% sand and 0.16% organic matter content, at 10-70% water holding capacity and at 10-20°C, but was suppressed at 30°C. Saprophytic survival of *R. solani* AG3 in soil requires an optimum moisture content in the range of 20-50% (Kyritsis, 2003). Ritchie *et al.* (2013) showed that sclerotia of *R. solani* AG3-PT could survive in soil for up to 18 months, although the percentage viability was reduced to about 20% after this period. The sclerotia buried in soil at 5-cm depth had the greatest viability (60%) compared to those buried at 20 cm (50%) after 18 months (Ritchie *et al.*, 2013).

Rhizoctonia solani can survive between two successive potato crops, or for longer periods between potato crops, through infection of alternative plant hosts. More than 100 plant species are susceptible to the different *R. solani* AGs (Ogoshi, 1996; Brierley *et al.*, 2013; Crop Protection Compendium, 2015). However, there has not been any published evidence of direct relationships between secondary host plants (including weeds) and *R. solani* diseases of potato.

1.2.5 Factors affecting *Rhizoctonia solani* diseases of potato

1.2.5.1 *Rhizoctonia solani* Anastomosis Groups

The ability of different *R. solani* AGs to cause disease on potato plants varies (Tsror, 2010; Brierley *et al.*, 2013). *Rhizoctonia solani* AG3-PT can cause disease on all underground plant parts, including cankers on stems, stolons and roots, tuber deformation, and severe black scurf on tubers. In addition, AG3-PT is the most virulent AG on potato compared with other AGs (Banville *et al.*, 1996; Tsror, 2010; Brierley *et al.*, 2013). Other *R. solani* AGs, such as AG2-1, AG4, AG5, AG9 and BNR, can cause cankers on stems and stolons to varying degrees. AG2-1 and AG5 cause mild black scurf on tubers, AG8 only infects the roots of potato plants, while AG2-1, AG5 and BNR can result in distorted tubers (Brierley *et al.*, 2013). In particular, Das *et al.* (2014) showed that AG2-1 isolates caused more severe tuber malformations than those incited by AG3-PT in New Zealand.

1.2.5.2 *Rhizoctonia solani* inoculum

Soil and seed tuber inoculum densities are similarly important factors affecting disease development on potato plants (Carling *et al.*, 1989; Banville *et al.*, 1996; Jeger *et al.*, 1996; Tsror (Lahkim) and Peretz-Alon, 2005; Tsror, 2010; Atkinson *et al.*, 2010, 2011; Brierley *et al.*, 2013; Ritchie *et al.*, 2013). An increase in *R. solani* AG3-PT inoculum levels, either from mycelium or sclerotia, can increase the severity of stem cankers and black scurf on progeny tubers, and reduce the number of host stems under controlled conditions (Kyritsis, 2003; Ritchie *et al.*, 2013). Severe damage occurs on potato plants if there are synergistic infections from soil and seed inocula compared with inoculum resulting from either source alone (Tsror (Lahkim) and Peretz-Alon, 2005; Brierley *et al.*, 2013).

The reported *R. solani* inoculum levels in soil or on tubers that result in disease on potato plants has varied, depending on the study. Some studies found that very small amounts of soil inoculum of AG3-PT, as hyphae (5×10^{-5} g/g of soil) or sclerotia (3×10^{-5} g/g of soil), could cause disease (Brierley *et al.*, 2013). Even infected seed tubers without any visible signs of disease, such as sclerotia or hyphae, can cause disease (Brierley *et al.*, 2013). The study of Tsror (Lahkim) and Peretz-Alon (2005) showed that using potato seed tubers with low *R. solani* surface contamination (3%) resulted in production of tubers with a similar disease severity compared with growing plants in soil containing *R. solani* inoculum. Since the current New Zealand phytosanitary regulations allow $\leq$ 5% of tubers in a seed

tuber line to have 5% of the tuber surface infested with *R. solani* (Potatoes New Zealand, 2014), seed tuber lines fulfilling this standard will act as potential inoculum sources for infections in subsequent crops and seasons.

It has been widely observed that *R. solani* hyphae are more uniformly distributed in soil than sclerotia (Brierley *et al.*, 2013). Therefore, hyphal inoculum will be more consistently detected in soil samples, if present. Quantitative PCR (qPCR)/Real-time PCR methods have been developed and applied to detect the presence of *R. solani* in potato seed tubers or in soil (Lees *et al.*, 2002; Ophel-Keller *et al.*, 2008; Brierley *et al.*, 2009; Brierley *et al.*, 2013; Woodhall *et al.*, 2013; Tegg *et al.*, 2014, 2015). Woodhall *et al.* (2012) developed a soil sampling strategy whereby 1 kg soil samples are initially taken, compared with the much smaller soil volumes (less than 10 g) used in other studies. Another study reported that qPCR method can detect a minimum of 8×10^{-7} g of sclerotia of AG3-PT/g of soil, or inoculum on tubers with no visible symptoms (Brierley *et al.*, 2013; Woodhall *et al.*, 2013).

1.2.5.3 Environmental conditions

Soil temperature is a critical factor influencing the severity of *R. solani* diseases of potato (Wharton and Wood, 2013). Mycelium growth of *R. solani* AG3-PT occurs over the range of 5-25°C (Anguiz and Martin, 1989; Ritchie *et al.*, 2009; Wharton and Wood, 2013), but is optimum at 20-25°C (Anguiz and Martin, 1989; Ritchie *et al.*, 2009). Kyritsis (2003) found that the optimum conditions for infection of potato by AG3-PT were 10°C and 40% soil water holding capacity. Carling and Leiner (1990) reported that AG3-PT caused more severe potato stem canker at 10°C than at 15-21°C, whereas AG5 only caused stem disease at 15-20°C. Ritchie *et al.* (2009) noted that the optimum temperature for mycelium growth of AG2-1 and AG3-PT was 20-25°C, both on media and in soil, while the optimum for mycelium growth of AG4 was 25-28°C on media (Anguiz and Martin, 1989), and growth of AG8 was favoured at 6-20°C in culture (Smiley and Uddin, 1993). *Rhizoctonia solani* AG3-PT sclerotia were observed to germinate between 20-30°C (at optimum pH of 5-6) in culture, but at 10-30°C in soil (Ritchie *et al.*, 2009). The mycelia of AG2-1 and AG3-PT were able to grow at pH ranging from 4 to 9, with optimum growth at pH 5.6. Ritchie *et al.* (2009) reported that sclerotia were produced over pH ranges of 4 to 8 for AG3-PT and 5 to 6 for AG2-1, with more sclerotia produced by AG3-PT than AG2-1 at all pHs tested. AG3-PT caused more severe stem canker on potato plants in sandy clay loam soil than in fine sand soil (Kyritsis, 2003).

The potato growing season in Canterbury, New Zealand is from October to May each year, when average total rainfall is 331 mm and average air temperature is 14°C (Oliveira, 2015). In a 2011 field study, Oliveira (2015) demonstrated that the mean daily temperature at planting time was 10-16°C, which is favourable for infection by AG3-PT. This indicated that the soil temperature in Canterbury is

likely to be a strong driver of infection and damage by *R. solani* AGs on potato plants during normal growing seasons.

1.2.6 Disease management strategies

1.2.6.1 Resistant potato cultivars

To date, there are no commercially available potato varieties that are resistant to *R. solani*, although resistant germplasm potentially exists in wild *Solanum* species (Wastie, 1994; Banville *et al.*, 1996; Brierley *et al.*, 2013). Lack of resistant varieties is partially due to difficulty in breeding potato cultivars for resistance to the multiple soilborne diseases, with approx. 40 species being important potato pathogens (Fiers *et al.*, 2012), as well as the requirement for new varieties to be high yielding and have superior quality for fresh market, processing or starch production (Secor and Gudmestad, 1999). *In vitro* studies using transgenic technologies have transformed potato plants with genes to enhance plant resistance, such as the *chitinase* gene from *Trichoderma harzianum,* the *β-1,3-glucanase* gene from *T. virens*, and the *rip30* gene (coding for ribosome inactivating protein) from barley. New selected transgenic lines of potato were shown to be resistant to *R. solani* under greenhouse conditions (Lorito *et al.*, 1998; Esfahani *et al.*, 2010; M'hamdi *et al.*, 2013).

1.2.6.2 Cultural practices

Favourable conditions for potato growth are generally also the preferred environment for *R. solani* (Fiers *et al.*, 2012). Reductions in initial inoculum can potentially lead to decrease in disease severity. Seed tuber-borne *R. solani* inoculm is considered to be important for potato disease, so pathogen-free seed tubers are the requisite for potato cultivation practices to reduce the initial disease inoculum. Seed tubers should be carefully inspected for *R. solani*, possibly using molecular techniques, and eradication of tubers having more than 5% of the surface covered with sclerotia is recommended before planting (Secor and Gudmestad, 1999; Tsror, 2010; Brierley *et al.*, 2013; Wharton and Wood, 2013). Soil or tuber DNA detection of the pathogens, AG2-1, AG2-2, AG3, AG3-PT, AG4 and AG8, can be carried out to evaluate disease risk prior to potato planting (Lees *et al.*, 2002; Ophel-Keller *et al.*, 2008; Brierley *et al.*, 2009; Budge *et al.*, 2009a; Woodhall *et al.*, 2013; Tegg *et al.*, 2014; Primary Industries and Regions South Australia, 2015; Sparrow *et al.*, 2015).

Continuous cropping of potato has been reported to promote inoculum accumulation of *R. solani* in soil (Wright *et al.*, 2015; Zhang *et al.*, 2015), and rotations with non-host crops have been shown to reduce *R. solani* disease pressure in following potato crops (Larkin *et al.*, 2012; Wright *et al.*, 2015). Crop rotation systems with 2 or 3 years between potato crops using crops such as barley, ryegrass, canola or rapeseed grown in the interval, were reported to reduce incidence of *R. solani* disease and other tuber diseases on potato tubers by 15-50% in Maine (USA) compared to successive potato cropping (Larkin *et al.*, 2012). A 10-year trial of potato rotations with other crops in New Zealand

showed that *R. solani* soil inoculum (DNA) in rotation treatments was reduced, and black scurf incidence was also reduced, compared with a potato monoculture treatment (Wright *et al.*, 2015). Based on long-term studies, Larkin *et al.* (2010) recommended that a 3-year rotation was suitable for potato production in Northeast USA, while Wright *et al.* (2015) proposed a 4-year rotation for potato cropping systems in New Zealand.

1.2.6.3 Agrichemicals

Several fungicides have been used to control *R. solani* on potato. The three main *Rhizoctonia*-specific fungicides are azoxystrobin (systemic broad-spectrum fungicide; chemical group: strobilurin), fludioxonil (systemic Basidiomycota-specific fungicide; chemical group: phenylpyrrole) and pencycuron (contact *Rhizoctonia*-specific fungicide; chemical group: phenylurea) (Djébali and Belhassen, 2010; Buysens *et al.*, 2015; New Zealand Novachem Agrichemical Manual, 2015). Other fungicides are thiophanate-methyl, and mancozeb (chemical group: dithiocarbamate) (Wharton and Wood, 2013). Azoxystrobin, and sometimes fludioxonil, are used for in-furrow applications at potato planting, while fludioxonil, pencycuron, thiophanate-methyl and mancozeb are applied for seed tuber treatments. These specific fungicides have been shown to effectively control *R. solani* infections of potato in the USA, Tunisia, India, Turkey and Canada (Bains *et al.*, 2002; Djébali and Belhassen, 2010; Wharton and Wood, 2013; Malik *et al.*, 2014; Özer and Bayraktar, 2015). In New Zealand, azoxystrobin (in furrow treatment), fludioxonil (seed tuber treatment) and pencycuron (seed tuber treatment) are officially registered for control of black scurf and stem canker on potatoes (New Zealand Novachem Agrichemical Manual, 2015).

Rhizoctonia solani isolates have different sensitivities to different fungicides (Kataria and Gisi, 1999). Some Tunisian *R. solani* isolates were very sensitive to fludioxonil and pencycuron, but highly resistant to azoxystrobin (Djébali *et al.*, 2014). In addition, AG1, AG2-1, AG2-2, AG3, and particularly AG4, AG6 and AG9 were highly sensitive to pencycuron, while there are no reports on the effectiveness of this fungicide towards AG5, AG7 and AG8 (Kataria and Gisi, 1999). Although the potential for *R. solani* to develop resistance to fungicides has been reported to be low, the continuous use of one fungicide active ingredient or mode-of-action group could result in development of resistance (Fungicide Resistance Action Committee, 2013). For example, azoxystrobin has been widely used and effectively controlled *R. solani* disease of potato in Tunisia (Djébali and Belhassen, 2010), but recently some *R. solani* isolates from potato tubers have been reported to be resistant to this chemical (Djébali *et al.*, 2014).

After the phasing out of methyl bromide in cropping systems in developed countries in 2005, and developing countries in 2015, metam sodium, which is converted to the active compound methyl isothiocyanate, and other related chemicals, have been used for soil fumigation in agriculture and horticulture. Due to the concerns of adverse environmental effects of chemical fumigants (Ibekwe *et*

al., 2001; Dungan *et al.*, 2003), reducing soil inoculum using environmentally-friendly methods such as biocontrol (Reddy, 2012; Larkin and Tavantzis, 2013) or soil biofumigation has been the focus of increased interest (Kirkegaard *et al.*, 1993; Angus *et al.*, 1994; Taylor, 2013).

1.2.6.4 Biological control

Biological control methodologies have been extensively studied for the management of soilborne diseases of potato (Bienkowski, 2012). Several studies have shown that beneficial soil microorganisms, including *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Verticillium biguttatum, Pythium oligandrum*, and non-pathogenic binucleate *Rhizoctonia*-like species, are able to control *R. solani* (Banville *et al.*, 1996; Hoitink and Boehm, 1999; Tsror (Lahkim) *et al.*, 2001; Garbeva *et al.*, 2006; Ikeda *et al.*, 2012; Olle *et al.*, 2015; Özer and Bayraktar, 2015). Olle *et al.* (2015) reported that composts used alone or in combination with beneficial microorganisms, such as *Trichoderma* spp., *Bacillus* spp., *Streptomyces* spp. and *Pseudomonas* spp., effectively suppressed *R. solani* diseases of potato. Some studies have shown that combinations of fungicides, such as pencycuron or fludioxonil, with the biocontrol agent *V. biguttatum,* successfully reduced black scurf on potato tubers (van den Boogert and Luttikholt, 2004). Recent studies noted that *Trichoderma* spp. were tolerant of isothiocyanates produced during biofumigation (Galletti *et al.*, 2008); thus, it is possible that combination of biofumigation and *Trichoderma* spp. could result in increased control efficiency against *R. solani*.

1.3 Biofumigation

1.3.1 Definition

The term biofumigation was initially coined by Kirkegaard *et al.* (1993) to describe the suppressive activity of volatile isothiocyanates and other related compounds, liberated from glucosinolate-producing *Brassica* plants, on soilborne plant pests, diseases and weeds (Angus *et al.*, 1994; Kirkegaard and Sarwar, 1998). This term has been broadened to other plants or microorganisms that can release volatile biocidal substances, such as *Allium* spp., Sudan grass, hybrid sorghum-Sudan grass, oat, and the endophytic fungi *Nodulisporium* spp. (Kirkegaard, 2009; Reddy, 2012; Suwannarach *et al.*, 2013).

The volatile antifungal activity of isothiocyanates in mustard oils against certain fungi was first mentioned in the study of Walker *et al.* (1937). Ellenby (1945) reported that *Brassica* root exudates had potential to inhibit the potato cyst nematode (*Globodera rostochiensis*). Due to the phasing out of the soil fumigant methyl bromide, and increased emphasis on reducing use of synthetic pesticides, there has been increased interest in biofumigation as an alternative for crop pest management (Dungan *et al.*, 2003; Martin, 2003; Kirkegaard, 2009).

The greatest number of glucosinolate-containing plants belongs to the Capparales, including members of the Brassicaceae, Capparidaceae and Moringaceae (Fahey *et al.*, 2001; Reddy, 2012). Of these, brassicaceous plants (mustards, radish) have mostly been studied, with these considered to have the best potential for biofumigation (Brown and Morra, 1997; Kirkegaard and Sarwar, 1998; Fahey *et al.*, 2001; Matthiessen and Kirkegaard, 2006; Kirkegaard, 2009; Reddy, 2012).

1.3.2 The glucosinolate-myrosinase system

The location of glucosinolates and myrosinases in plant tissues is not fully understood (Mithen, 2001; Kissen *et al.*, 2009). It has been widely recognised that glucosinolates and myrosinases are found in different cells (Koroleva *et al.*, 2000; Kissen *et al.*, 2009) or co-occur within cells in different subcellular compartments (Lüthy and Matile, 1984; Bones and Rossiter, 1996). Myrosinases have been found to occur in "myrosin cells" (Lüthy and Matile, 1984; Bones and Iversen, 1985; Bones and Rossiter, 1996; Mithen, 2001), while glucosinolates have been reported in cell vacuoles (Lüthy and Matile, 1984; Bones and Rossiter, 1996; Mithen, 2001), or in "S cells" which are rich in glucosinolates (Koroleva *et al.*, 2000; Kissen *et al.*, 2009).

Glucosinolates (β-thioglucoside N-hydroxysulfates) (GLSs/GLS) are secondary metabolites produced by plant cells, and comprise 1-13% of plant biomass (Fahey *et al.*, 2001; Gimsing and Kirkegaard, 2006). They are highly stable and water-soluble, and have limited biological activity. Based on their structures, the GLSs are in aliphatic, aromatic or indolic groups (Sarwar *et al.*, 1998; Fahey *et al.*, 2001; Kirkegaard, 2009). The isothiocyanates (ITCs/ITC) converted from the parent indolic GLSs, are very unstable (Fahey *et al.*, 2001), so are not used as biofumigants, unlike those from aliphatic and aromatic GLSs (Figure 1.17) (Fahey *et al.*, 2001; Kirkegaard, 2009). The profiles, contents and distribution of GLSs in plants vary greatly within and between species, cultivars, plant organs, developmental stages, seasons, harvest times, soil and weather conditions, and pest infections (Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998; Fahey *et al.*, 2001; Matthiessen and Kirkegaard, 2006; Kirkegaard, 2009; Dal Prá *et al.*, 2013). For instance, Chinese cabbage plants (*B. rapa* subsp. *campestris)* cultivated in soil with pH 7.6 had greater GLS content, including gluconasturtiin and gluconapin, than those grown in soil at pH 6.2 (Lee *et al.*, 2010). Plants can produce several types of GLSs, but there are generally less than 12 in a particular species (Fahey *et al.*, 2001). The intensive study of Sarwar and Kirkegaard (1998) showed that aromatic GLSs were dominant in roots, while abundant aliphatic GLSs were found in shoots (Table 1.1). Total GLSs usually reached maximum concentration in tissue at the early to middle flowering stages (Sarwar and Kirkegaard, 1998).

Myrosinases (β-thioglucosidases) are enzymes that often have greatest activity at temperatures above 30°C (Grevsen, 2010). For example, activities of these enzymes in Brussels sprouts and

mustard seeds are optimum at, respectively, 50°C and 60°C (Springett and Adams, 1989; Van Eylen *et al.*, 2006). The low soil temperatures in New Zealand potato growing areas (Oliveira, 2015) could reduce the rate of hydrolysis of GLSs by myrosinases if *Brassica* tissues are incorporated into soil. Besides being present in plants, myrosinases are also found in soil microorganisms, such as the fungus *Aspergillus* (Rakariyatham *et al.*, 2006).

Contact between GLSs and myrosinases through maceration of plant tissues is essential for the production of biologically active volatile compounds from fresh intact *Brassica* tissues (Figure 1.17). Hydrolysis of GLSs by endogenous myrosinases will form multiple bioactive compounds, such as ITCs, nitriles and thiocyanates, based on the original structures of the parent GLSs (Figure 1.17, Table 1.1). The bioactive hydrolysis products with greatest activity are ITCs formed at pH 5-7 (Brown and Morra, 1997; Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006), while thiocyanates are formed abundantly at pH >7 (Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006). At lower pH (2-5) or in the presence of Fe^{2+}, more nitriles are formed (Borek *et al.*, 1995; Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006). The simplest ITC is methyl ITC from the methyl GLS precursor which is not found in *Brassica* plants (Table 1.1) (Sarwar and Kirkegaard, 1998). The other ITCs, 2-Propenyl (Allyl) ITC and benzyl ITC, commonly found in *Brassica* spp., are presented in Table 1.1.

The ITCs are short-lived, but they have broad-spectrum biocidal activities through their irreversible interactions with sulfhydryl groups, disulfide bonds and amines in cellular amino acids and proteins, ITCs have also been shown to inactivate cell enzymes (Brown and Morra, 1997). Compared to their parental GLSs, ITCs are more hydrophobic and volatile (Fahey *et al.*, 2001; Mithen, 2001).

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Figure 1.17 The hydrolysis of glucosinolates (GLSs) under the action of myrosinase to produce multiple GLS-derived compounds (Mithen, 2001; Kirkegaard, 2009).

Table 1.1 Names and occurrence of isothiocyanates produced from different parental glucosinolates occurring in *Brassica* plants (modified from Sarwar *et al.*, 1998).

Isothiocyanate (ITC)	Glucosinolate precursor	Common occurrence
Aliphatic		
Methyl ITC	Glucocapparin	Capparales order, Metham sodium (synthetic fumigant)
2-Propenyl (Allyl) ITC	Sinigrin	*Brassica juncea*, *B. carinata, B. nigra*
3-Butenyl ITC	Gluconapin	*B. napus, B. campestris*
4-Pentenyl ITC	Glucobrassinapin	*B. napus, B. campestris*
Aromatic		
Benzyl ITC	Glucotropaeolin	*Sinapis* spp.
2-Phenylethyl ITC	Gluconasturtiin	*Brassica* roots

1.3.3 Application of biofumigation

Biofumigant crops and crop-derived materials have been used as harvested crops, green manures, cover crops in rotation cropping systems, or as intercrops, seed meals, dried plant pellets, or as extracted pure bioactive compounds (Kirkegaard, 2009; Reddy, 2012).

The effective concentrations of commercial chemicals used for soil sterilisation are 517 to 1,294 nmol/g of soil, depending on the crop and control requirement (Brown *et al.*, 1991; Brown and

Morra, 1997). Field experiments showed that the maximum concentration of detected GLSs was 100 nmol/g of soil, and the conversion efficiency into active compounds was approx. 60% (Gimsing and Kirkegaard, 2009). It has been suggested that these concentrations are unlikely to be effective for controlling soilborne diseases (Grevsen, 2014). However, the biofumigation process not only releases ITCs but also other bioactive compounds at lower concentrations, which are usually ignored in studies. This, in addition to the large biofumigant biomass which is incorporated into soil, may result in synergistic action between the main ITCs and other biocidal substances (Matthiessen and Kirkegaard, 2006; Gimsing and Kirkegaard, 2009; Kirkegaard, 2009; Motisi *et al.*, 2010). Matthiessen and Kirkegaard (2006) also suggested that biofumigation with *Brassica* green manures is not the only mechanism for controlling soilborne diseases. Some related mechanisms include improvement of soil characteristics, and enhancement of soil beneficial microorganisms, which in turn may suppress pathogen inoculum (Matthiessen and Kirkegaard, 2006; Motisi *et al.*, 2010; Yim *et al.*, 2016; Larkin *et al.*, 2017; Mazzola *et al.*, 2017).

Kirkegaard (2009) proposed strategies to optimise suppression of soilborne diseases in cropping systems using ITC-based biofumigation. These include: identification of biofumigants for specific diseases; the appropriate amount of fresh plant tissue biomass (recommended 5% w:w; approx. 5-6 kg of fresh tissues/m^2 of soil to 20-cm depth); suitable incorporation, including maceration, incorporation and watering; covering the soil to reduce the loss of bioactive volatile compounds; and at least a 2 week period between biofumigant incorporation and planting the next crop.

1.3.4 Edaphic factors affecting biofumigation

When incorporated into soils, GLSs within plant tissue are converted to ITCs by myrosinases, resulting in biofumigation. Therefore, the biofumigation activity will principally depend on edaphic conditions. The conversion of GLSs to ITCs in soil during biofumigation processes depends mainly on two important factors, the level of disruption of the plant tissues before incorporation into soil, and soil moisture content after burial of the plant tissues (Gimsing and Kirkegaard, 2009). If fresh plant tissues are not thoroughly macerated before incorporation into soil, ITC release efficiency from parental GLSs was reported to be only 1% or less after 24 hours under field conditions. However, increasing the level of disruption of the plant tissues, by freezing (at -19°C) and thawing before integration into soil, increased the efficiency of release of ITCs to 14% at soil field water holding capacity (-32 kPa), and 26% when the soil was waterlogged (saturated), at 2 hours after incorporation (Morra and Kirkegaard, 2002). In particular, with optimum tissue disruption by macerating plant tissues using a flail mulcher before incorporation into soil, and with soil moisture at 50-70% field capacity, 56% of the ITCs were released 30 minutes after incorporation into soil, and reached 79% after 6 hours (Gimsing and Kirkegaard, 2006). In addition, the concentrations of ITCs detected were positively correlated to the initial levels of GLSs (Morra and Kirkegaard, 2002; Matthiessen *et al.*,

2004; Gimsing and Kirkegaard, 2006). Fine disruption of mustard tissues (*B. juncea*) with high GLS content before incorporation into saturated soil resulted in the maximum levels of total ITCs detected in soil from 90.6 nmol/g of soil (Gimsing and Kirkegaard, 2006) to 100 nmol/g of soil (Matthiessen *et al.*, 2004). All of these studies were conducted in sandy loam soils with low organic matter contents (0.9% carbon, ≅ 1.5% organic matter) (Gimsing and Kirkegaard, 2009).

In addition, GLSs released from plant tissues can be leached from soil because they are highly solubile, or they can be degraded by soil microorganisms. GLSs were reported to be weakly absorbed in different soil types, including rich organic soils (Gimsing *et al.*, 2006, 2007a, 2007b; Gimsing and Kirkegaard, 2009). Adding myrosinases increased the ITC concentrations in soil (Gimsing and Kirkegaard, 2009). Some myrosinase-releasing microorganisms (Rakariyatham *et al.*, 2006) have been isolated from soil, so their application during the incorporation of macerated tissues could enhance the efficiency of ITC release. In addition, soil temperature has been shown to affect myrosinase activity, and influence conversion of GLSs to ITCs (Gimsing and Kirkegaard, 2009). Biodegradation of GLSs by microorganisms was observed in experiments using non-sterilised and sterilised soils. GLSs were not degraded, or degraded only by small amounts, in sterilised soil compared to high levels of degradation in non-sterilised soil, with degradation being dependent on diversity and level of soil microbial populations (Gimsing *et al.*, 2006; Poulsen *et al.*, 2008). Gimsing *et al.* (2007a) reported that incorporation of plant biofumigant tissues into soil at a depth of 30-65 cm reduced the conversion of GLSs to ITCs by 19-24% in comparison to incorporation into the soil near the surface (0-30 cm depth).

ITCs are usually lost from soil by volatility, and can persist in soil from a few days to 14 days (Gimsing and Kirkegaard, 2006, 2009). Unlike GLSs, ITCs are strongly absorbed and have reduced bioavailability in organic rich soils (Gimsing *et al.*, 2006; Poulsen *et al.*, 2008). ITCs with large molecular structures, such as 2-phenylethyl ITC, are more easily absorbed by organic matter compared to short chain of ITCs, such as 2-propenyl ITC (Gimsing and Kirkegaard, 2009). The study of Price *et al.* (2005) illustrated that allyl ITC could be degraded by soil microorganisms. The concentration of allyl ITC was found to be greater (1.71 µmol/l) when *B. juncea* freeze-dried tissues were incorporated into autoclaved soil compared with incorporation into non-autoclaved soil (0.4 µmol/l). In the same study, high soil temperature (45°C) increased the mobility of allyl ITC, and resulted in detection of 81% greater concentration of allyl ITC than that at 15°C (Price *et al.*, 2005). Additionally, ITCs in the forms of 3-butenyl, 4-pentenyl and 2-phenethyl applied to loamy and sandy soils were found to leach in soil water (Laegdsmand *et al.*, 2007), but usually at lower amounts than for GLSs (Gimsing and Kirkegaard, 2009).

1.3.5 Use of biofumigation to control *Rhizoctonia solani* causing potato diseases

The variable effectiveness of different pure ITCs against a potato *R. solani* isolate (unspecified AG) was observed in the *in vitro* study of Taylor *et al.* (2014). They prepared stock solutions of ITCs by dissolving in 50% ethanol, then 500 µL of each ITC solution was thoroughly mixed with 20 mL of liquid potato dextrose agar (PDA) prior being poured into Petri dishes. The results illustrated that benzyl ITC, 2-phenylethyl ITC, methyl ITC, or propyl ITC had greater suppression of *R. solani* mycelium growth than allyl ITC, naphthyl ITC, or isopropyl ITC (Taylor *et al.*, 2014). In addition, different *R. solani* AGs have been shown to respond to ITCs in distinct ways. For example, the concentrations of 2-phenylethyl ITC, dissolved in sterile distilled water and incorporated into PDA medium, which suppressed mycelium growth of AG3, AG4, and AG2-1 (all isolated from potato) by 50% were, respectively, 0.053 mM, 0.421 mM, and 0.902 mM (Smith and Kirkegaard, 2002). Sarwar *et al.* (1998) reported that volatile and soluble forms of pure ITCs, which were added to Petri plate headspaces or dissolved in PDA in flasks, had different efficacies against *in vitro* mycelium growth of a *R. solani* isolate (unspecified AG, isolated from a wheat field). These results showed that allyl ITC was ten times more toxic to mycelium growth of *R. solani* in its volatile form (concentration giving 50% suppression of mycelium growth (SD_{50}) = 0.63 µmol/l), than in the soluble form (SD_{50} = 7.1 µmol/l). In the soluble form, 2-phenylethyl ITC (SD_{50} = 3.3 µmol/l) was more than twice as effective at reducing mycelium growth of *R. solani* compared to allyl ITC (SD_{50} = 7.1 µmol/l) (Sarwar *et al.*, 1998).

There are several reports evaluating biofumigation to reduce *R. solani* disease in potato cropping systems, with studies from the USA and Australia showing efficiencies (Little *et al.*, 2004; Larkin and Honeycutt, 2006; Larkin and Griffin, 2007; Sexton *et al.*, 2007; Snapp *et al.*, 2007; Halloran *et al.*, 2008; Larkin *et al.*, 2010, 2011; Bernard *et al.*, 2014; Larkin and Halloran, 2014). The USA studies reported that biofumigant crops, cultivated as cover crops, green manures or harvest cover crops, reduced incidence of black scurf on tubers by 14-77%, and showed the varying effectiveness of different biofumigant crops (Larkin and Honeycutt, 2006; Larkin and Griffin, 2007; Halloran *et al.*, 2008). However, disease reduction with biofumigants has not always resulted in increased potato tuber yields in comparison with non-biofumigation (Larkin *et al.*, 2010). Although the Indian mustard *B. juncea* (unspecified cultivar) gave the highest suppression of *R. solani in vitro*, it was not effective at suppressing disease under field conditions compared with suppressions recorded from rapeseed (*B. napus* 'Dwarf Essex') and yellow mustard (*S. alba* 'IdaGold') (Larkin and Griffin, 2007). This illustrates that the efficiency of a biofumigant crop may differ for different pathogens in a cropping system. It is therefore essential to determine the most effective biofumigant crop for control of a particular pathogen, or pathogens.

1.3.6 Effects of biofumigation on soil microorganisms

Incorporation of biofumigant crops into soils have been reported to cause changes in soil microbial communities (Larkin *et al.*, 2010; Omirou *et al.*, 2011; Wang *et al.*, 2014). The increases in organic substrate availability due to the incorporation of biofumigant biomass have been attributed to modifications in soil microbial communities, rather than ITC toxicity (Omirou *et al.*, 2011). Compared to bacteria, fungi were reported to be strongly affected by biofumigation (Matthiessen and Shackleton, 2005; Omirou *et al.*, 2011; Wang *et al.*, 2014). Using the PCR-denaturating gradient gel electrophoresis (PCR-DGGE) technique, Omirou *et al.* (2011) observed that the community of ammonia-oxidizing bacteria was not affected by biofumigation or fumigation treatments induced by either incorporation of broccoli tissue (biofumigant) or pure 2-phenylethyl ITC or metham sodium (fumigant). However, the community structure of Ascomycete fungi was changed by the treatments. In particular, metam sodium reduced diversity of the Ascomycete community compared to other treatments. Another PCR-DGGE study showed that rapeseed (*B. napus* 'Dwarf Essex') meal applied as a biofumigant increased bacterial diversity, but decreased fungal diversity (Wang *et al.*, 2014). Yim *et al.* (2016), also using PCR-DGGE, found that fungi were more greatly affected than bacteria, by biofumigation with *B. juncea* 'Terra Plus' and *R. sativus* 'Defender'.

Several studies have concentrated on disease suppression induced by biofumigation, but have usually not considered effects on non-target soil microorganisms, which may also have roles in disease suppression (Omirou *et al.*, 2011). Cohen *et al.* (2005) and Cohen and Mazzola (2006) reported that biofumigation stimulated microbes involved in nitrification and nitrous oxide production, and consequently these enhanced functions suppressed soilborne diseases. However, other studies have shown that pure ITCs and other related compounds (Bending and Lincoln, 2000) as well as GLS-originating ITCs (Brown and Morra, 2009) inhibited nitrification in soil. Hyphal growth of arbuscular mycorrhizal fungi (germ tube branching and hyphal tuft formation) was reduced near *Brassica* roots in comparison to growth near of roots of tomato, tobacco or onion (Hayman *et al.*, 1975; Koide and Schreiner, 1992; Haramoto and Gallandt, 2004). In contrast, Ocampo (1980) did not observe any suppression of mycorrhizal colonisation from *Brassica* crops on subsequent planted hosts or intercropping plants. These results indicate that different *Brassica* crops, which do not form symbioses with mycorrhizae, may have variable effects on mycorrhizal fungi (Haramoto and Gallandt, 2004).

1.3.7 Potential issues with biofumigation for plant disease management

Like other crops, biofumigant *Brassica* crops are sensitive to unfavourable environmental conditions, and they can be affected by pests and diseases (Koike *et al.*, 2007; Clark, 2008). The New Zealand study of Johnstone *et al.* (n.d.) showed that biofumigant cultivars (*B. juncea* 'Caliente' and *Eruca*

sativa 'Nemat') were affected by winter weather, which reduced biofumigant biomass compared to that for annual ryegrass ('Moata'). Some *R. solani* AGs, including AG2-1, AG2-2, AG4 and AG9, have been shown to cause diseases on *Brassica* plants (Budge *et al.*, 2009b). Potato in rotations with *Brassica* crops could increase the risks of diseases caused by these *R. solani* AGs. In addition, Indian mustard (*B. juncea*) and turnip (*B. rapa*) were reported as favourable hosts of root-knot nematodes (*Meloidogyne* spp.), whereas arugula (salad rocket; *E. sativa* 'Nemat') was resistant to these nematodes (Edwards and Ploeg, 2014). Hence, effects on other pests or diseases must also be considered when selecting biofumigant plants. In addition, biofumigant plants require fertiliser and pesticide inputs to ensure high yields and prevention of pests and diseases, to ensure effective biofumigant potential (Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998; Johnstone *et al.*, n.d.). These requirements potentially add to the crop production costs, and may lead to reduced adoption of biofumigation by growers (Kirkegaard, 2009).

1.4 Aims and objectives of the present study

Although biofumigation has been studied overseas for ability to suppress soilborne diseases of potato (including *R. solani*), the factors that influence the success of disease control are still to be determined. The level of control achieved has been inconsistent between studies, with variable results obtained between *in vitro* laboratory research, shadehouse experiments and field trials. As well, anecdotal and promotional communications between potato growers and seed companies have seen biofumigant crops being increasingly used in potato production systems in New Zealand. Detailed research is required to identify the effects of biofumigant crop quality on effectiveness to suppress soilborne pathogens of potato.

The present study is focussed on *R. solani*. Experiments will be carried out to determine effects of edaphic and environmental factors on biofumigation, and to measure potential impacts of biofumigation on non-target soil microorganisms. To date, such information in New Zealand cropping conditions has been limited. The overall aim of this study was to determine the potential for suppression of *R. solani* infection of potato using biofumigant crops, and to determine if the level of disease control was sufficient to recommend widespread adoption of biofumigation for management of *R. solani* diseases in potato cropping systems.

The objectives of this study were:

Objective 1: To evaluate *in vitro* biofumigation potential of selected *Brassica* plants for growth suppression of *Rhizoctonia solani*.

Objective 2: To determine biofumigation potential, under different edaphic conditions, on suppression of *Rhizoctonia solani* AG3-PT.

Objective 3: To evaluate biofumigation potential of selected *Brassica* plants for suppression of *Rhizoctonia solani* infection of potato, under shadehouse conditions.

Objective 4: To evaluate effects of cover crops in potato rotation on soil microbial communities.

Chapter 2

In vitro biofumigation potential of *Brassica* plants against *Rhizoctonia solani* causing potato diseases

2.1 Introduction

Rhizoctonia solani, a soilborne pathogen, is a common causal agent for potato diseases worldwide (Banville *et al.*, 1996; Larkin and Griffin, 2007). This fungus is an important limiting factor for potato yields in Canterbury, New Zealand (Sinton *et al.*, 2013). There are several *R. solani* anastomosis groups (AG) which cause diseases on potato, including AG3-PT, AG2-1, AG2-2, AG4, AG5, AG7, AG8 and AG9, as well as binucleate *Rhizoctonia* strains (Banville *et al.*, 1996; Woodhall *et al.*, 2011, 2012, 2013; Wharton and Wood, 2013). In New Zealand, Das *et al.* (2014) reported that AG3-PT was the most prevalently isolated *R. solani* AG from black scurf symptomatic potato tubers, accounting for 85% of isolates obtained, followed by AG2-1 (14%) and AG-5 (1%). Several approaches have been proposed to control diseases caused by *R. solani*, including seed tuber or soil fungicide treatments (Özer and Bayraktar, 2015), crop rotations (Larkin *et al.*, 2012; Wright *et al.*, 2015), and biological agents (Olle *et al.*, 2015). In New Zealand, fungicides are the most commonly used disease control strategy for controlling soilborne pathogens on potatoes (Potatoes New Zealand, 2017). However, environmental concerns (Chung *et al.*, 2002; Beresford, 2010) and possible development of fungicide resistance (Djébali *et al.*, 2014), mean that alternative control strategies are required which can be incorporated into an integrated disease management system for potato crops.

Biofumigation to control soilborne pathogens, including *R. solani* strains, has been well documented in the studies of Kirkegaard *et al.* (1996), Kirkegaard and Sarwar (1998), Sarwar and Kirkegaard (1998), Sarwar *et al.* (1998), Charron and Sams (1999), Yulianti *et al.* (2006a, 2006b, 2007), Larkin and Griffin (2007), Snapp *et al.* (2007), Villalta *et al.* (2016) and Handiseni *et al.* (2016, 2017). The biofumigation potential of crops depends on many factors, including biomass, species/cultivars, tissue types, tissue amounts/concentrations, growth stages, application techniques and environmental factors (Kirkegaard *et al.*, 1996; Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998; Mattner *et al.*, 2008; Kirkegaard, 2009). Complex factors can affect biofumigation efficacy and these may result in inconsistency between the level pathogen suppression achieved *in vitro* and field disease reduction outcomes. Cultivars of brown mustard (*Brassica juncea*) are the most effective at suppressing *in vitro* mycelium growth of pathogens, including *R. solani* (Larkin and Griffin, 2007;

Handiseni *et al.*, 2016; Villalta *et al.*, 2016). However, Larkin and Griffin (2007) reported that mustards were more effective in field trials for reducing powdery scab (caused by *Spongospora subterranea*) and common scab (caused by *Streptomyces scabiei*) than for reducing disease caused by *R. solani*.

Responses to pure isothiocyanates varied not only between different plant pathogen species, but also between isolates of a species (Sarwar *et al.*, 1998; Smith and Kirkegaard, 2002). Additionally, Yulianti *et al.* (2006b) found that *R. solani* AG2-1 sclerotia and mycelium on colonised ryegrass seeds were more tolerant to volatiles released from *Brassica* crops than mycelium on agar plugs. Sclerotia or mycelia colonising plant debris are the main survival propagules of *R. solani* in soil (Papavizas, 1970; Coley-Smith and Cooke, 1971; Sneh *et al.*, 1996). Most biofumigation studies have used actively growing mycelium of *R. solani* on agar (Charron and Sams, 1999; Snapp *et al.*, 2007; Larkin and Griffin, 2007; Ríos *et al.*, 2016; Villalta *et al.*, 2016), while only a few studies have been carried out with other *R. solani* propagules such as sclerotia and mycelia colonised cereal grains (Yulianti *et al.*, 2006a, 2006b, 2007; Handiseni *et al.*, 2016, 2017). Thus, *in vitro* results may not reflect the biofumigation efficiency for reducing survival of propagules of the fungus in field conditions.

The aim of the studies described in this chapter was to determine the *in vitro* biofumigation potential of selected *Brassica* plants for suppression of *R. solani* AG3-PT and AG2-1 isolates. In addition, the effects of volatiles released from macerated biofumigant tissues on the viability and survival of different *R. solani* propagules, including actively growing mycelium on agar plugs, sclerotia and mycelium colonised barley grains, were investigated, initially on agar and then after incorporation into soil.

2.2 Materials and methods

2.2.1 *Rhizoctonia solani*

2.2.1.1 Anastomosis group identification

Six *R. solani* isolates (designated LUPP2515 to LUPP2520) from diseased potato tissue samples or nightshade (weeds), collected from potato fields in Canterbury, New Zealand were used in the experiments (Table 2.1). Four *R. solani* isolates (LUPP2521 to LUPP2524) were obtained from Plant & Food Research, Lincoln, New Zealand.

Table 2.1 Details and origin of the *Rhizoctonia solani* isolates used in this study.

Isolate	Anastomosis Group (AG)	Source location	Disease symptom, Organ, Host
LUPP2515	AG3-PT	South Canterbury	Black scurf, Tuber, Potato
LUPP2516	AG3-PT	South Canterbury	Canker, Stem, Potato
LUPP2517	AG3-PT	South Canterbury	Canker, Stem, Potato
LUPP2518	AG3-PT	South Canterbury	Black scurf, Tuber, Potato
LUPP2519	AG3-PT	South Canterbury	Black scurf, Tuber, Potato
LUPP2520	AG3-PT	South Canterbury	Canker, Root, Nightshade (weed)
LUPP2521	AG3-PT	South Canterbury	Canker, Root, Potato
LUPP2522	AG2-1	Lincoln	Hymenia, Stem, Potato
LUPP2523	AG3-PT	Lincoln	Black scurf, Tuber, Potato
LUPP2524	AG3-PT	Lincoln	Black scurf, Tuber, Potato

DNA for each of the *R. solani* isolates was extracted using cetyltrimethylammonium bromide (CTAB), and identified to anastomosis group (AG) by PCR-sequencing, following the method described by Das (2013). The *R. solani* ribosomal RNA region internal transcribed spacer (ITS) 1-5.8S-ITS2 was amplified using primers ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) and ITS5 (5´-GGAAGTAAAAGTCGTAACAAGG-3´). The PCR products were purified using the Qiagen PCR purification kit (Qiagen, Germany) and sequenced in two directions at the Bio-Protection Centre (Lincoln University) sequencing facility.

The forward and reverse sequences of each isolate were assembled using DNAMAN version 5.2.10 (Lynnon Biosoft, USA) to generate consensus sequences (Appendix A.2.1). These were then compared to those available in the GenBank database using Nucleotide BLAST program (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*), and the AG of each isolate was identified based on sequence identities (Appendix A.2.2). Nine of the isolates (LUPP2515 to LUPP2521, LUPP2523, LUPP2524) were identified as AG3-PT and one as AG2-1 (LUPP2522) (Table 2.1).

2.2.1.2 Inoculum preparation

The *R. solani* isolates were stored as mycelium on agar slopes at 4°C, and were sub-cultured on potato dextrose agar (PDA; Difco, Sparks, USA), incubated at 25°C for 7 days prior to setting up the *in vitro* assays described below.

Sclerotia of each *R. solani* isolate were produced using the method of Ritchie *et al.* (2013) with some modifications. AG3-PT isolates were sub-cultured in Petri plates containing malt yeast extract agar [MYA: 12 g agar; 15 g malt extract (Sigma-Aldrich), 5 g yeast extract (Sigma-Aldrich)], and the AG2-1 isolate on PDA. All plates were incubated at 25°C for 35 days for sclerotia formation. The plates were then stored at 4°C until used. The sclerotium mass from each plate was then harvested using

sterilised forceps and scalpel, transferred into a sterile plastic Petri dish and excess agar was removed. The sclerotium mass was then cut into small fragments, and sieved to obtain a fragment size range of 1-2 mm. The sieved sclerotia were stored at 4°C for a maximum of 7 days, and were examined under a stereo microscope (C-DSS230, Nikon Corporation, Tokyo, Japan) to ensure no hyphae had emerged (germination) before setting up experiments. The sclerotium fragments were also examined for germinability on PDA prior to setting up each experiment.

Rhizoctonia solani colonised barley grains of each isolate were prepared using the method of Bienkowski *et al.* (2010). Fifty g of barley grains was mixed with 50 mL V-8 juice (Campbell's Soups, Australia) (1:1 w:v) in a 250-mL Erlenmeyer flask, and the mixture was autoclaved twice (24 hours interval). After cooling, the grains were inoculated with five 7 mm *R. solani* mycelium discs cut from the actively growing colony margin of a PDA culture. Flasks inoculated with the different *R. solani* isolates were incubated at 25°C in the dark for 14 days, and stored at 4°C until used. The *R. solani* grains were examined for subsequent growth of mycelium on PDA before experiments were set up.

2.2.2 Biofumigant preparation

The ten crops to be evaluated are commercially sold as biofumigant/green manure/forage crops in New Zealand (Table 2.2). Seeds of the respective plants were placed on moistened filter papers in Petri dishes, and incubated for 72 hours at 20°C (12 hours light/12 hours dark cycle). Germinated seeds of each plant type were transplanted into five 3-L capacity plastic pots (five seeds per pot) each containing 2.2 kg of potting mix [220 litres of potting mixture contained 132 L of peat, 44 L of bulk pumice, 44 L of sterilised pumice, 660 g of Osmocote® N : P : K (16 : 3.5 : 10), 880 g of dolomite lime and 220 g of Hydraflo®], and grown under glasshouse conditions (Figure 2.1 A). After one week, one seedling of each cultivar was removed to retain four uniform and healthy plants in each pot. The plants were watered daily until harvested for experiments. The biofumigant plants were planted at three different times to provide material for the different experiments (Table 2.3). Temperature, humidity and light intensity conditions in the glasshouse were recorded using a HOBO data logger (Model U12-012, Onset Computer Corporation, USA), and these are summarised in Table 2.3.

Table 2.2 Details of the biofumigant species used in the experiments.

Species	Common name	Cultivar	Use
Brassica juncea	Oriental/brown mustard	'Caliente 199'	Biofumigation
Brassica juncea	Brown mustard	Unknown	Unknown
Eruca sativa	Arugula/Rocket salad	'Nemat'	Biofumigation
Sinapis alba (Brassica alba)	White mustard	'SKU 4295'	Green manure
Raphanus sativus	Forage radish	'Lunch'	Forage
Raphanus sativus	Fodder radish	Unknown	Forage
Brassica oleracea var. *acephala*	Kale	'Corka'	Forage
Brassica napus	Rapeseed	Unknown	Oilseed
Brassica napus ssp. *oleifera biennis*	Forage rape	'Titan'	Forage
Brassica campestris ssp. *Rapifera*	Leafy turnip	'Pasja II'	Forage

Seven of the plant types, including oriental mustard ('Caliente', 43 days after transplanting (DAT)), brown mustard (39 DAT), rocket salad ('Nemat', 60 DAT), white mustard ('SKU 4295', 37 DAT), forage radish ('Lunch', 56 DAT), fodder radish (unknown, 67 DAT) and rapeseed (unknown, 42 DAT) were harvested at the mid-anthesis growth stage, corresponding to growth stage 3.1-3.2 (Berkenkamp, 1973). The three other plant types [kale ('Corka', 68 DAT), forage rape ('Titan', 68 DAT) and leafy turnip ('Pasja II', 67 DAT)] were harvested at the vegetative growth stage. The shoots of each plant type were harvested and stored at -20°C. The roots were washed under running tap water to remove the potting mix, blotted dry on tissue papers and stored at -20°C. Prior to setting up experiments, the shoots and roots were cut into small pieces (1-2 cm) with secateurs and mixed thoroughly (Figure 2.1 B).

A **B**

Figure 2.1 Biofumigant plants grown in glasshouse (A), and frozen chopped *Brassica* tissues used for experiments (B).

Table 2.3 Temperature (°C), relative humidity (%), and light intensity (lux) (mean (minimum-maximum)) in the glasshouse during the growth of the three biofumigant plantings used in the different experiments.

Planting	Date	Temperature (°C)	Relative humidity (%)	Light intensity (lux)	Used for experiments described in
1st	26/11/2015 - 02/02/2016	21.5 (14.4-45.5)	65.4 (12.9-98.9)	6,790 (4.3-32,280)	Sections 2.2.4, 2.2.5, 2.2.6
2nd	04/02/2016 - 24/5/2016	20.6 (13.5-42.5)	64.1 (16.4-95.3)	5,543 (4.3-32,280)	Section 2.2.7
3rd	15/4/2016 - 01/7/2016	18.3 (12.5-35.4)	62.9 (18.4-86.1)	3,857 (4.3-32,280)	Sections 2.2.8, 2.2.9

2.2.3 Preliminary experiment to determine effects of two isothiocynate compounds on mycelium growth of *Rhizoctonia solani*

This experiment was conducted to evaluate the effects of different concentrations of pure isothiocyanates (ITCs/ITC) on mycelium growth of the *R. solani* isolate (LUPP2522, AG2-1), and to determine the ITC concentrations that gave 50% and 100% inhibition of mycelium growth were used for subsequent studies. Two pure ITCs, allyl (2-propenyl) ITC and 2-phenylethyl ITC (Sigma-Aldrich) were used in the experiment. The ITCs were dissolved in methanol to obtain different concentrations of allyl ITC (0, 0.04, 0.08, 0.12, 0.16, 0.2, 0.4, 0.6, 0.8 or 1 M) or 2-phenylethyl ITC (0, 0.06, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.5 or 2 M) (Table 2.4). A preliminary experiment with lower concentrations showed that 0.004-0.024 M of allyl ITC and 0.004-0.04 M of 2-phenylethyl ITC had no effects on growth of *R. solani* LUPP2522. A second preliminary experiment showed that 1-10 μL of methanol added to Petri plate lids did not inhibit the mycelium growth of this isolate.

Deep Petri plates (10 cm diameter, 2.5 cm depth), each containing 15 mL PDA, were each inoculated centrally with a disc (7 mm diameter) cut from the actively growing margin of a *R. solani* LUPP2522 colony in agar culture. Ten μL of each of allyl ITC or 2-phenylethyl ITC concentration (Sarwar *et al.*, 1998) were pipetted into the inverted lid of each inoculated Petri dish to achieve the final concentration required (Table 2.4). Each plate was immediately sealed with triple layers of white plastic film, and a layer of green masking tape. Untreated controls were included which consisted of plates inoculated with *R. solani* colonised agar plugs sealed to lids containing 10 μL of methanol. The plates were incubated at 25°C in the dark. There were three replicates for each ITC concentration, with the experiment laid out in a randomised complete block design. Growth of each *R. solani* colony growth was measured in two perpendicular directions at 72 hours after inoculation (the time when mycelium in the methanol controls reached the plate margins). The inhibitory efficiency (%) of the different ITC solutions was calculated using the formula (Kurt *et al.*, 2011):

IE (%) = $[(D_c - D_t)/D_c] \times 100$

Where:IE is the inhibitory efficiency

D_c is the colony diameter in the untreated control

D_t is the colony diameter for the different treatments

Table 2.4 Serial concentrations of allyl isothiocyanate and 2-phenylethyl isothiocyanate used.

Allyl isothiocyanate			2-phenylethyl isothiocyanate		
Stock (M)	Volume (µL/Petri dish)	Final concentration (µM/Petri dish)	Stock (M)	Volume/dish (µL/Petri dish)	Final concentration (µM/Petri dish)
0	10 (Methanol)	0	0	10 (Methanol)	0
0.04	10	0.4	0.06	10	0.6
0.08	10	0.8	0.10	10	1.0
0.12	10	1.2	0.20	10	2.0
0.16	10	1.6	0.40	10	4.0
0.20	10	2.0	0.60	10	6.0
0.40	10	4.0	0.80	10	8.0
0.60	10	6.0	1.00	10	10.0
0.80	10	8.0	1.50	10	15.0
1.00	10	10.0	2.00	10	20.0

2.2.4 Biofumigation effects of biofumigant plants on *Rhizoctonia solani* propagules

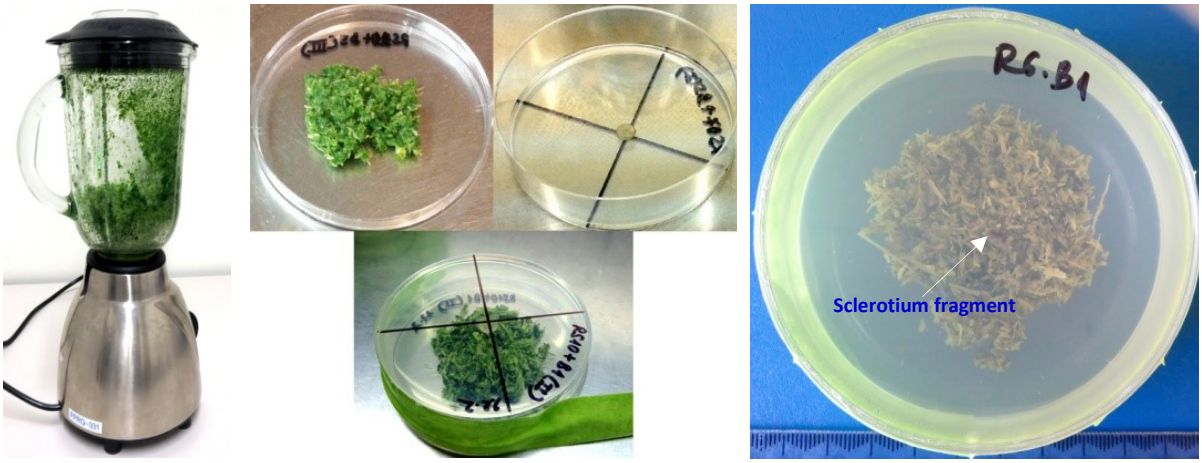
2.2.4.1 Mycelium growth from agar plugs

This experiment tested the biofumigation potential of ten biofumigant plant types (Table 2.2) to suppress mycelium growth of ten different *R. solani* isolates (Table 2.1). The frozen-chopped tissues (Section 2.2.2) from each of the biofumigant plant types were ground in a food blender for 30 seconds (Figure 2.2 A). Five grams of the finely macerated tissues were then placed into the inverted lid of a Petri dish containing PDA and centrally inoculated with a 7 mm-diameter fungal plug to ensure no contact between the plant tissue and the fungus. The plates were then immediately triple-sealed with plastic film and a layer of green masking tape (Figure 2.2 B). The concentrations of allyl (2-propenyl) ITC determined in Section 2.2.3 to give 50% (Allyl ITC_{50}) or 100% (Allyl ITC_{100}) suppression of mycelium growth of *R. solani* LUPP2522 were used as positive control treatments. Untreated controls without plant material or ITC were also included. The plates were incubated at 25°C in the dark for 72 hours. Four replicates were set up for each *R. solani* isolate, plant type p and

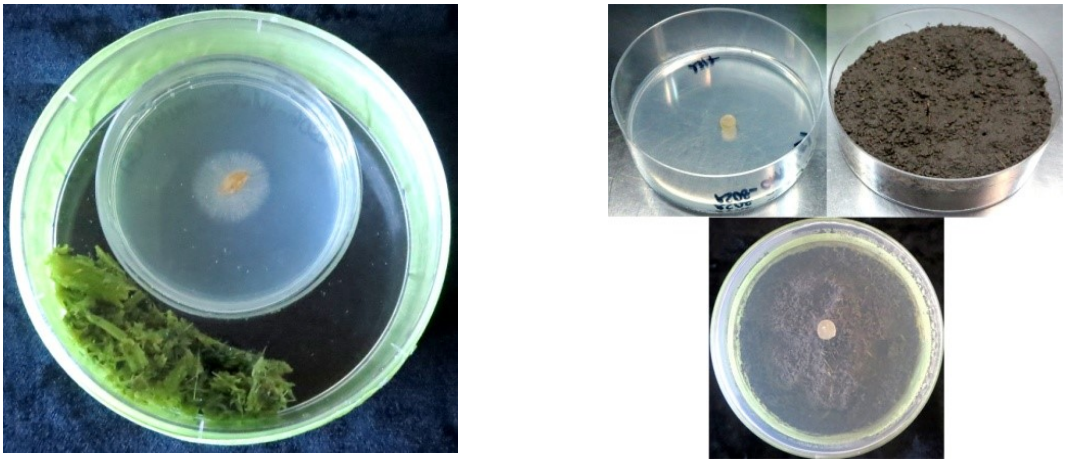
isothiocyanate treatment combination, and for the negative controls, with the experiment laid out in a randomised complete block design. Measurements and calculation of inhibitory efficiency were conducted as described for Section 2.2.3.

2.2.4.2 Sclerotium viability and subsequent mycelium growth from sclerotia

An experiment was designed to determine the inhibitory effects of volatile biocidal compounds from macerated biofumigant tissues on germination and viability of *R. solani* sclerotia. The experiment was carried out in a similar way to that described in Section 2.2.3, with ten biofumigant plant types (Table 2.2) and ten *R. solani* isolates (Table 2.1). Sclerotium fragments (as described in Section 2.2.1.2) were used to inoculate the Petri plates, with one sclerotium fragment placed in each Petri dish containing 15 mL of PDA. The concentration of allyl (2-propenyl) ITC determined in Section 2.2.3 to give 100% suppression (Allyl ITC_{100}) of *R. solani* LUPP2522 mycelium growth was used as a positive control treatment. The plates were triple-sealed with plastic film and a layer of green masking tape (Figure 2.2 C), and incubated at 25°C in dark for 72 hours. Four replicate plates were set up for each isolate and treatment combination, and the plates were arranged in a randomised complete block design. Germination of sclerotium fragments was assessed at 72 hours by determining the presence of hyphae growing from the sclerotia using a stereo microscope (C-DSS230, Nikon Corporation, Tokyo, Japan). A sclerotium was recorded as germinated where the length of the hyphae growing from the sclerotium was equal to or greater than the diameter of the sclerotium (Ritchie *et al.*, 2013). The mycelium growth of the colony originating from the sclerotium was measured to calculate inhibitory efficiency, as described for Section 2.2.3.



A B C



D E

Figure 2.2 Frozen chopped biofumigant tissues macerated in a food blender (A); macerated biofumigant tissues placed in the lid of a 2.5 cm deep Petri dish and sealed, with the base of the Petri dish containing PDA inoculated with a *Rhizoctonia solani* agar plug (B); 2.5 cm deep Petri dish containing macerated biofumigant tissues in the lid and a sclerotium fragment in the base (C); a small Petri plate inoculated with a *R. solani* colonised barley grain placed in a 2.5 cm deep Petri dish containing macerated biofumigant tissues (D); lid of a 2.5 cm deep Petri dish containing soil incorporated with macerated biofumigant tissues and the base of the dish containing PDA inoculated with a *R. solani* agar plug (E).

2.2.4.3 Subsequent mycelium growth from *Rhizoctonia solani* colonised barley grains

An experiment was carried out with ten biofumigant plant types (Table 2.2) and ten *R. solani* isolates (Table 2.1). A *R. solani* colonised barley grain was centrally placed in the lid of a small Petri plate (6 cm diameter, 1.5 cm depth) containing 7 mL of PDA. The opened lid of the plate was placed into a deep Petri plate (10 cm diameter, 2.5 cm depth) containing 5 g of macerated biofumigant plant tissue. The deep plate was immediately sealed with triple layers of plastic film and a layer of green masking tape (Figure 2.2 D). The concentration of allyl (2-propenyl) ITC, determined in Section 2.2.3 to give 100% suppression (Allyl ITC_{100}) of *R. solani* LUPP2522 mycelium growth was used as a positive control treatment. Mycelium growth from the barley grains was assessed at 72 hours using a stereo microscope. Mycelium growth of the colony originating from each barley grain was measured to calculate inhibitory efficiency, as described in Section 2.2.3.

2.2.5 Biofumigation effects of different amounts of biofumigant plant tissues on *Rhizoctonia solani* propagules

Based on the results of Section 2.2.4, the five most effective biofumigant plant types, including 'Caliente' mustard, brown mustard, 'Nemat' rocket salad, kale and leafy turnip, were selected for this experiment. The experiment examined the responses of three propagules (mycelia, sclerotia or colonised barley grains) of representative *R. solani* isolates to different amounts of macerated biofumigant tissues. *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) were selected to represent the range of responses observed to biofumigant plant types and ITCs. The experiments were set up as described in Sections 2.2.4.1, 2.2.4.2 and 2.2.4.3 (above), but with three different amounts (1 g, 5 g and 10 g) of macerated biofumigant tissues for the selected plant types placed in the inverted Petri dish lids. The effects of the biofumigant tissues on mycelium growth, sclerotium viability and subsequent mycelium growth from sclerotia, and *R. solani* viability and subsequent mycelium growth from colonised barley grains, were determined as described in Sections 2.2.4.1, 2.2.4.2 and 2.2.4.3.

2.2.6 Biofumigation effects of biofumigant tissue types on *Rhizoctonia solani* propagules

This experiment evaluated the effects of different types of macerated tissues on *R. solani* propagules. 'Caliente' mustard, brown mustard, 'Nemat' rocket salad, kale and leafy turnip plants were prepared as described above (Section 2.2.2). Three macerated tissue treatments were tested, including roots, shoots and whole plants (shoots + roots, 1:1 w:w). The effects of the three tissue treatments were tested on the three propagule types (mycelia, sclerotia or colonised barley grains) of two *R. solani* isolates [LUPP2519 (AG3-PT) and LUPP2522 (AG2-1)]. The experiment was set up as described in Sections 2.2.4.1, 2.2.4.2 and 2.2.4.3, with 5 g of macerated root, shoot or root +shoot tissues.

2.2.7 Biofumigation effects of different flowering times of biofumigant plants on *Rhizoctonia solani* propagules

This experiment evaluated the effects of different flowering times of the biofumigant plant types on *R. solani* propagules. Whole plants, including roots and shoots, were harvested at the growth stages of first flower emergence, or at 50% or 100% anthesis. The experiment was set up with the three propagule types (mycelia, sclerotia and colonised barley grains) of two *R. solani* isolates [LUPP2519 (AG3-PT) and LUPP2522 (AG2-1)], and the three most effective biofumigant plants ('Caliente' mustard, brown mustard, 'Nemat' rocket salad) selected from results obtained in the Section 2.2.4 experiment. The experiment was set up as described in Sections 2.2.4.1, 2.2.4.2 and 2.2.4.3, with 5 g of macerated plant tissue.

2.2.8 Biofumigation effects on *Rhizoctonia solani* propagules of different amounts of biofumigant plant tissues amended in soil

2.2.8.1 Field soil preparation

Field soil was collected from Lincoln University site (S 43°38'54.0384", E 172°27'43.0308"), air-dried, sieved through 2 mm mesh, and stored at 4°C until used. The soil was the loam type (12.5% clay, 42.4% silt, 45.1% sand), of 63.3% water holding capacity (WHC), contained 7.3% organic matter, and pH_{H2O} 5.9. The methods used are described in Appendices B.2.1-B.2.4.

2.2.8.2 Experimental design

The experiment was set up and assessed in the same way as described for Section 2.2.5, except that the macerated biofumigant tissues was incorporated into soil at 1, 5 or 10% (w:w). The three most effective biofumigant plant types, including 'Caliente' mustard, brown mustard and 'Nemat' rocket salad, were selected for this experiment against three propagule types (mycelia, sclerotia and colonised barley grains) of two *R. solani* isolates (LUPP2519 (AG3-PT) and LUPP2522 (AG2-1)).

The air-dried soil (100 g) amended with the macerated plant tissue was placed in the base of a large (9 cm) deep Petri dish, and then moistened with water to obtain 70% soil WHC. The plate base was then sealed to another base of a large Petri dish containing 15 mL PDA inoculated with a 7 mm *R. solani* mycelium plug as described in Section 2.2.4.1 (Figure 2.2 E). The experiment was repeated using sclerotia as described in Section 2.2.4.2, and *R. solani* colonised barley grains as described in Section 2.2.4.3. Unamended soil was set up as the experimental control. The sealed Petri dishes were incubated at 25°C to 7 days, after which the mycelium growth was measured as described in Section 2.2.4.1.

2.2.9 Conversion and survival of *Rhizoctonia solani* propagules in soil amended with different amount of biofumigant plant tissues

This experiment examined the response of three different *R. solani* propagules to different amounts of macerated *Brassica* tissues incorporated into soil, and whether this stimulates the conversion of mycelium inoculum into sclerotia or *vice versa*.

2.2.9.1 Experimental design

Three types of propagules (mycelia, sclerotia or infected barley grains) of one *R. solani* AG3-PT isolate (LUPP2519) and one AG2-1 isolate (LUPP2522) were prepared as described in Section 2.2.1.2, and used for the experiment. Each propagule form of the pathogen was placed into a nylon fabric mesh bag (5 x 5 cm) (Schweizer Seidengaze-fabrik AG, Thanl, Switzerland) with a pore size of 20 μm. The bags contained 10 mycelium agar plugs (7 mm diameter), 10 sclerotia, or 10 *R. solani* colonised barley grains. The bags were then heat-sealed.

'Caliente' mustard, brown mustard and 'Nemat' rocket salad plants were grown as described in Section 2.2.2 until the mid-flowering growth stage. The whole plants were then harvested and tissues (roots and shoots) were prepared as described in Section 2.2.2. Three different amounts of tissues were incorporated into the air-dried soil (Section 2.2.8.1).

The macerated biofumigant tissues were thoroughly incorporated into the air-dried soil at 3 concentrations, 1, 5 or 10% (w:w), and mixed by hand. One kg of the biofumigant incorporated soil was used for each 2 L polypropylene square plastic box (Stowers, New Zealand). Half of the biofumigant incorporated soil (0.5 kg) was weighed and put into the box, the mesh bags containing the different *R. solani* propagules were then randomly placed on the soil surface, and the remaining biofumigant incorporated soil (0.5 kg) was placed in the boxes to cover the bags. Each box contained one bag for each propagule type of each isolate, resulting in six bags in total. The appropriate amount of tap water was added to each box to achieve 70% soil WHC. The box lids were closed tightly for two weeks, and then loosely thereafter. The soil water content was maintained at 70% WHC based on weighing of the boxes at weekly intervals, and adding water if required. The experiment was set up in a completely randomised block design with three replicates per isolate, propagule and biofumigant treatment combination, in a 22°C growth room. The mesh bags were harvested after 28 days of incubation to evaluate survival and subsequent mycelium growth of the propagules and propagule conversion rates.

2.2.9.2 Assessments

The collected mesh bags were thoroughly washed under running tap water, and were then soaked three times in sterile water. The bags were then put on sterile tissue paper until dry, and stored at

4°C before being assessed within 14 days. The content of each bag was emptied into a sterile Petri dish. There was no reduction in the total numbers of agar plugs, sclerotia or colonised barley grains in nylon mesh bags after 28 days incubation. There were sclerotia present on the retrieved agar plugs, and therefore the sclerotium conversion proportions per mesh bag were measured. The retrieved agar plugs were placed onto water agar to assess viability. For the sclerotia bags, there were hyphae around the retrieved sclerotia inside the bags. For the colonised barley grains bags, no sclerotia were observed, but there were mycelium masses outside the bags at harvest, and the retrieved colonised barley grains were flattened.

To determine the conversion of mycelia on the agar plugs into sclerotia, the presence of sclerotia per agar plug was recorded. For the sclerotia and barley grain bags, the number of sclerotia and barley grains recovered from the bags were counted.

The retrieved agar plugs, sclerotia or barley grains were placed onto water agar (1.5%) plates amended with chloramphenicol (100 μg/mL) and benomyl (1 μg/mL) (Paulitz and Schroeder, 2005). After 3 days incubation at 25°C, the subsequent mycelium growth of *R. solani* from the agar plugs, sclerotia or colonised barley grains was observed under a stereo microscope and compound microscope (CX41RF, Olympus Corporation, Tokyo, Japan). The percentage of agar plugs having subsequent mycelium growth per mesh bag, and the proportions were calculated of sclerotium germination or colonised barley grains having subsequent mycelium growth.

2.2.10 Data analyses

The raw data from each experiment were firstly validated for the assumption normal distribution using GenStat software (Version 18.1.0.17005; VSN International Ltd, Hemel Hempstead, United Kingdom). The histogram of residues, fitted-value plot, normal plot and half-normal plot were examined for normal distribution. If the data were normally distributed, they were directly used for analysis of variance (ANOVA), otherwise they were transformed prior to analysis to fulfil the assumption of normality.

For data from Section 2.2.3, the mean inhibitory efficiencies of each ITC concentration were calculated and the mean differences were compared between concentrations using one-way ANOVA with means (different concentrations) separated using Tukey's Honestly Significant Difference (HSD) test at $P = 0.05$. The effective dose (ED) which inhibited mycelium growth of *R. solani* LUPP2522 by 50% (ED_{50}) and 100% (ED_{100}) at 95% confidence limits were estimated based on the slope and the intercept of the regression equations (Smith and Kirkegaard, 2002) using the simple linear regression model from the GenStat software.

For data from Section 2.2.4, the mean inhibitory efficiency (%) of mycelium growth from agar plugs and subsequent mycelium growth from sclerotia or colonised barley grains, the percentage

sclerotium germination, and the proportions of mycelium growth from colonised barley grains were used to compare differences using two-way ANOVA, with means (biofumigant plant type, *R. solani* isolate, and interaction of biofumigant type x *R. solani* isolate) separated using Tukey's HSD test at *P* = 0.05.

For the data from Sections 2.2.5, 2.2.6, 2.2.7 and 2.2.8, the mean inhibitory efficiency (%) for mycelium growth from agar plugs and subsequent mycelium growth from sclerotia or colonised barley grains, the percentages sclerotium germination, and the proportions of mycelium growth from colonised barley grains were used to compare differences using general ANOVA with means (biofumigant plant type, *R. solani* isolate, biofumigant amount/tissue type/flowering time, and interactions of biofumigant type x *R. solani* isolate, biofumigant type x biofumigant amount/tissue type/flowering time, *R. solani* isolate x biofumigant amount/tissue type/flowering time, biofumigant type x *R. solani* isolate x biofumigant amount/tissue type/flowering time) separated using Tukey's HSD test at *P* = 0.05.

For the data from Section 2.2.9, percentages of sclerotia presence per mesh bag containing agar plugs, and proportions of subsequent mycelium growth from agar plugs, sclerotia or colonised barley grains were used to compare differences, using general ANOVA with means (biofumigant plant type, *R. solani* isolate, biofumigant amount, and interaction of biofumigant type x *R. solani* isolate, biofumigant type x biofumigant amount, *R. solani* isolate x biofumigant amount, biofumigant type x *R. solani* isolate x biofumigant amount) separated using Tukey's HSD test at *P* = 0.05.

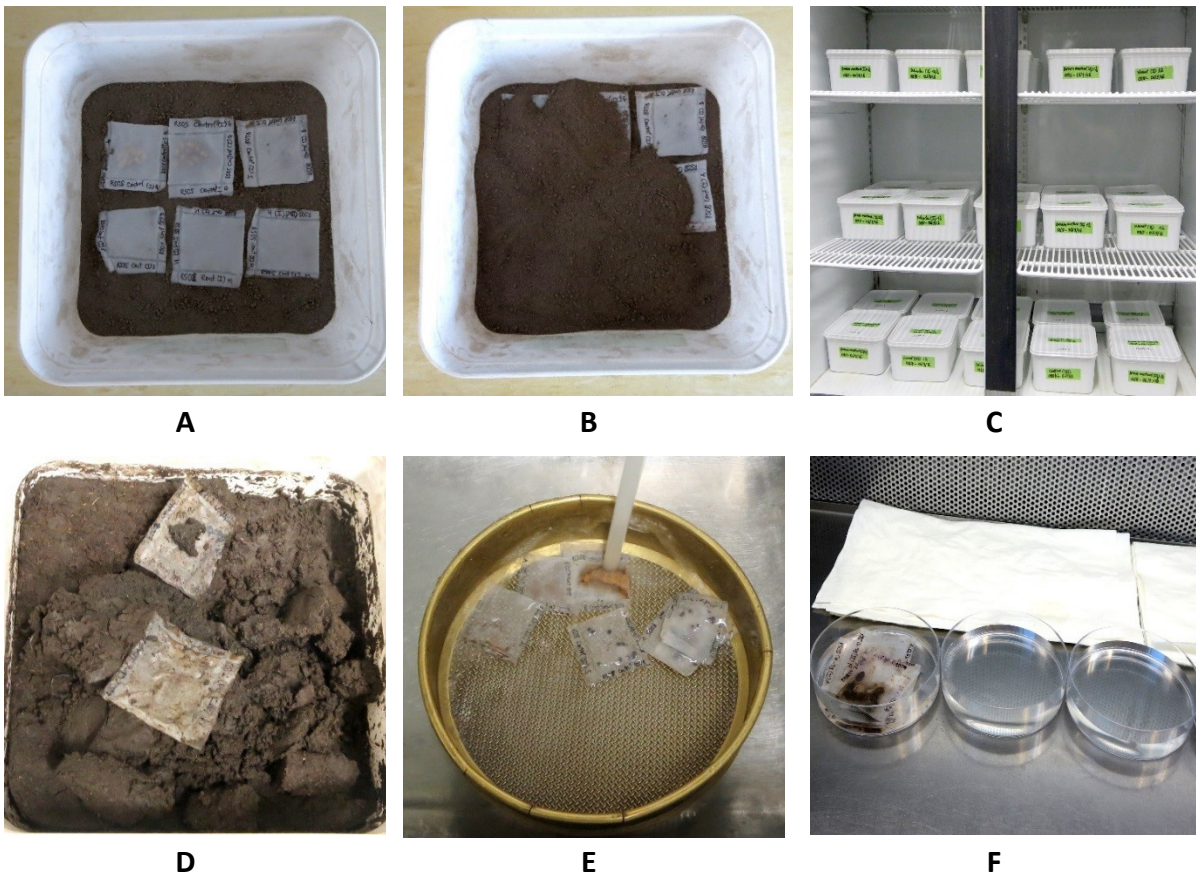


Figure 2.3 Nylon mesh bags containing *Rhizoctonia solani* propagules randomly laid out on the surface of half of the biofumigant amended soil in a plastic box (A); the rest of biofumigant amended soil placed on top of the bags (B); the boxes arranged in a randomised block design in a 22°C growth room (C); the nylon bags harvested after 28 days incubation (D), and washed under running tap water (E) and sterile water (F).

2.3 Results

2.3.1 Suppression of *Rhizoctonia solani* mycelium growth by two isothiocyanate compounds

Allyl ITC (AITC) and 2-phenylethyl ITC (2-PEITC), in the volatile phase, had different effects on the mycelium growth of *R. solani* LUPP2522 (Tables 2.5 and 2.6). The inhibitory effect of AITC on mycelium growth was proportional to increasing concentration levels (Table 2.5, Appendix C.2.1). The relationship between the inhibitory efficiency (IE) and AITC concentration (CAITC) was described by a linear regression equation IE (%) = 10.49 x CAITC + 0.58 (R^2 = 0.98, $P < 0.001$) (Figure 2.4, Appendices C.2.2 and C.2.3). The concentrations of AITC which inhibited 50% or 100% of mycelium growth ($AITC_{50}$ and $AITC_{100}$) were calculated as, respectively, 4.71 and 9.48 µM.

Table 2.5 Mean inhibitory efficiency (%) in relation to unamended controls for different concentrations of allyl isothiocyanate against mycelium growth of *Rhizoctonia solani* LUPP2522 after 3 days growth on PDA.

Concentration (µM)	Inhibitory efficiency (%)
0	0 (0 f)
0.4	1.1 (0.11 e)
0.8	7.5 (0.27 d)
1.2	11.3 (0.34 d)
1.6	18.8 (0.43 c)
2.0	21.9 (0.47 c)
4.0	51.6 (0.72 b)
6.0	60.7 (0.78 b)
8.0	89.1 (0.94 a)
10.0	100 (1 a)
P	<0.001
Transformed MSD (*P*=0.05)	0.08

(*) Means within column followed by the same letter are not significantly different according to Tukey's HSD test at *P* = 0.05 using a minimal significant difference (MSD) level. Data were sqrt(X/100) transformed (in parentheses) prior to statistical analysis, and are presented as backtransformed means.

The volatile toxic effect of 2-PEITC on mycelium growth was low at all the tested concentrations (Table 2.6, Appendix C.2.4), so it was not appropriate to construct a regression equation for inhibitory efficiency (IE) of 2-PEITC.

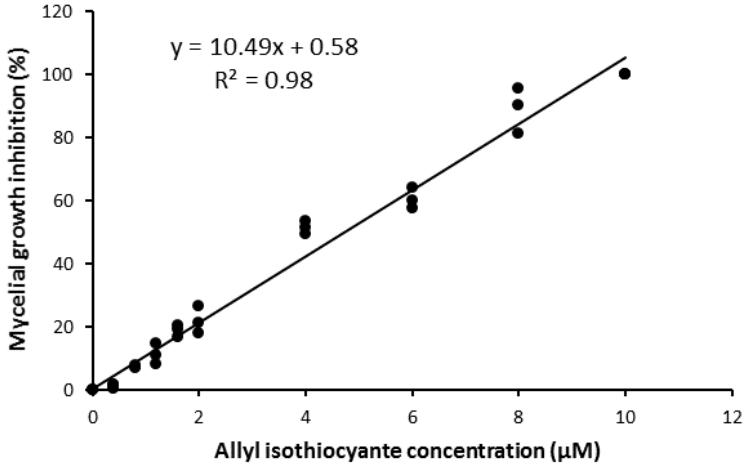


Figure 2.4 The linear relationship between allyl isothiocyanate concentrations and mycelium growth inhibition (%) for *Rhizoctonia solani* LUPP2522.

Table 2.6 Mean inhibitory efficiency (%) in relation to the unamended control for different concentrations of 2-phenylethyl isothiocyanate against mycelium growth of *Rhizoctonia solani* LUPP2522 after 3 days growth on PDA.

Concentration (µM)	Inhibitory efficiency (%)
0	0 (0 d)
0.6	8.0 (0.28 c)
1.0	14.9 (0.39 b)
2.0	13.4 (0.37 b)
4.0	23.5 (0.48 a)
6.0	27.9 (0.53 a)
8.0	28.9 (0.54 a)
10.0	23.7 (0.49 a)
15.0	28.6 (0.54 a)
20.0	26.8 (0.52 b)
P	<0.001
Transformed MSD (*P*=0.05)	0.07

(*) Means within column followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05 using a minimal significant difference (MSD) level. Data were sqrt(X/100) transformed (in parentheses) prior to statistical analysis, and are presented as backtransformed means.

2.3.2 Biofumigation effects of biofumigant plant types on *Rhizoctonia solani* propagules

2.3.2.1 Mycelium growth from agar plugs

Statistical analyses of these data are presented in Appendix C.2.5 and the results are summarised in Table 2.7. There was a significant interaction (P<0.001) between treatment and *R. solani* isolate on the inhibition of mycelium growth. Volatiles from the macerated tissue of 'Caliente' mustard completely inhibited the mycelium growth of all isolates, while volatiles from brown mustard and 'Nemat' arugula macerated tissue completely inhibited the mycelium growth of eight isolates, but suppressed mycelium growth of isolates LUPP2522 by 96.7% for brown mustard and 51.5% for 'Nemat' arugula, and LUPP2523 by 79.7% for brown mustard and 47.8% for 'Nemat' arugula. Volatiles from the macerated tissues of white mustard and forage rape had the least inhibitory effects, inhibiting the mycelium growth of isolate LUPP2523 by the most (19.8 and 14.4% inhibition, respectively). The positive control, AITC50, completely suppressed mycelium growth of isolates LUPP2521 and LUPP2524 and inhibited the mycelium growth of all the other isolates by 50.8-95.6%. AIT100 completely suppressed mycelium growth of seven isolates, with mycelium growth of isolate LUPP2516 (88.3% inhibition) being the least sensitive to AITC100.

Across all *R. solani* isolates, there was a significant effect (P<0.001) of biofumigant treatment on the mycelium growth inhibition (Figure 2.5, Appendix C.2.5). Volatiles released from the macerated tissue of 'Caliente' mustard gave the greatest inhibition of growth (100% inhibition), which was significantly different to all other treatments apart from brown mustard (99.7% inhibition). Mycelium inhibition by AITC100 was not significantly different from that with volatiles of 'Caliente' and brown mustards but significantly greater than from all the other biofumigant treatments. 'Nemat' arugula, 'Corka' kale and leafy turnip were the next most effective biofumigants types at suppressing mycelium growth (86.4-89.9% inhibition), and were not significantly different from each other. Volatiles from white mustard (44.6 % inhibition) and forage rape (38.8% inhibition) had the least effects on the mycelium growth.

Across all treatments, there was a significant effect (P<0.001, Appendix C.2.5) of *R. solani* isolate on mycelium growth inhibition (Figure 2.6). Isolate LUPP2523 was the least sensitive to inhibition by volatiles from biofumigant treatments (58.4% inhibition), compared with all other isolates. This was followed by isolate LUPP2522 (65.9% inhibition) which was significantly different from the other isolates. In contrast, isolate LUPP2524 was the most sensitive to volatiles from biofumigants and pure AITC (86.3% inhibition).

Table 2.7 Mean mycelium growth inhibition (%) from agar plugs, in relation to the unamended controls, of ten *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissue of ten biofumigant plant types (5 g/Petri dish) and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) inhibition.

Treatment	Mycelium growth inhibition (%) [1]									
	LUPP2515	LUPP2516	LUPP2517	LUPP2518	LUPP2519	LUPP2520	LUPP2521	LUPP2522	LUPP2523	LUPP2524
AITC50	95.6	67.6	70.7	86.2	73.4	59.3	100	50.8	79.0	100
AITC100	100	88.3	94.8	100	100	95.3	100	100	100	100
'Caliente' mustard	100	100	100	100	100	100	100	100	100	100
Brown mustard	100	100	100	100	100	100	100	96.7	79.7	100
'Nemat' arugula	100	100	100	100	100	100	100	51.5	47.8	100
'Corka' kale	95.0	90.8	92.1	88.8	93.9	91.0	92.7	76.0	56.8	96.7
'Pasja II' leafy turnip	92.3	89.4	92.1	91.1	91.7	94.1	91.4	78.8	50.2	93.0
'Lunch' radish	79.4	80.6	80.7	80.1	76.6	80.7	72.1	65.7	60.2	89.7
Fodder radish	61.6	58.3	60.9	61.9	54.6	61.1	57.6	54.2	43.4	58.0
Rapeseed	53.3	61.2	62.8	62.1	61.7	57.1	34.8	49.8	50.0	71.3
White mustard	41.1	52.1	45.6	43.4	51.3	44.4	41.9	31.3	19.8	75.5
Forage rape	35.8	46.3	39.7	33.8	51.4	38.7	39.9	36.2	14.4	51.6
LSD (*P*=0.05) of Biofumigant type x *Rhizoctonia solani* isolate = 8.5										

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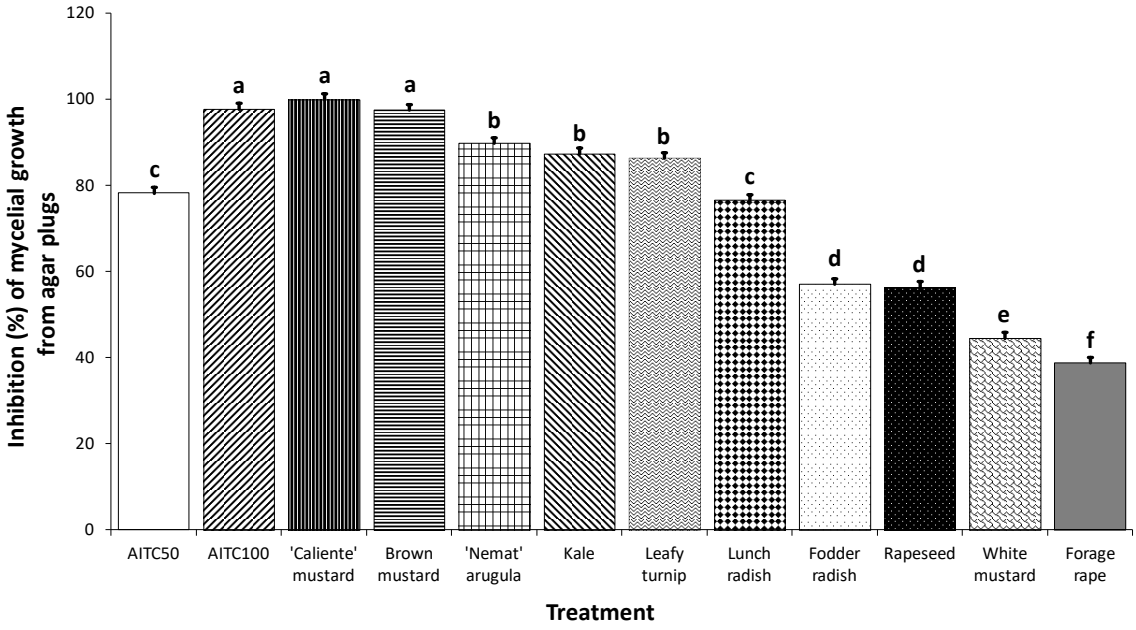


Figure 2.5 Mean mycelium growth inhibition (%) from agar plugs, in relation to the unamended controls, of ten *Rhizoctonia solani* isolates (data averaged across ten isolates) after 3 days exposure to volatiles from macerated tissue of ten biofumigant plant types (5 g/Petri dish) and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) inhibition. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.7.

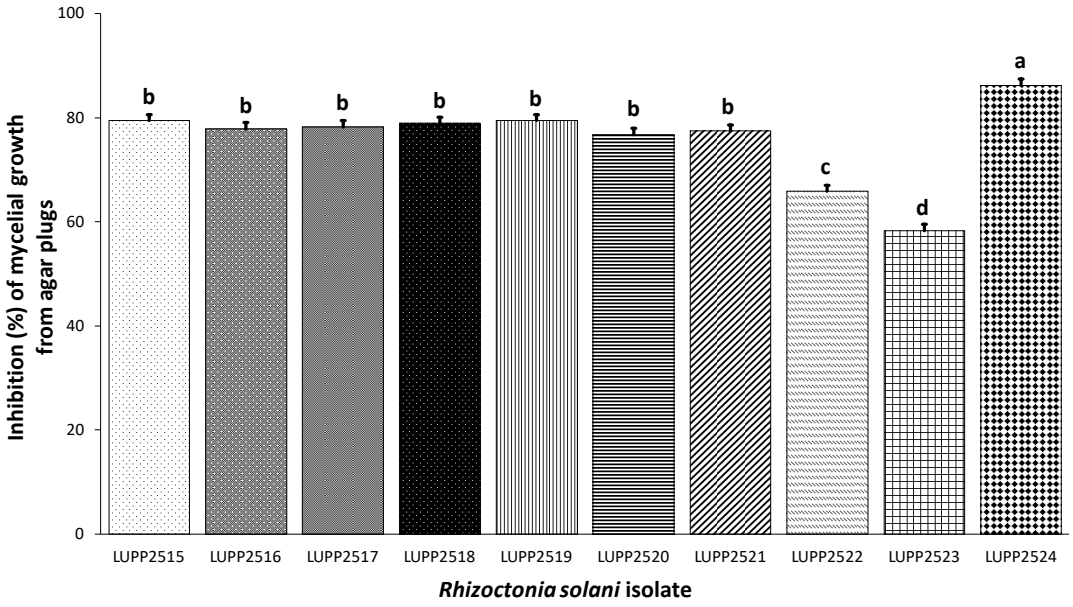


Figure 2.6 Mean mycelium growth inhibition (%) from agar plugs, in relation to the unamended controls, of ten *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissue of biofumigant plant types (5 g/Petri dish) and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) inhibition (data averaged across ten plant types and allyl ITC concentrations). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.4.

2.3.2.2 Sclerotia

Statistical analyses of sclerotium germination data are shown in Appendix C.2.6, and the results are summarised in Appendix C.2.32. There was a significant interaction effect (P<0.001) between isolate and treatment on sclerotium germination. AITC100 significantly reduced sclerotium germination of *R. solani* isolates LUPP2523 (75%) and LUPP2524 (50%), but did not have any effect on the germination of sclerotia of any other isolate (all being 100%). No other biofumigant type treatments reduced the sclerotium germination of any of the isolates. There was a significant effect (P<0.001) of treatment on sclerotium germination. AITC100 treatment reduced sclerotium germination (by 7.5 %) compared with all the other treatments, with none of the biofumigant types suppressing sclerotium germination. There was significant effect (P=0.043) of isolate on sclerotium germination for the different *R. solani* isolates. Sclerotium germination of *R. solani* LUPP2524 was significantly reduced 4.8% in comparison with other isolates (0% reduction), except for LUPP2523 (2.3% reduction).

The effects of the biofumigant treatments on subsequent growth of mycelium from sclerotia were also assessed, and these are presented in Appendix D.2.1.

2.3.2.3 Colonised barley grains

Statistical analyses of the effects on the mycelium growth from barley grains colonised by different *R. solani* isolates after exposure to different biofumigant treatments is presented in Appendix C.2.8, and the results are summarised in Appendix C.2.33. There was a significant interaction effect (P=0.003) between isolate and treatment on the percentage of colonised barley grains from which mycelia grew onto agar. AITC100 significantly reduced the percentage of colonised barley grains from which mycelium grew onto the agar for *R. solani* isolates LUPP2515 (45.7% grains colonised), LUPP2516 (71.9%), LUPP2521 (45.7%) and LUPP2523 (71.9%), but did not affect any of the other isolates. No other biofumigant types had any effects on the percentage of colonised barley grains positive for mycelium growth for any of the *R. solani* isolates. There was a significant effect (P<0.001) of treatment on the percentage of colonised barley grains positive for mycelium growth. AITC100 treatment reduced the percentage of colonised barley grains positive for mycelium growth (17.2% germination reduction) compared with all other treatments, with none of the biofumigant types suppressing the percentage of colonised barley grains positive for mycelium growth. There was no significant effect (P=0.136) of isolate on the percentage of colonised barley grains positive for mycelium growth for the different *R. solani* isolates (95.2-100% colonised).

The effects of the biofumigant treatments on subsequent growth of mycelium from colonised barley grains were also assessed, and these are presented in Appendix D.2.2.

2.3.3 Biofumigation effects of different amounts of biofumigant plant types on *Rhizoctonia solani* propagules

2.3.3.1 Mycelium growth from agar plugs

Statistical analyses of data are presented in Appendix C.2.10, and the mean inhibition of mycelium growth from the agar plugs with the different biofumigant treatments are summarised in Appendix C.2.34. There was a significant interaction effect (P<0.001) between treatment, amount and isolate on the mycelium inhibition (Figure 2.7, Appendix C.2.34). 'Caliente' mustard (at 5 g and 10 g) and AITC100 completely inhibited the mycelium growth of both *R. solani* isolates, being significantly different to 'Caliente', brown mustards and 'Nemat' arugula at lower amounts (1 g), and 'Corka' kale and 'Pasja II' leafy turnip at all concentrations. For *R. solani* isolate LUPP2519 there was no significant difference between the mycelium inhibition caused by brown mustard and 'Nemat' arugula (at 5 or 10 g) compared with 'Caliente' mustard (at 5 g or 10 g) and AITC100 (mean inhibition = 100%). However, for *R. solani* isolate LU2522 the mycelium inhibition caused by brown mustard and 'Nemat' arugula (87.7% at 5 g and 93.6% at 10 g for brown mustard, and 43.9% at 5 g and 59.3% at 10 g for 'Nemat' arugula) wwas significantly less compared with 'Caliente' mustard at 5 g or 10 g and AITC100. In addition, for LUPP2522, volatiles from 'Corka' kale (63.4% inhibition at 5 g and 70.2% at 10 g) and 'Pasja II' leafy turnip (71.2% at 5 g and and 76.3% at 10 g) inhibited the mycelium growth to greater extents than 'Nemat' arugula (43.9% at 5 g and 59.3% at 10 g).

There was a significant interaction effect (P<0.001) between amount and isolate on the mycelium inhibition. This was mainly associated with a different response of the *R. solani* isolates to AITC100 (100% inhibition for both isolates) compared with the different amounts of the biofumigant plant types, where at all biofumigant amounts the mycelium growth of *R. solani* LUPP2522 was significantly more inhibited compared to *R. solani* LUPP2519. There was a significant interaction effect (P<0.001) between treatment and isolate on the mycelium suppression. 'Caliente' mustard gave the greatest mycelium inhibition of both *R. solani* isolates, 89% inhibition of *R. solani* LUPP2519 and 83.8% of *R. solani* LUPP2522, but significantly less than AITC100 (100% inhibition).

There was a significant interaction effect (P<0.001) between treatment and amount on the mycelium inhibition. For 'Caliente' mustard there was no significant difference in the *R. solani* mycelium growth inhibition at 5 g or 10 g (100% inhibition for both amounts), whereas, for the other biofumigant treatments, inhibition of mycelium growth was significantly greater at 10 g than with 5 g.

There was a significant overall effect (P<0.001) of treatment on mycelium suppression. 'Caliente' mustard had the greatest mycelium growth inhibition (86.4%) compared with the other biofumigants, followed by brown mustard (80.4%). There was a significant effect (P<0.001) of amount on the mycelium inhibition. Biofumigant at 10 g had the greatest inhibitory efficiency (87.4%) compared with at 5 g (82.7% inhibition) or 1 g (37.1% inhibition). There was a significant

effect (P<0.001) of isolate on the mycelium suppression. *Rhizoctonia solani* LUPP2519 was more sensitive to volatiles from the biofumigants than *R. solani* LUPP2522, with 77.7% inhibition for LUPP2519 and 60.6% inhibition for LUPP2522.

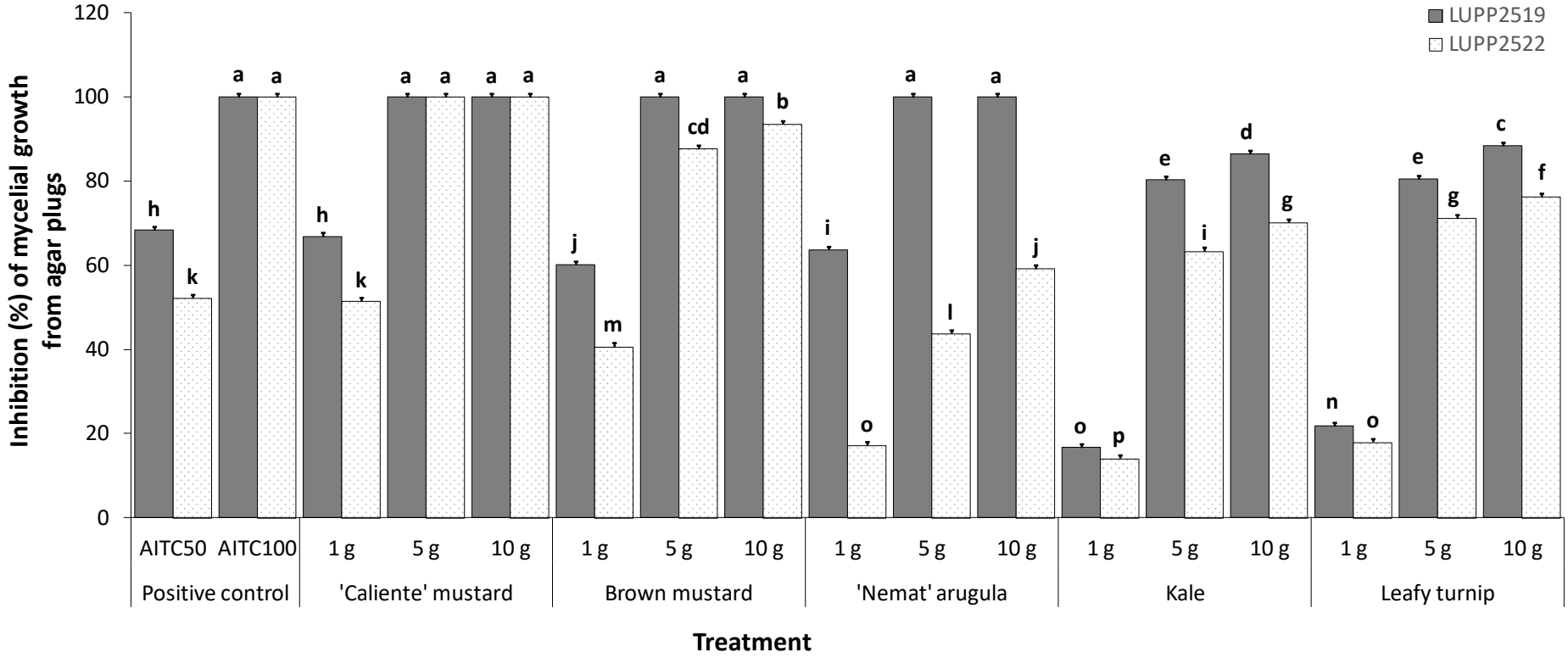


Figure 2.7 Mean inhibition of mycelium growth (%) from agar plugs, in relation to the unamended controls, of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant plant types at 1, 5 or 10 g per Petri dish, and allyl ITC at concentrations to provide 50% (AITC50) or 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (P = 0.05). Error bars indicate LSD (P=0.05) = 1.5.

2.3.3.2 Sclerotia

Statistical analyses of sclerotium germination data are presented in Appendix C.2.11, and the results are summarised in Appendix C.2.35. There were no significant interaction effects on sclerotium germination between treatment, amount and isolate (*P*=0.602; 45.7-100% sclerotium germination), amount and isolate (*P*=0.543; 45.7-100% germination), treatment and isolate (*P*=0.121; 45.7-100% germination) or treatment and amount (*P*=0.565; 71.8-100% germination). There were no significant effects of treatment (*P*=0.076; 71.9-100% germination) or isolate (*P*=0.155; 90.2-94.3% germination) on the sclerotium germination. There was, however, a significant effect (*P*=0.016) of amount on the sclerotium germination. AITC100 had the greatest effect on reducing the sclerotium germination of *R. solani* isolates (28.1% reduction of sclerotium germination), which was significantly greater than all treatments apart from 10 g of biofumigant material (17.2% germination reduction).

The effects of different amounts of biofumigant plant types on subsequent growth of mycelium from sclerotia were also assessed, and these are presented in Appendix D.2.3.

2.3.3.3 Colonised barley grains

Statistical analyses of percentages of barley grains positive for mycelium growth are presented in Appendix C.2.13, and the results are summarised in Appendix C.2.37. There was a significant interaction effect (*P*=0.007) between treatment, amount and isolate on the percentage of colonised barley grains from which mycelium grew onto agar. For isolate LUPP2519, only volatiles from 'Nemat' arugula at 10 g (45.7%) had any effect on the proportion of colonised barley grains producing mycelium. For isolate LUPP2522, only 'Caliente' and brown mustards at 10 g (71.8% for both) had any effects on the proportion of colonised barley grains producing mycelium. No other biofumigant treatment inhibited the proportion of colonised barley grains producing mycelium growth on agar (100% for all the other types). There was a significant effect (*P*=0.003) of amount on the proportion of colonised barley grains producing mycelium on agar. Biofumigants at 10 g significantly reduced the proportions of the colonised barley grains producing mycelium (88.5%) in comparison with all the other biofumigant treatments (100%).

The effects of different amounts of biofumigant crops on subsequent growth of mycelium from colonised barley grains were also assessed, and these are presented in Appendix D.2.4.

2.3.4 Biofumigation effects of biofumigant tissue types on *Rhizoctonia solani* propagules

2.3.4.1 Mycelium growth from agar plugs

Statistical analyses of mycelium growth data from agar plugs are presented in Appendix C.2.15 and the results are summarised in Appendix C.2.39. There was a significant interaction effect (P<0.001) between treatment, tissue type and isolate on the mycelium growth inhibition. Volatiles from all three 'Nemat' arugula tissue types significantly suppressed mycelium growth of isolate LUPP2519 (100% inhibition) compared with isolate LUPP2522 (38.8% inhibition for roots, 66.0% for shoots and 60.4% inhibition for roots + shoots). For 'Caliente' mustard, shoots or shoots + roots inhibited the mycelium growth of both *R. solani* isolates (100% inhibition for all) compared with roots only (89.9% for LUPP2519 and 69% for LUPP2522). For brown mustard, shoot tissue inhibited the mycelium growth of isolate LUPP2519 (100% inhibition) compared with all other treatments (56.1-91.9% inhibition), with shoots alone or in combination with roots resulting in significantly greater inhibition of the mycelium of isolate LU2522 (100% for shoots and 91.9% for roots + shoots) in comparison with isolate LUPP2522 (91.1% for shoots and 80% for roots + shoots). Similarly, all tissue types for 'Corka' kale and 'Pasja II' leafy turnip resulted in greater inhibition of mycelium of isolate LU2519 compared with LUPP2522 (Figure 2.8).

There was a significant interaction effect (P<0.001) between tissue type and isolate on the mycelium growth inhibition. The combination of roots and shoots gave greater inhibition of the mycelium growth of isolate LUPP2519 (90.6%) than for isolate LUPP2522 (72.3%), and root or shoot tissues separately for both isolates, but significantly less than AITC100 for both isolates (100% inhibition). There was no significant difference in the effects of volatiles from root and shoot tissue on the mycelium growth of isolate LUPP2519 (87.8-88.3%), but for LUPP2522 shoot tissue (71.7% inhibition) gave greater inhibition of mycelium growth than root tissue (51.3%) (Appendix C.2.39).

There was a significant interaction effect (P<0.001) between treatment and isolate. There was no significant difference in the inhibition of mycelium growth of the two *R. solani* isolates by AITC100, which completely inhibited mycelium growth of both isolates. In contrast, for all other treatments mycelium growth of isolate LUPP2519 (69.2-100% inhibition) was more inhibited compared with that of the isolate LUPP252 (45.3-89.7% inhibition) (Appendix C.2.39).

There was a significant interaction effect (P<0.001) between biofumigant plant type and tissue type on the mycelium growth. For 'Caliente' mustard, shoot tissue alone, and in combination with root tissue, gave greater inhibition of mycelium growth compared with root tissue alone, with no significant difference between shoot tissue and shoot + root tissue. In contrast, for brown mustard and 'Nemat' arugula, shoot tissue reduced mycelium growth compared with root tissue alone, and

shoot + root tissue, and for 'Corka' kale and 'Pasja II' leafy turnip, shoot +aroot tissue was more effective at inhibiting mycelium growth compared with either tissue type alone (Appendix C.2.39).

There was a significant effect (P<0.001) of biofumigant crop on mycelium growth. Volatiles from 'Caliente' mustard significantly inhibited the mycelium growth (93.2% inhibition) compared with all other biofumigant plant types, apart from AITC100 (100% inhibition), followed by brown mustard (81.6% inhibition) and 'Nemat' arugula (77.5% inhibition) (Appendix C.2.39). There was a significant effect (P<0.001) of biofumigant tissue type on the mycelium growth. The combination of root + shoot of the biofumigants inhibited *R. solani* mycelium growth (81.5%) compared with those from shoots (79.8% inhibition) or roots (69.8% inhibition), but was significantly less than the effect from AITC100 (100% inhibition) (Appendix C.2.39). There was a significant effect (P<0.001) of *R. solani* isolate on the mycelium growth. *Rhizoctonia solani* LUPP2522 was less sensitive to biofumigants (65.2% inhibition) in comparison with isolate LUPP2519 (88.9% inhibition) (Appendix C.2.39).

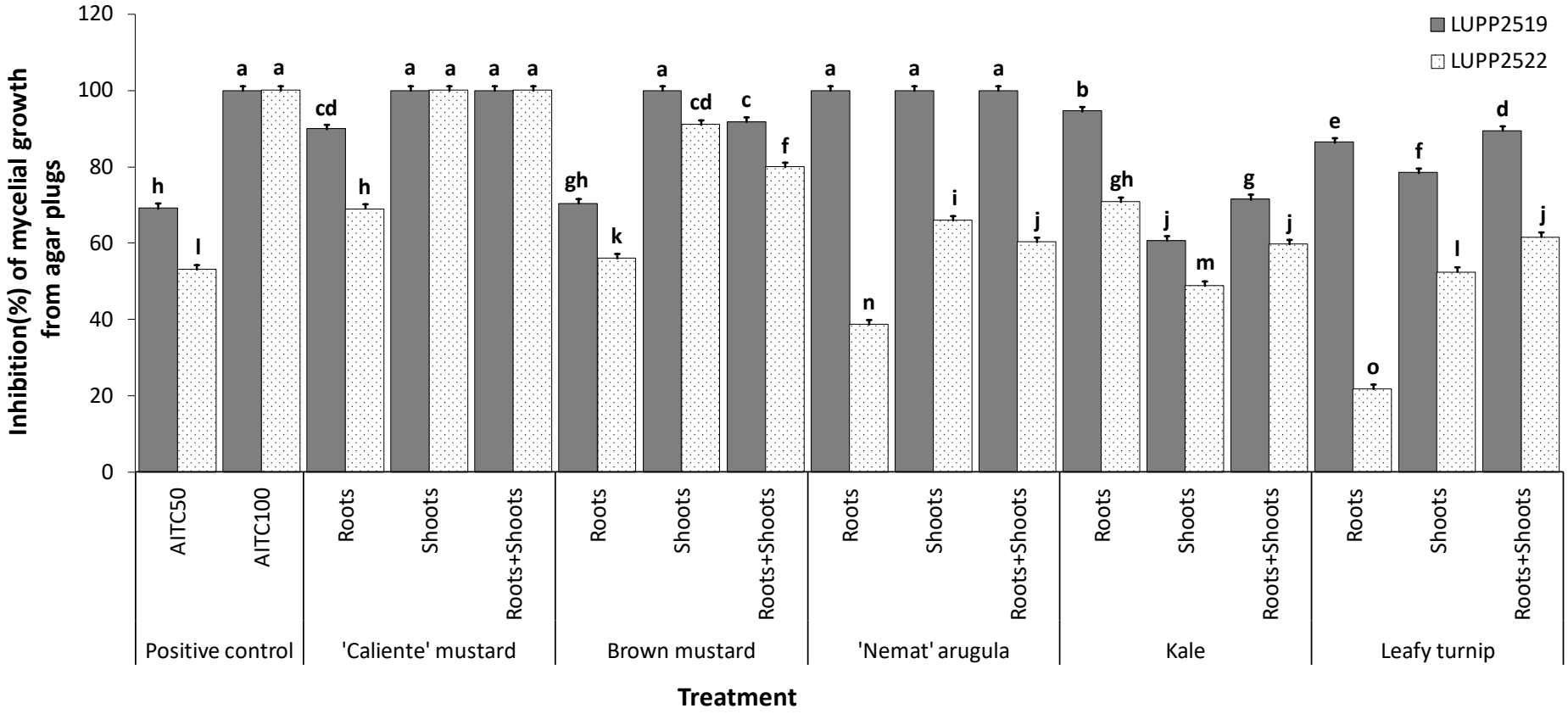


Figure 2.8 Mean mycelium inhibition (%) from agar plugs, in relation to the unamended controls, of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots + shoots) of five biofumigant plant types, and allyl ITC at concentrations to provide 50% (AITC50) or 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (P=0.05). Error bars indicate LSD (P=0.05) = 2.3.

2.3.4.2 Sclerotia

Statistical analyses of sclerotium germination data are presented in Appendix C.2.16, and the results are summarised in Appendix C.2.40. There were no significant interaction effects on sclerotium germination between treatment, tissue type and isolate (*P*=0.914) (45.7-100% germination rate), between tissue type and isolate (*P*=0.499) (45.7-100% germination rate), between treatment and isolate (*P*=0.518) (45.7-100% germination rate), or between treatment and tissue type (*P*=0.914) (58.5-100% germination rate). There were no significant effects on the sclerotium germination of tissue type (*P* = 0.499) (58.5-100% inhibition rate) or isolate (*P*=0.081) (45.7-100% germination rate). However, there was a significant effect (*P*<0.001) of treatment on the sclerotium germination. AITC100 reduced sclerotium germination (58.5%) compared with all other treatments, with no significant difference in germination rate between all other treatments (90.4-100%).

The effects of different tissue types of biofumigants on the subsequent growth of mycelium from sclerotia were also assessed, and these are presented in Appendix D.2.5.

2.3.4.3 Colonised barley grains

Statistical analyses of the percentages of colonised barley grains having mycelium growth on agar are presented in Appendix C.2.18, and the results are summarised in Appendix C.2.42. There were no significant interaction effects on the percentage of colonised barley grains with mycelium growth on agar, between treatment, tissue type and isolate (*P*=1.000) (71.8-100% grains with mycelium growth), between tissue type and isolate (*P*=1.000) (71.9-100% grains with mycelium growth), or between treatment and tissue type (*P*=1.000) (85.6-100% grains with mycelium growth).

There was a significant interaction effect (*P*=0.024) between treatment and isolate. AITC100 reduced the proportion of barley grains colonised by LUPP2519 with mycelium growth (71.9%), but had no significant effect for LUPP2522 (100%).No other biofumigant treatment affected the percentage of colonised grains with mycelium growth onto agar (100% for all treatments). There was no significant effect of tissue type on the percentage of colonised barley grains with mycelium growth onto agar (*P*=1.000) (85-100% grains with mycelium growth) or isolate (*P*=0.320) (100% grains with mycelium growth).There was also a significant effect (*P* = 0.024) of treatment on the percentage of colonised barley grains with mycelium growth. AITC100 significantly reduced the proportion of grains positive for mycelium growth (85.6%) compared with all the other treatments, which did not have any effects on the percentage of grains positive for mycelium growth (all being 100%).

The effects of different tissue types of biofumigants on the subsequent growth of mycelium from colonised barley grains were also assessed, and these are presented in Appendix D.2.6.

2.3.5 Biofumigation effects of different flowering times of biofumigant plant types on *Rhizoctonia solani* propagules

2.3.5.1 Mycelium growth from agar plugs

Statistical analyses of data of mycelium growth from agar plugs are presented in Appendix C.2.20, and the results are summarised in Appendix C.2.44. There was a significant interaction effect (P<0.001) between biofumigant crop, biofumigant flowering time and *R. solani* isolate on the inhibition of mycelium growth from agar plugs. For 'Caliente' mustard there was no difference in the level of mycelium inhibition for the two *R. solani* isolates achieved with the tissue harvested at the three flowering times, with all tissue completely inhibiting both *R. solani* isolates. In contrast, there were significant differences in the levels of mycelium inhibition achieved from the different 'Nemat' arugula or brown mustard tissues, with all tissue harvested at the three flowering times giving greater inhibition of LUPP2519 (AG3-PT) (100% inhibition in all treatments apart from 86.9% for brown mustard at first flower emergence) compared with the same treatment on LUPP2522 (AG2-1) (55.3-87.9% inhibition from brown mustard, and 20.9-38.1% inhibition from 'Nemat' arugula) (Figure 2.9).

There was a significant interaction effect (P<0.001) between biofumigant flowering time and *R. solani* isolate on mycelium growth. Biofumigant crops harvested at three flowering times had significantly greater inhibition of the mycelium growth for LUPP2519 compared with LUPP2522. For LUPP2519, biofumigant crops at mid or full anthesis, and the positive control AITC100, reduced mycelium growth (100% inhibition) compared with first flowering tissues (95.7% inhibition) . However, for LUPP2522, the biofumigants harvested at mid anthesis (72.2% inhibition) gave greater mycelium growth inhibition compared with tissue at first flowering (64.5% inhibition) or full flowering (69.6%).

There was a significant interaction effect on the mycelium growth (P<0.001) between biofumigant plant type and *R. solani* isolate. There was no significant difference between the level of mycelium growth inhibition from 'Caliente' mustard and the positive control AITC100 between the two *R. solani* isolates. However, brown mustard and 'Nemat' arugula were both more effective at inhibiting the mycelium growth of LUPP2519 (95.7-100%) than for LUPP2522 (30.5-75.7%).

There was a significant interaction effect (P < 0.001) on the mycelium growth between biofumigant plant type and flowering time. For 'Caliente' mustard there was no significant difference in the amount of mycelium growth inhibition when it was harvested at the three anthesis stages, which were not significantly different to the positive control AITC100 (100% for all these treatments). In contrast, inhibition of mycelium growth was greater from brown mustard harvested at mid or full flowering (92 and 93.9% inhibition, respectively) than from first flowering (71.1%). 'Nemat' arugula at first and mid flowering stages gave greater inhibition of the mycelium growth (69 and 66.3% inhibition, respectively) compared with the full anthesis stage (60.5% inhibition).

There was a significant effect (P<0.001) of biofumigant plant type on the mycelium growth, with 'Caliente' mustard and AITC completely inhibiting mycelium growth (100% inhibition), followed by brown mustard (85.7%, and 'Nemat' arugula (65.3%). There was also a significant effect (P<0.001) of biofumigant flowering time on the mycelium growth, with mid flowering tissues giving greater inhibition (86.1%) compared with that from first (80.1% inhibition) or full (84.8% inhibition) flowering times. There was a significant effect (P<0.001) of *R. solani* isolate on the mycelium growth, with LUPP2519 being more sensitive to volatiles from biofumigant types (98.2% inhibition) than LUPP2522 (68.9% inhibition).

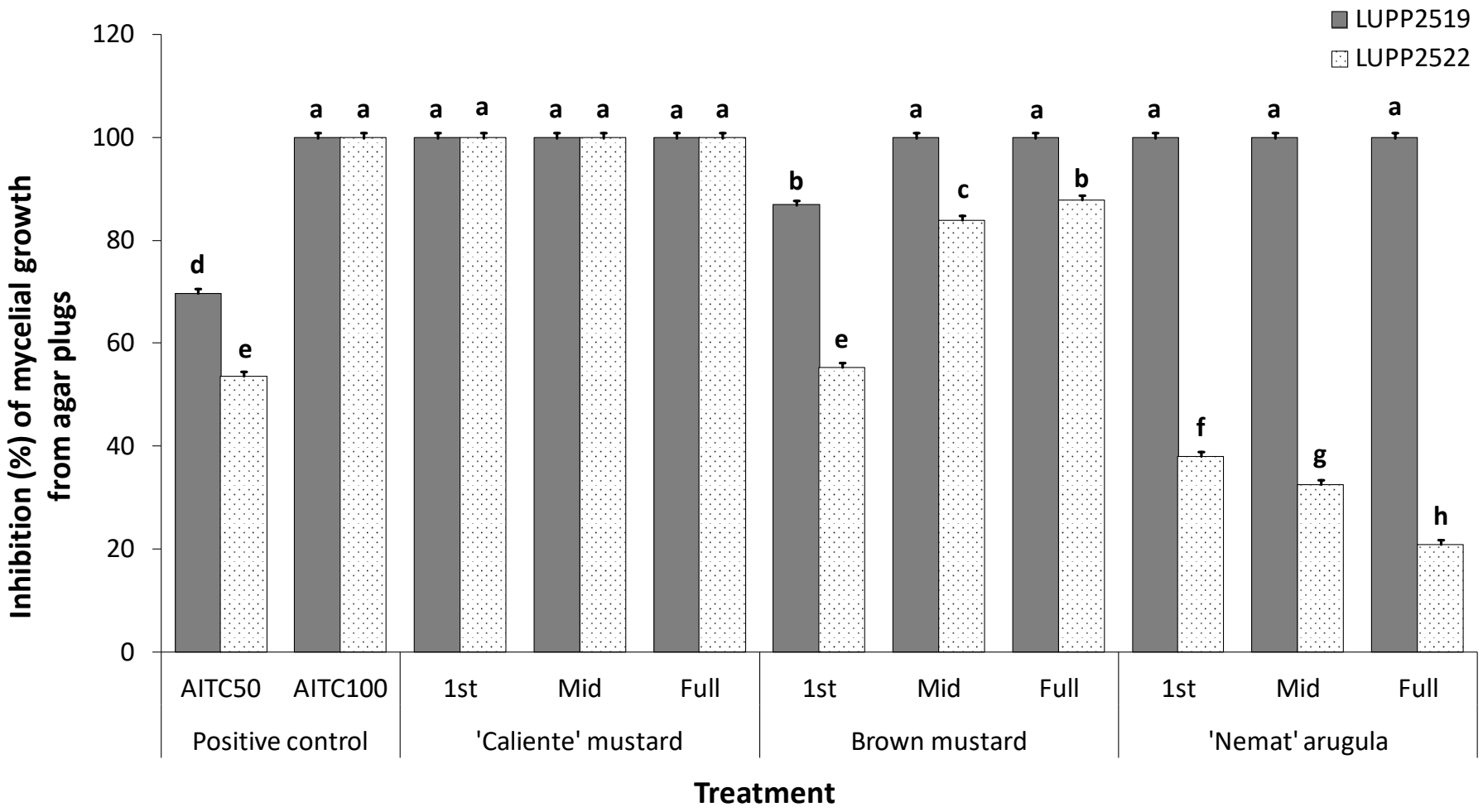


Figure 2.9 Mean mycelium inhibition (%) from agar plugs, in relation to the unamended controls, of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissues of three biofumigant crops harvested at first, mid or full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) inhibition. Bars with the same letter are not significantly different based on Tukey's HSD test (*P* = 0.05). Error bars indicate LSD (*P*=0.05) = 1.8.

2.3.5.2 Sclerotia

Statistical analyses of percentages of sclerotium germination are presented in Appendix C.2.21, and the results are summarised in Appendix C.2.45. There were no significant interaction effects between treatment, flowering time and isolate (*P*=0.275) (45.7-100% germination), or between flowering time and isolate (*P*=0.080) (45.7-100% germination). There was, however, a significant interaction effect (*P*=0.052) between treatment and flowering time, with AITC100 and brown mustard tissues at full flowering reducing the germination of *R. solani* (averaged two isolates, 71.8%) in comparison with all the other treatments (100%), apart from 'Caliente' mustard at mid flowering and 'Nemat' at first flowering (85.6%). There was a significant interaction effect (*P*=0.017) between treatment and isolate, with AITC100 significantly reducing the germination of LUPP2519 sclerotia (45.7% germination) compared with all the other treatments (90.4-100%). In comparison, there were no significant effects of any treatment on the germination of LUPP2522 sclerotia (80.9-100%). There were also no significant effects of treatment (*P*=0.179) (71.9-100% germination), flowering time (*P*=0.748) (90.4-100% germination), or isolate (*P*=0.353) (93.2-93.8% germination) on the sclerotium germination.

The effects of biofumigant flowering times on the subsequent growth of mycelium from sclerotia were also assessed, and iare presented in Appendix D.2.7.

2.3.5.3 Colonised barley grains

Statistical analyses of the proportions of colonised barley grains having mycelium growth on agar are presented in Appendix C.2.23, and the results are summarised in Appendix C.2.47. There were no significant interaction effects on the percentage of colonised barley grains with mycelium growth onto agar, between treatment, flowering time and isolate (*P*=0.266) (71.8-100% colonised barley grains with growth), between flowering time and isolate (*P*=0.270) (90.4-100% grains with growth), between treatment and isolate (*P*=0.700) (90.4-100% grains with growth), or between treatment and flowering time (*P*=0.266) (85.6-100% grains with growth). There was no significant effect on the percentage of grains with mycelium growth on agar, of treatment (*P*=0.700) (95.2-100% grains with growth), flowering time (*P*=0.270) (100% grains with growth), or isolate (*P*=0.321) (96.8-100% grains with growth).

The effects of flowering times of biofumigants on subsequent growth of mycelium from colonised barley grains was also assessed, and these are presented in Appendix D.2.8.

2.3.6 Biofumigation effects on *Rhizoctonia solani* propagules of different amounts of biofumigant plant types amended in soil

2.3.6.1 Mycelium growth from agar plugs

Statistical analyses of the inhibition of mycelium growth from agar plugs are presented in Appendix C.2.25, and the results are summarised in Appendix C.2.49. There was a significant interaction effect (P<0.001) between biofumigant plant type, biofumigant amount and *R. solani* isolate on the mycelium growth. 'Caliente' mustard at 5 and 10% completely inhibited mycelium growth of *R. solani* LUPP2519 (100% inhibition), and this was significantly different compared with all the other treatments. The next most effective treatments were brown mustard at 10% for LUPP2519 (89.8% inhibition) and 'Caliente' mustard at 5 and 10% for LUPP2522 (84.8-87.3% inhibition) (Figure 2.10).

There was a significant interaction effect (P<0.001) on the mycelium growth, between biofumigant amount and *R. solani* isolate. For LUPP2519, volatiles released from 10% biofumigant tissues gave greater inhibition of mycelium growth (90.6%), compared with 5% or 1%, with 5% (83.8%) giving greater inhibition than 1% (59.1%). In contrast, for LUPP2522 there was no significant difference in the effect of biofumigants at 10% (76.7% inhibition) or 5% (77.3% inhibition), with both incorporation amounts giving greater inhibition of mycelium growth than 1% (39.4%).

There was a significant interaction effect (P<0.001) on mycelium growth between biofumigant type and *R. solani* isolate. For 'Caliente' mustard, brown mustard and 'Nemat' arugula, growth of LUPP2519 was significantly more inhibited than that of LUPP2522.

There was a significant interaction effect (P<0.001) on the mycelium growth between biofumigant type and amount. For 'Caliente' mustard, growth inhibition was 92.4% from 5% tissue incorporation in soil, and 93.7% from 10% incorporation. For 'Nemat' arugula inhibition was 70.3% from 5% incorporation and 72.2% from 10% incorporation. , and the two biofumigant types did not differ significantly. For both types these inhibition rates were greater than at the 1% incorporations (58.6% for 'Caliente' and 46.5% for 'Nemat'). In contrast for brown mustard, mycelium growth inhibition at 10% incorporation (85.2%) was greater than at 5% (78.9%) or 1% incorporation (46.5%), with that at 5% being significantly greater than that at 1%.

There was a significant effect (P<0.001) of biofumigant plant type on the mycelium growth. 'Caliente' mustard gave the greatest mycelium growth (81.6%), followed by brown mustard (70.2%). There was also a significant effect (P<0.001) of *R. solani* isolate on the mycelium growth. *Rhizoctonia solani* LUPP2519 was more sensitive to volatiles from biofumigants (77.9% inhibition) compared to LUPP2522 (64.5% inhibition).

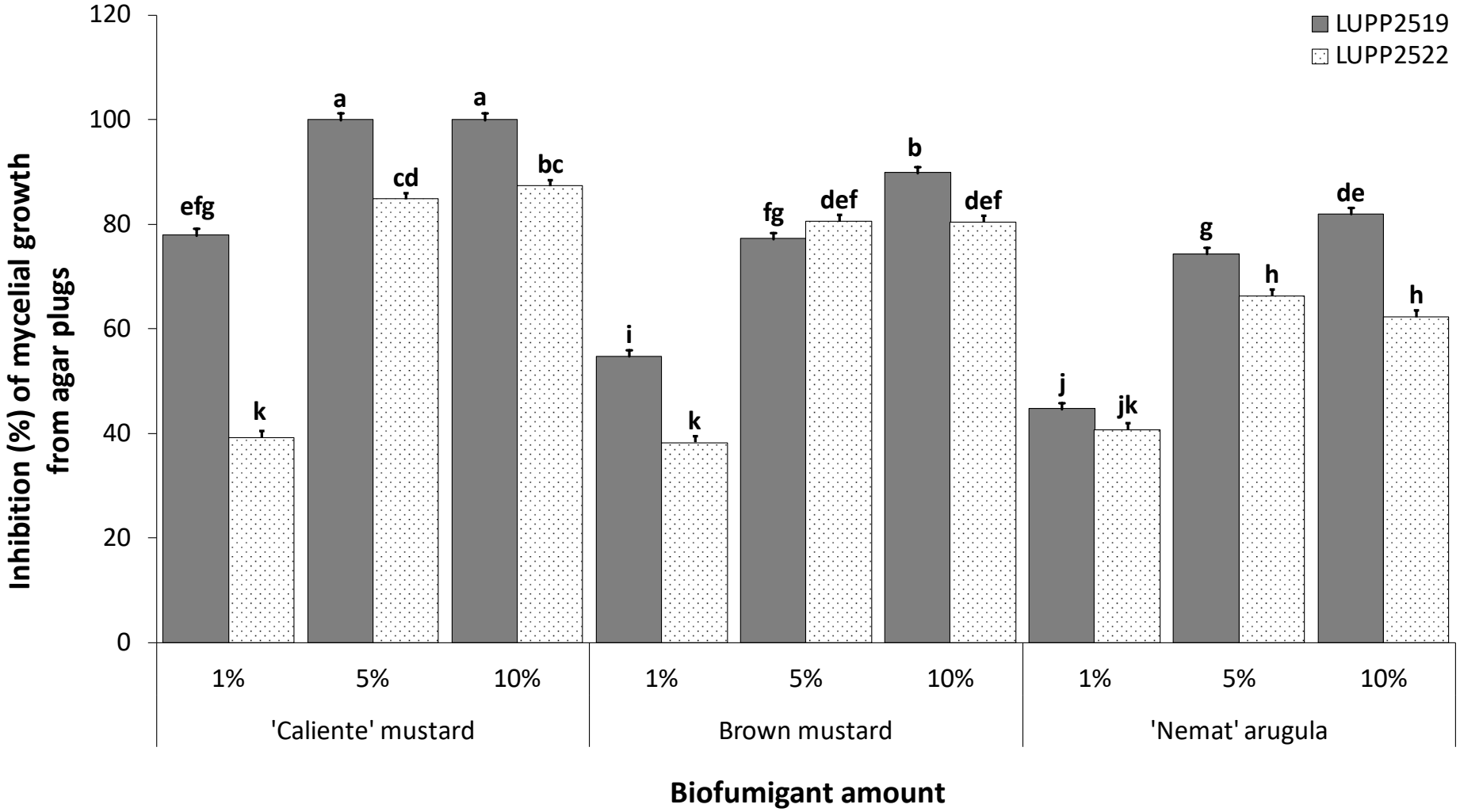


Figure 2.10 Mean mycelium growth inhibition (%) from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 or 10% (w:w). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.4.

2.3.6.2 Sclerotia

All sclerotia had 100% germination after seven days exposure to volatiles from biofumigants. The effects of different amounts of biofumigant crops amended in soil on the subsequent growth of mycelium from sclerotia was assessed, and is presented in Appendix D.2.9.

2.3.6.3 Colonised barley grains

All (100%) of the colonised barley grains were positive for mycelium growth onto agar after seven days exposure to volatiles from biofumigants. The effects of different amounts of biofumigant crops amended in soil on subsequent growth of mycelium from colonised barley grains was assessed, and is presented in Appendix D.2.10.

2.3.7 Changes and survival of *Rhizoctonia solani* propagules in soil amended with different amounts of biofumigant plant types

2.3.7.1 Mycelia from agar plugs

Statistical analyses of sclerotium conversion proportions per mesh bag are presented in Appendix C.2.28, and the results are summarised in Appendix C.2.52. There was a significant interaction effect (P<0.001) on the conversion of mycelium into sclerotia after 28 days incubation of mesh bags containing mycelium agar plugs in soil amended with the different biofumigant treatments, between biofumigant type, biofumigant amount and *R. solani* isolate. For the untreated control, no sclerotia were observed for either isolate after 28 days incubation. For *R. solani* LUPP2519 (AG3-PT), 'Caliente' mustard at 1% incorporation gave greater conversion of mycelium into sclerotia (sclerotia present in 40% of plugs per mesh bag) compared with at 5% (10% of plugs with sclerotia) and 10% (0% of plugs with sclerotia). For *R. solani* LUPP2522 (AG2-1), burial of agar plug-containg bags in soil amended with 'Caliente' mustard at 5 or 10% incorporation gave greater conversion to sclerotia (sclerotia on 23.2-26.5% of plugs) compared with at 1% (16.8% of plugs with sclerotia). For brown mustard and 'Nemat' arugula, only incorporation at 1% gave sclerotium formation (sclerotia on 10-13% of plugs) from both of the *R. solani* isolates, with no significant difference between the isolates. Mycelium did not convert into sclerotia for any of the remaining treatments, including for brown mustard and 'Nemat' arugula incorporated at 5% or 10% for both isolates, and for 'Caliente' mustard incorporated at 10% for isolate LUPP2519 (Figure 2.11).

There was a significant interaction effect (P<0.001) on the conversion of mycelium from agar plugs into sclerotia between biofumigant amount and *R. solani* isolate. For biofumigants at 1%, sclerotium formation was greater for isolate LUPP2519 (sclerotia on 19.6% of plugs per bag) compared with LUPP2522 (sclerotia on 12.1% of plugs). In contrast, for biofumigants at 5% or 10%, sclerotium

formation was greater for LUPP2522 (3.2% at 5% incorporation and 2.8% of plugs at 10% incorporation) compared with LUPP2519 (respectively 1.1% and 0% of plugs with sclerotia per bag).

There was a significant interaction effect (*P*<0.001) on the conversion of mycelium from agar plugs into sclerotia between biofumigant crop and *R. solani* isolate. For 'Caliente' mustard sclerotium formation was greater from isolate LUPP2522 (22.0% of plugs with sclerotia) than from LUPP2519, with both isolates developing more sclerotia than from any other treatment. In contrast, for brown mustard and 'Nemat' arugula sclerotium formation was low (1.1-1.5%) with there being no significant difference between the isolates. There was a significant interaction effect (*P*<0.001) on the production of sclerotia from agar plugs between biofumigant type and amount. 'Caliente' mustard at 1% incorporation increased sclerotium conversion from mycelium agar plugs (27.6% of plugs with sclerotia) compared with incorporation at at 5% giving 17.5% of plugs with sclerotia, and 10% giving 0% of plugs with sclerotia. For brown mustard and 'Nemat' arugula, sclerotia were only formed with treatment with 1% amendment (10.0% of plugs with sclerotia for brown mustard and 11.5% for 'Nemat' arugula). No sclerotia were produced with 5 and 10% incorporation, or from the untreated control treatments.

There was a significant effect (*P*<0.001) of biofumigant plant type on the sclerotium production on the agar plugs, with greater production of sclerotia from the 'Caliente' mustard treatment(16% of plugs with sclerotia) compared with brown mustard or 'Nemat' arugula (respectively, 1.1% and 1.3%). There was also a significant effect (*P*<0.001) of biofumigant amount on the conversion of mycelium into sclerotia, with biofumigants at 1% incorporation increasing the number of plugs with sclerotia (15.7% of plugs). There was a significant effect (*P*<0.001) of *R. solani* isolate on the conversion of mycelium into sclerotia, with the number of plugs with sclerotia being greater for isolate LUPP2522 (sclerotia on 5.3% of plugs) compared with isolate LUPP2519 (sclerotia on 3.4% of plugs).

Statistical analyses of theproportions of retrieved agar plugs producing subsequent mycelium growth onto agar are presented in Appendix C.2.29, and the results are summarised in Appendix C.2.53. There was a significant interaction effect (*P*<0.001) on subsequent mycelium growth from retrieved agar plugs between biofumigant plant type, biofumigant amount and *R. solani* isolate. For both isolates, the proportion of the retrieved plugs with subsequent mycelium growth from the 'Caliente' and brown mustards and from 'Nemat' arugula at 1% incorporation, did not differ significantly from the untreated controls, with all these treatments giving more subsequent mycelium growth (Figure 2.12). There was no interaction effect on the subsequent mycelium growth from retrieved agar plugs between biofumigant amount and *R. solani* isolate (*P*=0.243), or between biofumigant type and *R. solani* isolate (*P*=0.524).There was a significant interaction effect (*P*<0.001) on subsequent mycelium growth from retrieved agar plugs between biofumigant plant type and biofumigant amount. For

‘Caliente’ mustard, the proportion of the agar plugs with subsequent mycelium growth was greater with incorporation of the tissue at 1% (57.7%) compared with 5% (43.3%) or 10% incorporation (7.0%), with 5% being greater than 10%. In contrast, there was no difference between 5% and 10% incorporation of brown mustard and ‘Nemat’ arugula, although the proportion of agar plugs with mycelium growth was greater with incorporation of the tissue at 1% (68.7% for brown mustard and 58.8% for ‘Nemat’) compared with 5% (23.2% for brown mustard and 9.6% for ‘Nemat’) and 10% incorporation (17.5% for brown mustard and 10.0% for ‘Nemat’).

There was a significant effect (P<0.001) of biofumigant treatment on the subsequent mycelium growth from retrieved agar plugs. All treatments (23.3-35.4%) reduced the proportions of agar plugs with mycelium compared with the untreated control (64.5%). ‘Nemat’ arugula (23.3%) reduced the proportion of plugs with mycelium growth compared with both ‘Caliente’ mustard (35.4%) and brown mustard (33.4%).

There was a significant effect (P<0.001) of biofumigant amount on subsequent mycelium growth from retrieved agar plugs, with the 5% incorporation rate (24.0% of plugs) and 10% rate (11.2%) reducing the proportions of plugs with mycelium growth compared with the untreated controls (64.5%). Biofumigants incorporated at 1% (61.8%), or at 10% gave lower proportions of plugs with mycelium growth compared with 5%. Biofumigants at 1% had no effect on the proportions of plugs with mycelium growth compared with the untreated control. There was a significant effect (P<0.001) of *R. solani* isolate on subsequent mycelium growth from retrieved agar plugs, with more isolate LUPP2519 plugs (36.9%) producing mycelium compared with isolate LUPP2522 (25.3%).

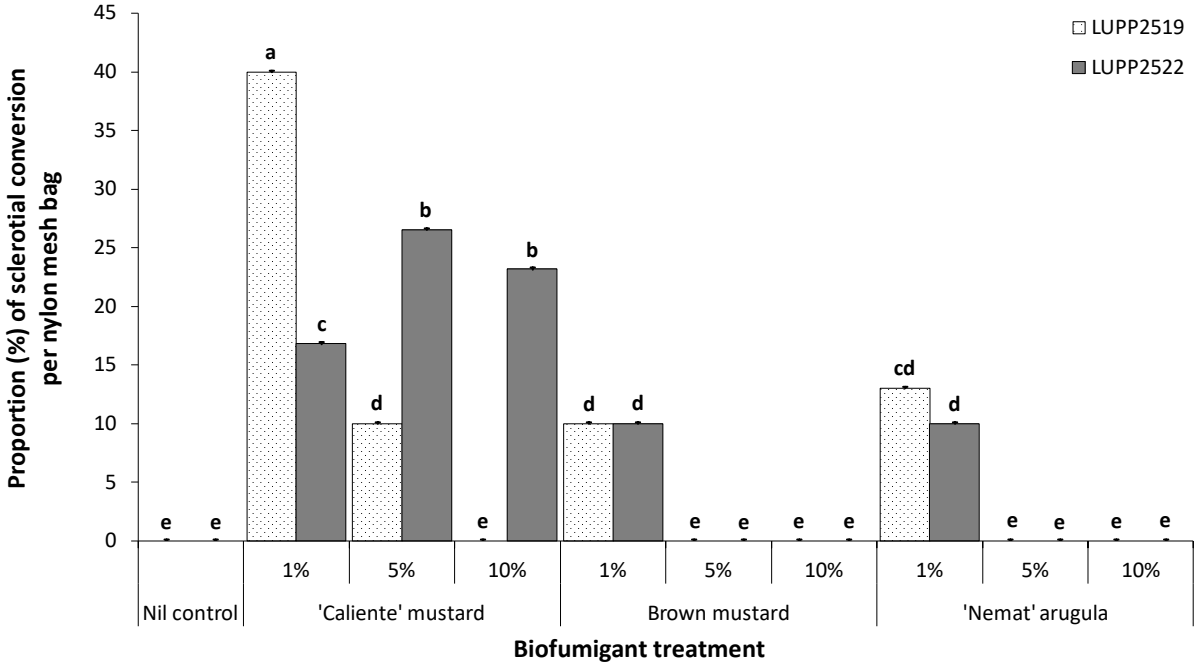


Figure 2.11 Mean proportions of the retrieved agar plugs with sclerotia (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) or LUPP2522 (AG2-1) per mesh bag, buried in soil amended with macerated tissue of three biofumigant plant types incorporated into soil at 1, 5 or 10% (w:w), after 28 days incubation. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 0.3.

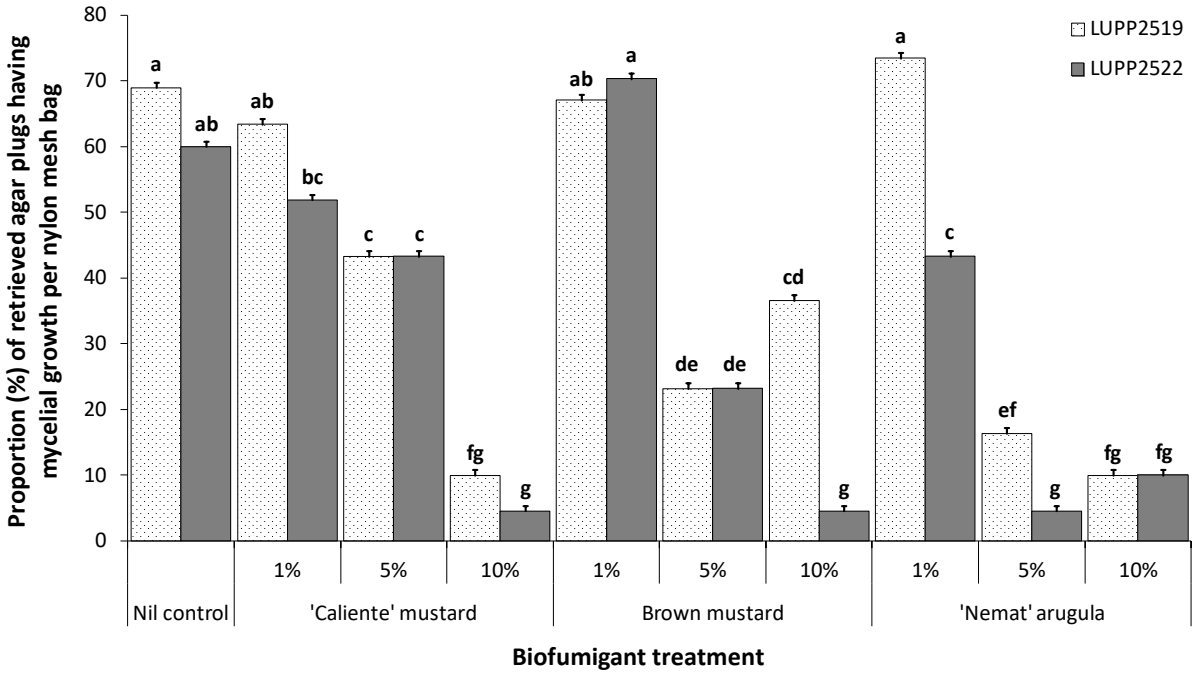


Figure 2.12 Mean proportions (%) of retrieved agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) or LUPP2522 (AG2-1) having mycelium growth per mesh bag, buried in soil amended with macerated tissue of three biofumigant plant types incorporated into soil at 1, 5 or 10% (w:w), after 28 days incubation. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 1.6.

2.3.7.2 Sclerotia

Statistical analyses of the germination proportions of retrieved sclerotia are presented in Appendix C.2.30, and the results are summarised in Appendix C.2.54. There was a significant interaction effect (P<0.001) of biofumigant plant type, biofumigant amount and *R. solani* isolate on the proportion of sclerotia which subsequently germinated after 28 days incubation of bags containing the sclerotia in soil amended with the different biofumigant treatments. For isolate LUPP2519, sclerotium germination was greater for sclerotia recovered from the 'Caliente' mustard treatments at 1% and 5% incorporation, from brown mustard at all three incorporation amounts, and 'Nemat' arugula at 1%, compared with the untreated control (Figure 2.13). 'Nemat' arugula at 10% incorporation was the only treatment that reduced sclerotium germination compared with the untreated control. In contrast, for isolate LUPP2522, only sclerotia recovered from the brown mustard treatment at 1% had greater compared with the untreated control. Sclerotia recovered from the 'Caliente' mustard treatments at 1 or 10% incorporation, brown mustard at 5% and 10%, and 'Nemat' arugula all reduced sclerotium germination compared with the unamended soil (control). There was no significant interaction effect on the sclerotium germination between biofumigant amount and *R. solani* isolate (P=0.11).

There was a significant interaction effect (P<0.001) on the sclerotium germination between biofumigant crop and *R. solani* isolate. For *R. solani* LUPP2522, 'Caliente' mustard and 'Nemat' arugula both reduced sclerotium germination compared with the untreated control and brown mustard treatments. In contrast, for isolate LUPP2519, no treatment significantly reduced sclerotium germination compared with the untreated control (76.8%), with 'Caliente' mustard (91.1% germination) and brown mustard (99.9%) giving greater sclerotium germination than the untreated control and 'Nemat' arugula (78.8%).

There was a significant interaction effect (P<0.001) on the sclerotium germination between biofumigant plant type and biofumigant amount. 'Caliente' mustard (65.1% germination) and 'Nemat' arugula (9%) incorporated at 10% were the only treatments which reduced sclerotium germination. Sclerotium germination was greater for sclerotia recovered from 'Caliente' mustard at 5%, brown mustard at 1% and 5%, and 'Nemat' arugula at 1%, compared with the untreated control.

There was a significant effect (P<0.001) of biofumigant plant type on the sclerotium germination. 'Nemat' arugula inhibited the sclerotium germination (60.3%) compared with all other treatments, with brown mustard (94.9%) having greater sclerotium germination compared with the untreated control (78.4%). There was a significant effect (P<0.001) of biofumigant amount on the sclerotium germination, with the biofumigants at 10% incorporation reducing sclerotium germination (52.7%) compared with the untreated control (78.4%), and biofumigants at 1% incorporation (93.4%) or 5% (89.0%) resulting in greater germination than the control. There was a significant effect (P<0.001) of

R. solani isolate on the sclerotium germination, with isolate LUPP2519 sclerotia (92.6% germination) being less sensitive to the biofumigants compared with isolate LUPP2522 (65.3% germination).

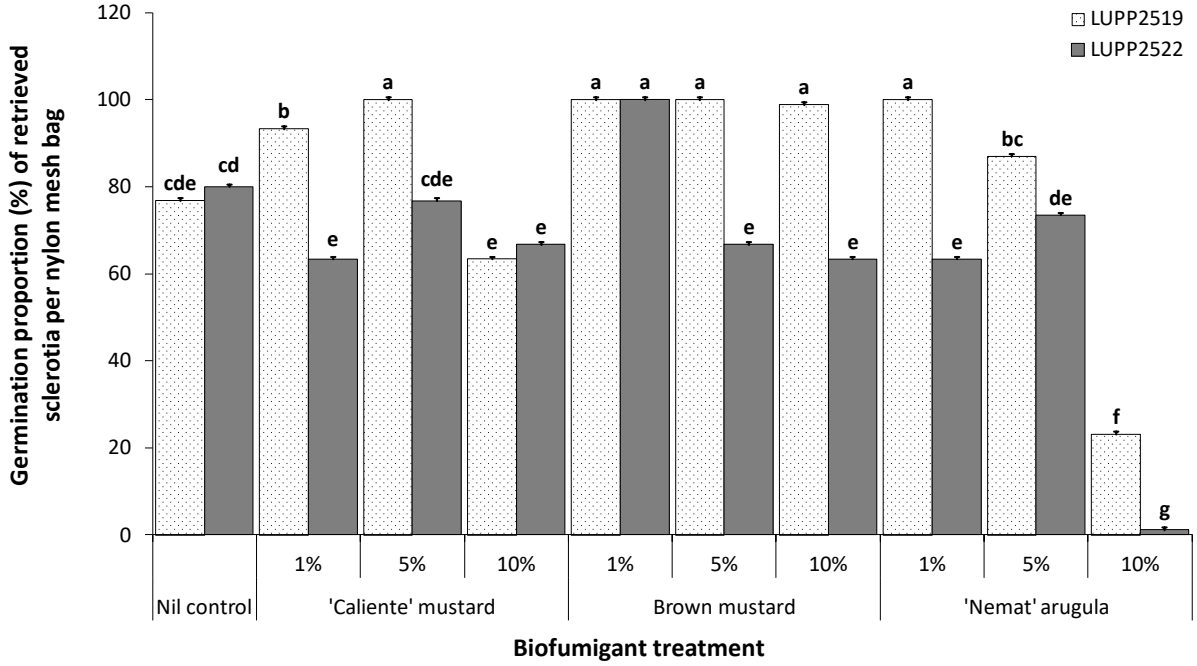


Figure 2.13 Mean germination proportions (%) of retrieved sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) per mesh bag, buried in soil amended with macerated tissue of three biofumigant crops incorporated into soil at 1, 5 or 10% (w:w), after 28 days incubation. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 1.3.

2.3.7.3 Colonised barley grains

Statistical analyses of the proportions of retrieved colonised barley grains having subsequent mycelium growth on agar are presented in Appendix C.2.31, and the results are summarised in Appendix C.2.55. There was no significant interaction effect on proportions of retrieved colonised barley grains from which mycelia grew onto agar between biofumigant plant type, biofumigant amount and *R. solani* isolate (*P*=0.393), between biofumigant amount and *R. solani* isolate (*P*=0.398) (0-40% grains positive for mycelium growth), or between biofumigant type and *R. solani* isolate (*P*=0.958) (8-40% grains positive for mycelium growth).

There was a significant interaction effect (*P*=0.027) between biofumigant plant type and biofumigant amount on the percentage of retrieved colonised barley grains from which mycelia grew onto agar. All treatments apart from brown mustard at 1% incorporation reduced the proportions of colonised grains positive for mycelium growth, compared with the untreated control. Of these treatments 'Caliente' mustard, brown mustard and 'Nemat' arugula at 10% completely inhibited mycelium

growth from colonised grains, and these were significantly different from all the other treatments (Figure 2.14).

There was a significant effect (P<0.001) of biofumigant plant type on the percentage of retrieved colonised barley grains from which mycelia grew onto agar. All three biofumigant types inhibited subsequent mycelium growth from the retrieved grains (8.5-11.3% grains positive for mycelium growth), compared with the untreated control (38.3%). There was a significant effect (P<0.001) of biofumigant amount on the percentage of retrieved colonised grains from which mycelia grew onto agar. Biofumigants at 10% incorporation completely suppressed subsequent mycelium growth from the retrieved grains (all being 0%), followed by 5% (14.8%) and 1% (27.5%), which were significantly different from the untreated control (38.3%). There was no significant effect (P=0.075) of *R. solani* isolate on the percentage of retrieved grains from which mycelia grew, being 9.1% for isolate LUPP2519 and 10.6% for isolate LUPP2522.

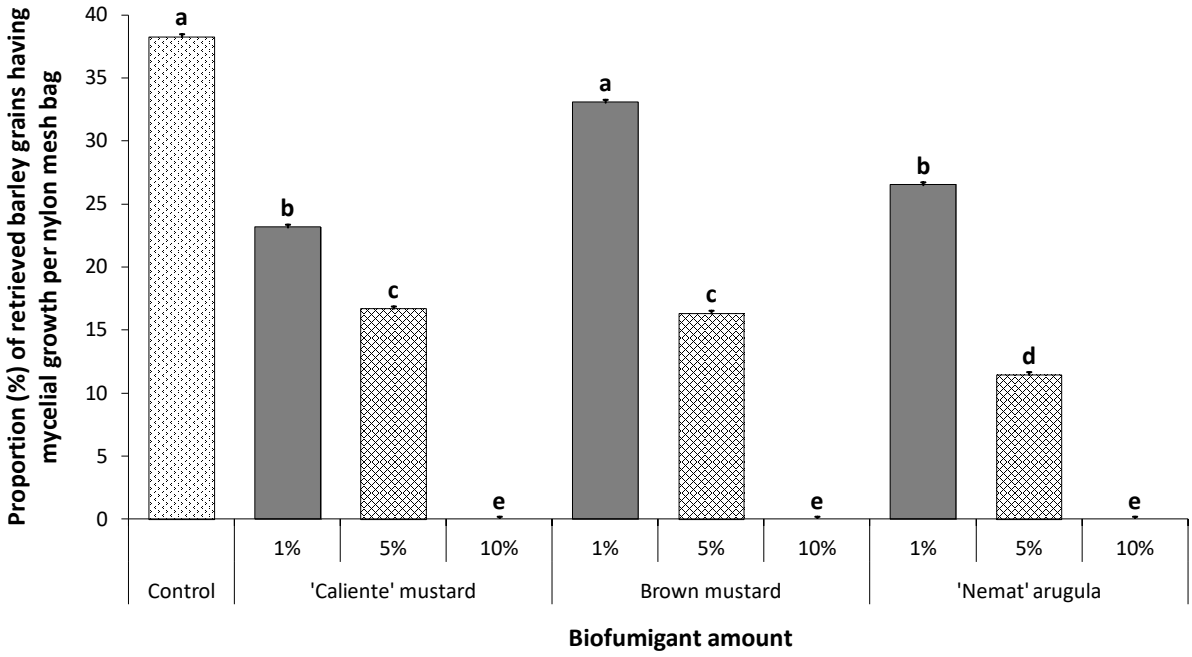


Figure 2.14 Mean proportions (%) of retrieved barley grains colonised by *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) (data averaged across both isolates), having mycelium growth in mesh bags buried in soil amended with macerated tissue of three biofumigant crops incorporated into soil at 1, 5 or 10% (w:w), after 28 days incubation. Bars with the same letter are not significantly different based on Tukey's HSD test (P=0.05). Error bars indicate LSD (P=0.05) = 0.5.

2.4 Discussion

The results obtained from these experiments have highlighted the biofumigation potential of selected biofumigant crops for reducing potato diseases caused by *R. solani* belonging AG3-PT and AG2-1. However, biofumigation efficiency was dependent on biofumigant cultivars/species, amount of biofumigant tissues, tissue type and plant flowering times, and whether macerated biofumigants were incorporated into soil or not. In addition, the results illustrate that the conversion of *R. solani* mycelium into sclerotia was enhanced at 1% and 5% soil incorporation of 'Caliente' mustard and 1% of brown mustard and 'Nemat' arugula. 'Nemat' arugula at 10% was the only treatment which significantly reduced *R. solani* sclerotium germination. 'Caliente' mustard, brown mustard and 'Nemat' arugula at 10% incorporation in soil completely inhibited the subsequent mycelium growth from *R. solani* colonised barley grains. Across all the experiments, AITC50 and AITC100 gave similar results for suppression of mycelium growth, sclerotium germination and colonised barley grains positive for the mycelium growth.

Allyl ITC (AITC) was highly toxic to the mycelium growth of *R. solani* LUPP2522, while 2-phenylethyl ITC (2-PEITC) had low effectiveness in reducing the mycelium growth of *R. solani* AG2-1 (LUPP2522). These results agree with the previous study of Sarwar *et al.* (1998), who found that the mycelium growth of one *R. solani* isolate causing bare patch in wheat (unknown AG) was more sensitive to AITC than 2-PEITC in a headspace (volatile activity) experiment, whereas 2-PEITC was more effective at inhibiting mycelium growth compared with AITC when dissolved in PDA. Yulianti *et al.* (2006b) also reported that 2-PEITC was more toxic to mycelium growth from agar plugs of one *R. solani* isolate (AG2-1) causing damping-off of canola seedlings than AITC, while mycelium growth from sclerotia and infested ryegrass seeds was more sensitive to AITC than 2-PEITC in the volatile phase. However, the effects of the isothiocyanate compounds dissolved in agar were not assessed in the present study, as the main purpose was to identify an effective positive control for the following suite of experiments. From the results of this study AITC was chosen as the most appropriate positive control.

The concentration of AITC that suppressed 50% mycelium growth (ED_{50}) of *R. solani* LUPP2522 was 4.71 µM, while the ED_{50} of 2-PEITC was not calculated because of low suppressive efficiency on mycelium growth. This ED_{50} concentration was greater than that determined by Sarwar *et al.* (1998) on the mycelium growth of one *R. solani* isolate (from wheat, unknown AG), where the ED_{50} was calculated as 0.63 µM. That study and the the present research differ in the experimental methods used. Sarwar *et al.* (1998) used 250 mL capacity Erlenmeyer flasks containing 20 mL of PDA for vapour phase experiments, while the present research used deep Petri plates containing 15 mL PDA, creating different head space volumes for volatile compounds. The variation in results from these studies could also be due to differences in the *R. solani* isolates used, and their relative sensitivity to

AITC. The present study used an isolate from potato, while Sarwar *et al.* (1998) used one from wheat (unknown AG). Since *R. solani* AG2-1 isolates are also reported to infect *Brassica* species (Budge *et al.*, 2009b), they may have developed tolerance towards isothiocyanates, which may explain the decreased sensitivity to AITC.

The different *R. solani* isolates (nine isolates of AG3-PT and one of AG2-1) used in this study varied in their sensitivity to pure AITC concentrations. In a study evaluating 2-phenylethyl ITC (2-PEITC) directly dissolved into PDA, inhibition of mycelium growth of ten *R. solani* AG2-1 isolates (from potato, wheat, medic or canola), AG3 (from potato), AG4 (from potato), AG5 (from soybean), AG8 (from barley or wheat roots) and AG9 (unknown host), Smith and Kirkegaard (2002) also found that the isolates varied in sensitivity to 2-PEITC, with the mycelium growth suppression ED_{50}s ranging from 0.053 (AG3) to 0.902 mM (AG2-1), and mycelium inhibition ED_{90}s ranging from 0.094 (AG3) to 1.576 mM (AG2-1). Similar results were obtained in the present study, where the AG3-PT isolates were generally more sensitive to the concentration which suppressed the AG2-1 isolate (LU2522) by 50%. However, as only one AG2-1 isolate was included in the present study, this result may be due to the specific tolerance of the AG2-1 isolate assessed rather than AG2-1 isolates in general being less sensitive to AITC's. In addition, the sensitivity to 2-PEITC of four *R. solani* AG2-1 isolates tested by Smith and Kirkegaard (2002) varied, with ED_{50}s ranging from 0.220 to 0.902 mM and ED_{90}s from 0.321 to 1.576 mM for 2-PEITC. Similar results were seen in the present study, where the sensitivity of the nine AG3-PT isolates to AITC varied. At particular concentrations some isolates were completely inhibited (100%) while others were only inhibited by 59%.

All the selected biofumigant plant types had biofumigation effects on the mycelium growth from propagules of the *R. solani* isolates, but to differing degrees. The two mustard genotypes of *Brassica juncea*, 'Caliente 199' and brown cultivars, were the most effective at reducing the mycelium growth from agar plugs, sclerotia or colonised barley grains. These results concur with those from previous studies. Charron and Sams (1999) reported that 10 g of macerated leaf tissue per Petri dish, from Indian mustard (*B. juncea* 'unknown') resulted in 72.6% inhibition of the mycelium growth from agar plugs of a *R. solani* isolate (unknown AG) causing tomato disease, which was significantly greater than that from kale (*B. oleracea* 'Blue Scotch Curled'), cabbage (*B. oleracea* 'Charmant'), broccoli (*B. oleracea* 'Premium Crop') and second mustard (*B. juncea* 'Florida Broadleaf'). An *in vitro* study by Larkin and Griffin (2007) showed that 1 g per Petri dish of chopped Indian mustard tissue (*B. juncea* 'unknown') resulted in the complete suppression of the mycelium growth from agar plugs of a *R. solani* isolate (isolated from diseased potato plants or tubers, unknown AG) in comparison with canola (*B. napus*, 'Hyola 401'), rapeseed (*B. napus* 'Dwarf Essex'), radish/oilseed (*Raphanus sativa* 'unknown'), turnip (*B. rapa*' Purple Top'), yellow mustard (*Sinapis alba* 'IdaGold'), ryegrass (*Lolium multiflorum*, 'Lemtal'), oat (*Avena sativa*) or barley (*Hordeum vulgare*). The study of Snapp *et al.*

(2007) found that volatiles from 10 g of macerated tissue of oriental mustard (*B. juncea* 'Pacific Gold') per 400 mL capacity glass container gave greater inhibition of the mycelium growth of a composite *R. solani* strain (six AG3 isolates) causing potato disease compared with that from cereal rye (*Cereale secale* 'Wheeler') after two days exposure. Moreover, at 0.5 g of freeze-dried shoot tissue per Petri dish, *B. juncea* ('Caliente 199') mustard completely inhibited the mycelium growth of a *R. solani* isolate (AG2-1) in comparison with a rape/turnip mixture ('BQ-Mulch'; *B. napus/B. campestris*) (Villalta *et al.*, 2016). The highly inhibitory efficiency of mustards, e.g 'Caliente 199', in the present and other studies has been attributed to high levels of allyl glucosinolate in the plant tissues, and release of high levels of volatile AITC which are inhibitory to fungal mycelium growth (Ríos *et al.*, 2016; Villalta *et al.*, 2016). More than 90% of the volatiles released from macerated *Brassica* mustard tissue were AITC (Charron and Sams, 1999). Similarly, for the oomycete pathogen *Phytophthora cinnamomi*, biofumigant crops such as *B. juncea*, *B. carinata*, *B. nigra* and *Armoracia rusticana* rich in allyl glucosinolates were more effective at inhibiting mycelium growth compared with biofumigant plants containing other aliphatic or aromatic glucosinolates. In the present study, most of the biofumigants contained dominant aliphatic glucosinolates, including 'Caliente' mustard, brown mustard, 'Nemat' arugula, 'Lunch' radish, fodder radish, 'Corka' kale, rapeseed, 'Titan' forage rape and 'Pasja II' leafy turnip (Kirkegaard and Sarwar, 1998; Ngala *et al.*, 2014), while only white mustard had a high proportion of aromatic glucosinolates (Kirkegaard and Sarwar, 1998).

'Nemat' arugula was less effective at reducing the mycelium growth from propagules of *R. solani* isolates compared with the two *Brassica* mustards, but was more effective than the other biofumigant plants tested. Two other biofumigant plants, 'Corka' kale and 'Pasja II' leafy turnip, also had the intermediate effects on the mycelium growth of *R. solani* propagules compared with the other crops tested. These results are in agreement with the studies of Kirkegaard and Sarwar (1998), Sarwar and Kirkegaard (1998), Charron and Sams (1999), Villalta *et al.* (2016) and Handiseni *et al.* (2016). These authors also reported that those plants had intermediate glucosinolate concentrations compared with other biofumigant crops, including *B. juncea* mustards, and thus produced less active isothiocyanates from macerated tissues.

Biofumigation efficiency of 'Caliente' mustard, brown mustard, 'Nemat' arugula, 'Corka' kale and 'Pasja II' leafy turnip on mycelium growth from propagules of the *R. solani* isolates AG3-PT and AG2-1 increased with the increasing amounts of the biofumigant crop used and incorporated into soil. These results are similar to those reported by Yulianti *et al.* (2006a, 2006b), where incorporation of tissues of *B. napus* 'Karoo', *B. napus* 'B1', *B. napus* 'B2', *B. nigra* 'unknown' or a brassicaceous weed (*Diplotaxis tenuifolia*) into Lancelin soil at 10% (w:w) was more effective at suppressing the mycelium growth of a *R. solani* AG2-1 isolate from three propagule types (mycelium from agar plugs, sclerotia or mycelium colonised ryegrass seeds) compared with at incorporation at 1%. Mattner *et al.* (2008)

also reported that for *R. fragariae*, the cause of black root rot of strawberry, 2 to 4 g per Petri dish of macerated tissue of a biofumigant mixture (*B. rapa*/*B. napus* 'BQ-Mulch') resulted in greater suppression of mycelium growth compared with lower amounts of tissue (0 to 1 g per Petri dish). Handiseni *et al.* (2016) reported that exposing the mycelium of a *R. solani* AG1A-1A isolate (causing rice sheath blight) to increasing concentrations (from 0.2 g to 3.2 g per Petri dish) of frozen macerated shoot tissue of 'Caliente 199' mustard, or incorporated into soil from 0.2 to 3.2% (w:w), resulted in related increases in inhibition of the mycelium growth. In the present study, the greatest amounts of plant tissues used (5 and 10 g per Petri plate or 5 and 10% w:w in soil) were greater than those in the study of Handiseni *et al.* (2016). In general, for the most effective biofumigant plant types, 'Caliente' mustard, brown mustard and 'Nemat' arugula, increasing the tissue amounts from 5 to 10 g (or 5 to 10% in soil) did not result in increased suppressive effects, especially for the *R. solani* AG3-PT isolate. In contrast, for the less effective biofumigants, 'Corka' kale and 'Pasja II' leafy turnip, increasing the tissue amounts from 5 to 10 g resulted in concurrent decreases in mycelium growth.

For four biofumigant plant types tested, 'Caliente' mustard, brown mustard, 'Nemat' arugula and 'Pasja II' leafy turnip, volatiles from macerated shoot tissue or root plus shoot tissues resulted in greater inhibition of mycelium growth from propagules of both the AG3-PT and AG2-1 *R. solani* isolates compared with inhibition from macerated root tissue. The current data is in agreement with the report of Kirkegaard *et al.* (1996), where shoot tissue of Indian mustard (*B. juncea* '99Y-1-1') was more effective at inhibiting the mycelium growth of five soilborne pathogens of cereals (*Gaeumannomyces graminis* var*. tritici, R. solani, Fusarium graminearum, Pythium irregulare* and *Bipolaris sorokiniana*) than root tissues. *Brassica* roots were shown by Kirkegaard and Sarwar (1998) to mainly contain aromatic glucosinolates, which are the precursors of aromatic ITCs, while *Brassica* shoots were rich in aliphatic glucosinolates, the precursors of aliphatic ITCs. Aromatic ITCs are less volatile and, therefore, less toxic to the mycelium growth of *R. solani* compared with aliphatic ITCs in the gaseous/vapour phase (Sarwar *et al.*, 1998), as was tested in the current research. The current results contrast with those of Mattner *et al.* (2008), where macerated roots of a *B. rapa*/*B. napus* mixture were six times more inhibitory to mycelium growth of *R. fragariae* than macerated shoots. This was suggested to be associated with the greater concentrations and more diverse range of isothiocyanates produced from *B. rapa*/*B. napus* macerated roots in comparison with those in macerated shoots (Mattner *et al.*, 2008). In contrast, the macerated root plus shoot tissue of 'Pasja II' leafy turnip were more effective at reducing mycelium growth of both the *R. solani* AG3-PT and AG2-1 isolates, compared with root or shoot tissue alone. The results concurred with those of Snapp *et al.* (2007) that combination of roots and shoots of *B. juncea* ('Pacific Gold') were the most effective for suppressing *R. solani* mycelium growth, followed by shoots. In contrast to the other biofumigant plants tested, for 'Corka' kale the root tissue was more effective than shoot tissue alone or in combination with root tissue for inhibiting mycelium growth of both *R. solani* isolates. This result was

similar to that reported by Mattner *et al.* (2008) for *R. fragariae* inhibition by *B. rapa*/*B. napus* mixture. Because roots partly contribute to the total glucosinolates present in *Brassica* plants (Kirkegaard and Sarwar, 1998), the experiments carried out with shoots only (Charron and Sams, 1999; Villalta *et al.*, 2016; Handiseni *et al.*, 2016, 2017) may not reflect the actual biofumigation potential of crops against plant pathogens.

'Caliente' mustard harvested at three flowering stages did not affect the biofumigant potential for suppression of *R. solan*i mycelium growth. However, brown mustard harvested at mid and full flowering stages, and 'Nemat' arugula harvested at first and mid flowering stages were, overall, more effective at reducing *R. solani* mycelium growth. These results are similar to those reported by Kirkegaard and Sarwar (1998) and Sarwar and Kirkegaard (1998), who reported that the total glucosinolate levels in *Brassica* tissues peak at mid flowering growth stages, leading to potentially greater biofumigation capacity due to the release of greater amounts of the active isothiocyanates from plant tissue. In addition, 'Caliente' mustard harvested from the three growth stages was more effective at suppressing the mycelial growth of *R. solani* LUPP2522 (AG2-1) than brown mustard and 'Nemat' arugular. This could be due to 'Caliente' mustard tissues having higher glucosinolate levels, thus producing more volatile toxic compounds compared to brown mustard and 'Nemat' arugula tissues. Further research is required to determine total glucosinolates as biofumigation potential in each biofumigant plant.

Sclerotia of *R. solani* were tolerant to biofumigation. Papavizas (1970) noted that sclerotia are one of the main survival propagules of *R. solani* in soil, and that survival results from the specialised sclerotium cell wall structures with loosely arranged and melanised monilioid cells (Coley-Smith and Cooke, 1971; Willetts, 1971; Hyakumachi *et al.*, 1987). These ensure that sclerotia remain dormant or recalcitrant in soil under adverse conditions such as toxic volatiles released from macerated biofumigants.

Within the *R solani* colonised barley grains it is likely that some of the mycelium will be more mature than that on agar plugs which were taken from the actively growing outer edges of PDA colonies. Barley grain mycelium may have thickened hyphal walls (Papavizas, 1970) and high levels of melanin (Hyakumachi *et al.*, 1987). This may result in these hyphae being more tolerant to toxic isothiocyanate volatiles released from biofumigant tissues. Further, even if the volatiles can reduce the viability of mycelium on the outer regions of the barley grains, mycelium within the inner areas of each grain could be protected from the toxic effects of these volatiles, and be able to resume growth after the toxic volatiles have dissipated. Dormant *R. solani* mycelium requires the presence of nutrient base to support initial growth (Papavizas, 1970). The nutrient reserves provided by ryegrass seed was proposed to enable the increased growth of *R. solani* mycelium from colonised ryegrass seeds compared with that from agar plugs (Yulianti *et al.*, 2006b). Since this pathogen survives in soil

between crops as sclerotia or on colonised plant tissue (Papavizas, 1970), with mycelium growth from these propagules being responsible for primary infection of young potato plants, the importance of nutrient source should be confirmed in further studies.

The current *in vitro* studies have shown that maximum efficacy of biofumigant tissues was seen after 3 days, whereas when they were incorporated into soil the effects were the greatest after 7 days. This difference could be explained by the released biofumigant volatiles being trapped in soil, in soil texture components (Bending and Lincoln, 1999; Price *et al.*, 2005) and/or in soil organic matter (Gimsing *et al.*, 2006; Poulsen *et al.*, 2008). They could also be degraded by soil microbial activity (Price *et al.*, 2005). Dhingra *et al.* (2004) showed that mycelium growth of *R. solani* was completely inhibited by 50 µL/L of pure AITC in the absence of soil, but when AITC was incorporated into soil, a greater concentration of (150 or 200 µL/kg of soil) was required to reduce the viability of propagules, and reduce *R. solani* disease on subsequently planted snap bean and cabbage seedlings. Several other studies have also demonstrated that when mustard tissue is incorporated into soil AITC emission rapidly reduced after 72-96 hours (3-4 days), and was not detected after 10 days (Borek *et al.*, 1995; Morra and Kirkegaard, 2002; Price *et al.*, 2005; Mazzola and Zhao, 2010; Handiseni *et al.*, 2016). Thus, if the volatiles do not reduce the viability of the propagules in the first 24 hours (when concentrations are greatest) then the ability to reduce mycelium growth would rapidly decline, as was observed in the present study.

A low concentration (1% w:w) of macerated biofumigant tissues, particularly for 'Caliente' mustard, conversion of mycelium inoculum into sclerotia was accelerated for both the *R. solani* AG3-PT (LUPP2519) and AG2-1 (LUPP2522) isolates. In addition, for the AG2-1 *R. solani* isolate burial in soil amended with 'Caliente' mustard at 5% or 10% resulted in the greatest sclerotium conversion compared with all other treatments. These results were partially similar to those from f the *in vitro* study of Yulianti *et al.* (2006a), who tested seven green manure species/cultivars, including *B. nigra* '91046', *B. napus* 'B1', *B. napus* 'B2', *B. napus* 'Karoo', oat (*A. sativa* 'Mortlock'), lupin (*Lupinus angustifolius* 'Gungurru') and a brassicaceous weed (*D. tenuifolia*). Only *D. tenuifolia* and *B. nigra* '91046' at high amounts (10% in a Lancelin soil) reduced the conversion of *R. solani* (AG2-1) mycelium into sclerotia, potentially due to biofumigant effects. For non-brassicaceous crops (oat, lupin) or other *Brassica* crops, greater biomass increased sclerotium numbers, which was probably due to increased saprophytic growth of *R. solani*. However, Handiseni *et al.* (2017) reported contrasting results, where volatiles from frozen macerated shoot tissues of 'Caliente 199' mustard incorporated into soil at 0.2 to 3.2% (w:w) resulted in fewer sclerotia forming from mycelium inoculum of *R. solani* AG1-IA, compared with the untreated soil. These results suggested that at low levels of biofumigant incorporation, these biofumigant plants may not only be ineffective at suppressing mycelium growth of *R. solani* isolates but also potentially increase the risk of converting the active mycelium into sclerotia, as observed in the

present study and in other research (Yulianti *et al.,* 2006a; Handiseni *et al.,* 2017). These sclerotia act as survival propagules, allowing the pathogen to survive under adverse soil conditions (including biofumigation), which was observed in the present study.

Burial of sclerotia and colonised barley grains in soil amended with 10% biofumigant reduce pathogen viability, with sclerotia being more tolerant than barley grains, but not as susceptible as mycelium inocula. As described earlier, this was probably due to the resistant sclerotium structure and melanisation of the hyphae in these propagules. The mechanism of sclerotium formation from the actively growing mycelium in soil observed for *R. solani* in this study, and in the research of Yulianti *et al.* (2006a) and Handiseni *et al.* (2017), has not been well-elucidated (Banville *et al.*, 1996; Ritchie *et al.*, 2013). Most of the literature has reported that sclerotium formation is primarily induced by novel compounds secreted during hos plant (potato) senescence, and sclerotia are abundant after potato shoot (haulm) death. However, the compounds responsible have not been identified (Dijst, 1990; Sumner, 1996; Banville *et al.*, 1996; Ritchie *et al.*, 2013). Some studies have shown that sclerotium differentiation in *R. solani* is induced under *in vitro* conditions by oxidative stresses. Sclerotium formation was promoted when oxidative substrates, including H_2O_2, ethylenediaminetetraacetic acid, and Fe^{2+} were added to agar media. Conversely, sclerotium biogenesis was inhibited when agar media was supplemented with reducing compounds, such as ascorbic acid, β-carotene, dimethyl sulphoxide, p-nitrosodimethylaniline, ethanol, benzoate, salicylate and thiourea (Georgiou *et al.*, 2000; Georgiou and Petropoulou, 2001; Patsoukis and Georgiou, 2007; Papapostolou and Georgiou, 2010a, 2010b). These results suggest that some oxidative substances released during the incorporation of macerated biofumigant tissue at different concentrations could trigger active mycelium on agar plugs to convert into sclerotia, as was observed in the present study.

This study showed that, overall, incorporation of the biofumigants into soil at 10% (w:w) was effective at reducing the viability of the *R. solani* propagules. The results contrast with the *in vitro* results indicating that volatiles released from macerated biofumigant tissue did not kill the sclerotia or inoculum in colonised barley grains after 3 or 7 days exposure. Biofumigant tissues mostly contain aliphatic glucosinolates (abundant in shoots) and aromatic glucosinolates (abundant in roots) (Kirkegaard and Sarwar, 1998). Aliphatic ITCs are more volatile and toxic to the mycelium growth of fungi in the gaseous phase than aromatic ITCs, while aromatic ITCs are more suppressive to the mycelium growth when amended into substrates such as agar (contact phase) in comparison with aliphatic ITCs (Sarwar *et al.*, 1998; Kurt *et al.*, 2011). Different pathogen propagules may also vary in their sensitivity to the two glucosinolate groups. Kurt *et al.* (2011) reported that the aromatic ITC benzyl ITC gave greater inhibition of germination of *S. sclerotiorum* sclerotia compared to aliphatic ITCs and other aromatic ITCs tested, while the aliphatic ITC methyl ITC gave greatest inhibition of

mycelium growth. Additionally, in the present study, the mesh bags containing the *R. solani* propagules were buried into soil amended with macerated biofumigant tissue, so any released ITC compounds could affect to the propagules through gaseous and contact phases, potentially acting synergistically to increase the biofumigation efficiency, compared with that seen with only the gaseous phase tested in the *in vitro* experiments.

Data from the present study also revealed that biofumigant crops were only effective at reducing the viability of *R. solani* propagules when incorporated into soil at high amounts (10% w:w). In contrast, Handiseni *et al.* (2017) reported that 'Caliente 199' shoots incorporated into soil at lower concentrations (0.2 to 3.2% w:w) were effective at reducing mycelium growth and sclerotium formation from mycelium agar plugs of the rice sheath blight pathogen *R. solani* AG1-IA, and 1.6 to 3.2% incorporation rates reduced sclerotium viability. These contradictory results could be due to differences in the susceptibility of different *R. solani* strains, different planting conditions of biofumigants or different soils. The 'Caliente' plants used by Handiseni *et al.* (2017) were grown in a greenhouse at 14°C (night)-36°C (day). The present study experiment used biofumigants grown over autumn-winter (Table 2.3) in a glasshouse with lower mean temperature and mean humidity and much lower light intensity, compared with plants grown over spring and summer, and used for the other experiments. The glucosinolate levels in biofumigant plant tissues, as precursors of the active isothiocyanate compounds, have been shown to be dependent on environmental conditions during plant growth (Sarwar and Kirkegaard, 1998). Another study showed that day length, photosynthetic photon flux and temperature had the greatest influences on the levels of total glucosinolates (GLS) and glucoraphanin (4-methylsulfinylbutyl GLS) in broccoli (*Brassica oleracea* var. *italica*), Brussels sprouts (*B oleracea* var. *gemmifera*), cabbage (*B oleracea* var. *capitata*), cauliflower (*B oleracea* var. *botrytis*) and kale (*B oleracea* var. *acephala*) (Charron *et al.*, 2005). In addition, some studies have illustrated that temperature had greater effects on total glucosinolate levels than day length (Charron and Sams, 2004; Steindal *et al.*, 2013). Ngala *et al.* (2014) reported that *B. juncea* 'Caliente 99' planted in summer had 50 µmol/g biomass of total GLS and 49 µmol/g biomass of allyl GLS, compared with 11 µmol/g biomass of total GLS and 10 µmol/g biomass of allyl GLS in *B. juncea* planted in winter. Therefore, the biofumigants harvested in the present study may have possessed lower concentrations of glucosinolates, and thus needed to be applied at the greater amounts to effectively inhibit *R. solani* propagules. The present research used an air-dried loamy soil, containing 12.5% clay, 42.4% silt, 45.1% sand, 7.3% organic matter, was of 63.3% water holding capacity and pH_{H2O} = 5.9. In the Handiseni *et al.* (2017) study, the Texas soil used contained 64% clay, 32% silt, 3% sand, 1.8% organic matter, and was of pH = 6.3). Thus, soil conditions may contribute to the variation of results between the two studies.

Overall, selected biofumigant plant types, particularly ‘Caliente’ mustard, brown mustard and ‘Nemat’ arugula had potential to inhibit *R. solani* inocula, both in the *in vitro* and in the soil experiments. However, biofumigation efficiency depended on plant type, biofumigant amount, and planting conditions. In addition, soil conditions are reported to have different effects on both *R. solani* inoculum survival and biofumigation effectiveness. Thus, it is necessary to conduct further studies to evaluate biofumigation potential towards *R. solani* inocula, for promising biofumigant plant types under different edaphic conditions.

Chapter 3

Influence of edaphic conditions on biofumigation potential for suppression of *Rhizoctonia solani* AG3-PT inoculum

3.1 Introduction

Since the effective soil fumigant, methyl bromide, has been phased out in crop production systems, biofumigation has been extensively studied as a potential alternative strategy for management of soilborne pests, pathogens, nematodes, and weeds (Kirkegaard *et al.*, 2000; Bello *et al.*, 2004; Larkin and Griffin, 2007; Snapp *et al.*, 2007; Njoroge *et al.*, 2008; Mattner *et al.*, 2008; Ukeh *et al.*, 2010; Martínez *et al.*, 2011; Ojaghian *et al.*, 2012a, 2012b; Ngala *et al.*, 2014; Rudolph *et al.*, 2015). Biofumigation plants used as cover or cash crops in potato cropping systems in the USA have potential for control of *Rhizoctonia solani* and other soilborne pathogens causing diseases of potato plants and tubers (Specht and Leach, 1987; Larkin and Honeycutt, 2006; Larkin and Griffin, 2007; Halloran *et al.*, 2008; Bernard *et al.*, 2014; Larkin, 2013; Larkin and Halloran, 2014; Larkin *et al.*, 2010, 2011, 2012, 2017). Comparable research has not been carried out in New Zealand.

The potential of biofumigation depends on the biofumigant plant types, biofumigant amounts and the target pathogens (Smith and Kirkegaard, 2002; Kirkegaard, 2009). In addition, biofumigation efficiency mostly relies on amounts of biofumigant tissues macerated, incorporation efficiency into crop soils, and watering and covering of biofumigant-treated soils. Fine disruption of biofumigant crops and sufficient soil moisture (50-70% field capacity) can increase levels of isothiocyanates (ITCs), the bioactive compounds produced in biofumigant plants, from 1% to 79% (Smith and Kirkegaard, 2002; Gimsing and Kirkegaard, 2006). As the biofumigation processes occurs in soil, edaphic conditions can greatly influence their efficiency, through effects on glucosinolates (GLSs) and ITCs. GLSs and ITCs can be degraded by soil microorganisms (Price *et al.*, 2005; Gimsing *et al.*, 2006; Gimsing *et al.*, 2007a, 2007b; Gimsing and Kirkegaard, 2009). Organic-rich soils strongly absorb ITCs, and reduce their bioavailability (Gimsing *et al.*, 2006; Poulsen *et al.*, 2008). Soil pH strongly affects ITC formation, and consequently influences biofumigation effectiveness. ITCs, the most bioactive products, are formed at soil pH 5-7, while lower activity compounds such as thiocyanates are formed at soil pH >7, and nitriles at pHs of 2-5 or in the presence of Fe^{2+} (Borek *et al.*, 1994; Brown and Morra, 1997; Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006). Price *et al.* (2005) reported

that measured allyl ITC concentration in a sandy loam soil was greater than that in a clay loam soil. Understanding soil conditions before biofumigation implementation could, therefore, improve biofumigation efficiency managing soilborne plant pathogens.

Besides the brief pathogen suppressive effects by bioactive volatiles (Gimsing and Kirkegaard, 2006, 2009), incorporation of macerated biofumigant tissues into soil can also enhance soil microbial activities, particularly those of beneficial microorganisms (Yulianti *et al.*, 2007; Larkin *et al.*, 2010; Mazzola *et al.*, 2017). Yulianti *et al.* (2007) reported that incorporation of *Diplotaxis tenuifolia* and *Brassica nigra* into a Lancelin soil at 5% (w:w) increased soil microbial activity (measured as fluorescein diacetate activity or FDA) more than incorporation at 1% (w:w), or in an untreated control. In addition, there was no damping off caused by *Rhizoctonia solani* (AG2-1) on canola seedlings sown in soil into which *D. tenuifolia* or *B. nigra* tissue had been incorporated at 5% (w:w) after 6 months incubation (Yulianti *et al.*, 2007).

In some cases, increased soil microbial activity from biofumigation may result in suppression of pathogen inoculum (Matthiessen and Kirkegaard, 2006; Motisi *et al.*, 2010; Yim *et al.*, 2016; Larkin *et al.*, 2017; Mazzola *et al.*, 2017). For instance, biofumigation stimulated microbes (*Streptomyces* spp.) involved in nitrification and nitrous oxide production, and consequently, these enhanced functions suppressed Rhizoctonia root rot of apple (Cohen *et al.*, 2005; Cohen and Mazzola, 2006). These changes may also account for pathogen control efficiency together with the short term effects of toxic volatiles.

The aim of the experiments reported in this chapter was to determine biofumigation potential of biofumigant plant types selected from results described in Chapter 2 ('Caliente' mustard, brown mustard and 'Nemat' arugula), under different edaphic conditions, for suppression of *Rhizoctonia solani* AG3-PT. The soil factors considered were soil type, pH, temperature and water holding capacity.

3.2 Materials and methods

3.2.1 *Rhizoctonia solani* AG3-PT inoculum

The inoculum of a *R. solani* AG3-PT isolate (LUPP2519) was prepared on barley grains as described in Section 2.2.1 (Chapter 2). Barley grains colonised by the fungus were spread on plastic trays and placed under sterile airflow in a laminar flow hood until dried (3-4 days) (Figure 3.1). The dried grains were then ground using a food blender, and then passed through a 2-mm mesh sieve to obtain consistently sized fragments. The sieved material was stored at 4°C before use in experiments.

Figure 3.1 *Rhizoctonia solani* colonised barley grains spread on a tray and air-dried in a laminar flow hood.

3.2.2 Biofumigants

The three most effective biofumigant cultivars for suppression of *R. solani* isolates, as determined by Chapter 2, were used for the following experiments. The biofumigant plant types were 'Caliente' mustard, brown mustard and 'Nemat' arugula. The biofumigants were planted in potting mix (as described in Section 2.2.2) under glasshouse or shadehouse conditions (Table 3.1), harvested at the mid flowering stage, and the harvested plant material was stored at -20°C until used.

Table 3.1 Temperature (°C), relative humidity (%), and light intensity (lux) (mean (minimum-maximum)) in the glasshouse/shadehouse, during growth of biofumigant and potato plants. These parameters were recorded using a HOBO data logger (Model U12-012, Onset Computer Corporation USA).

Crop	Temperature (°C)	Relative humidity (%)	Light intensity (lux)	Plants used in
Biofumigant (glasshouse)	18.3 (12.5-35.4)	62.9 (18.4-86.1)	3,857 (4-32,280)	Sections 3.2.4.1, 3.2.4.2, 3.2.4.3, 3.2.4.4
Biofumigant (shadehouse)	22.7 (15.4-42.8)	65.5 (14.6-94.6)	6,927 (4-32,280)	Section 3.2.4.5
Potato (shadehouse)	19.9 (12.2-37.3)	68.1 (18.5-92.7)	5,525 (4-32,280)	Section 3.2.4.5

3.2.3 Soil preparation

Field soils (0-20 cm depth) (Table 3.2) were collected, air-dried, passed through a 2-mm mesh sieve and stored at 4°C until used, which was within 2-3 months from collection. Soil characteristics were determined, including texture (Appendix B.2.1, Chapter 2), water holding capacities (Appendix B.2.2, Chapter 2), organic matter contents (Appendix B.2.3, Chapter 2), and pHs (Appendix B.2.4, Chapter 2) (Table 3.2).

Table 3.2 Characteristics of the field soils used in experiments.

Location	Coordinates	Soil texture (%)			Soil type (USDA)	pH_{H2O}	Water holding capacity (%)	Organic matter (%)	Used in
		Sand	Silt	Clay					
Lincoln University	S 43°38'54.0384" E 172°27'43.0308"	45.1	42.4	12.5	Loam	5.9	63.3	7.3	Sections 3.2.4.1, 3.2.4.2, 3.2.4.3, 3.2.4.4, 3.2.4.5
West Melton	S 43°32'19.2264" E 172°22'57.1836"	78	15	7	Loamy sand	5.7	10.4	3.4	Section 3.2.4.1
Lincoln	S 43°38'52.1484" E 172°28'21.4248"	22	51	27	Clay loam	6.1	54.0	5.0	Section 3.2.4.1

3.2.4 Experimental designs

A series of experiments were carried out to determine the effects of biofumigation as influenced by different edaphic factors, including texture, pH, temperature, and water holding capacity, on *R. solani* AG3-PT inoculum levels determined using quantitative polymerase chain reaction (qPCR) techniques.

3.2.4.1 Influence of soil type on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum and soil microbial activity

Three soil types, including loam, loamy sand and clay loam (Table 3.2), were used in this experiment. Air-dried soil was inoculated with *R. solani* colonised barley grains (Section 3.2.1) at 5% (w:w), and mixed thoroughly. Inoculated soil was then placed into 350 mL capacity polypropylene pots (Stowers, New Zealand), at 250 g per pot. Fine macerated biofumigant tissues (as described in Section 2.2.4.1, Chapter 2) were incorporated and thoroughly mixed into the inoculated soil at 5% (w:w). The soil in the pots was then watered to 70% water holding capacity. The pots were tightly sealed with lids for 14 days and then the lids were loosened. The pots were weighed every 7 days and water was added

to maintain the water holding capacity at 70%. For each soil type, pots containing *R. solani* uninoculated soil or *R. solani* inoculated soil without added biofumigant crop tissues were also set up as experimental controls. The experimental treatments were replicated three times and arranged in completely randomised block design (Figure 3.2). The pots were incubated for 28 days at 22°C in a growth room.

3.2.4.2 Influence of soil pH on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum and soil microbial activity

This experiment evaluated effects of soil pH (4.8, 5.8 or 6.6) on biofumigation. This pH range represented typical pH range for New Zealand agricultural soils (Rooney *et al.*, 2007). The pH of the soil collected from the Lincoln University field (loam soil, pH_{H2O} = 5.9, Table 3.2) was adjusted to experimental pHs of 4.8, 5.8 and 6.6 as experimental treatments, using the method described in Appendix A.3.1 (Figure A.3.1). The experiment was set up as described in Section 3.2.4.1.

3.2.4.3 Influence of soil temperature on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum and soil microbial activity

This experiment used the soil collected from Lincoln University field (Table 3.2). The experiment was set up as described in Section 3.2.4.1, except that the experimental treatments were incubation at three temperatures of 10°C, 15°C or 20°C, in controlled temperature incubators (Sanyo, Japan) (Figure 3.2).

Figure 3.2 Pots were arranged in a randomised block design in a SANYO incubator (in this case, set at 15°C.

3.2.4.4 Influence of soil water holding capacity on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum and soil microbial activity

This experiment used the soil collected from the Lincoln University field (Table 3.2). The experiment was set up as described in Section 3.2.4.1, except that the soil was adjusted to three water holding capacities (WHC) as experimental treatments, of 40% WHC, 70% WHC or 100% WHC (as described in Appendix B.2.2, Chapter 2).

3.2.4.5 Influence of soil water holding capacity and temperature on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum and soil microbial activity

Based on the results from the experiments described in Sections 3.2.4.1, 3.2.4.2, 3.2.4.3 and 3.2.4.4, a further experiment was conducted to evaluate the effects of biofumigation on *R. solani* AG3-PT inoculum, using 'Caliente' mustard, under a combination of soil conditions, including two water holding capacities and two temperatures. The experiment used the loam soil, collected from the Lincoln University field (Table 3.2).

The air-dried soil was mixed with *R. solani* AG3-PT (LUPP2519) inoculum (Section 3.2.1) at 5% (w:w). Inoculated soil was placed into 700 mL capacity plastic pots (Stowers, New Zealand) at 650 g per pot, and 5% (w:w) of finely macerated 'Caliente' mustard biofumigant crop tissue (as described in Section 2.2.4.1, Chapter 2) was thoroughly mixed into the soil in each pot, using a sterilised spoon. The pots were irrigated with tap water to obtain 40% or 70% WHC, and the pots were immediately sealed with plastic film and lids. The pots were incubated at 15 or 22°C for 28 days, and were weighed every 7 days and water was added to maintain the required WHCs. After 14 days incubation, the plastic film was removed and the pot lids were loosened. The experiment was set up as a 2 x 2 factorial experiment with treatments of one biofumigant ('Caliente' mustard), two levels of water holding capacity (40% or 70%), and two levels of temperature (15°C or 22°C), with three replicates for each treatment. For each soil temperature x water holding capacity treatment combination, pots containing *R. solani* non-inoculated soil or *R. solani* inoculated soil without biofumigation tissue were also included as experimental controls.

A further control with heat-treated *R. solani* colonised barley grain fragments was also included to evaluate whether qPCR detected DNA from non-viable *R. solani* AG3-PT propagules in the soil. The heated inoculum was prepared by spreading a thin layer (2-3 mm) of *R. solani* colonised barley grain material (Section 3.2.1) on aluminium trays and placing the trays into an oven at 70°C for 8 hours. Samples of the heat-treated inoculum were placed on potato dextrose agar (PDA) plates each hour to determine that the *R. solani* AG3-PT inoculum was not viable before being used in the experiment.

After 28 days incubation, the pots were planted with seed tubers of potato 'Jersey Benne' (supplied by Morton Smith-Dawe Ltd, New Zealand). The seed tubers were treated with 0.5% formaldehyde

solution for 5 minutes (Falloon, 2008), then dried completely. The treated seed tubers were then kept placed in the dark at room temperature until sprouted. One sprouted seed tuber was placed centrally in each pot and covered with treated soil. The pots were set up in a completely randomised factorial design under shadehouse conditions (Table 3.1), and were watered as required to apply the designated soil water holding capacity treatments. Thirty five days after planting, the resulting potato plants were harvested, and the plants were washed thoroughly and assessed for *R. solani* disease on the stems.

The severity of Rhizoctonia stem canker was evaluated using a 0-6 scale (Gibson and Falloon, 2016) (Appendix A.3.2), where 0 = no symptoms; 1 = 1-10% of stem area covered with lesions; 2 = 10-30%, 3 = 30-50%, 4 = 50-80%, 5 = 80-100% of stem area covered with lesions, and 6 = stem dead. The disease severity (DS) was calculated using the following formula (Atkinson *et al.*, 2010):

$$\mathrm{DS}\ (\%) = \frac{\sum_{i=0}^{m}(n_i \times i)}{N \times m} \times 100$$

Where: **i =** the i^{th} disease score

m = the greatest disease score

n_i = number of stems in the i^{th} disease score

N = total number of stems

Potato plant height (cm) and total dry biomass (dried at 60°C for 3 days) were measured.

3.2.3 Soil sampling

For the experiments described in Sections 3.2.4.1, 3.2.4.2, 3.2.4.3 and 3.2.4.4, soil samples (30 g) were taken from five positions in each plastic pot, using a cork borer (1 cm diameter), after 28 days incubation (Figure 3.3). For the experiment described in Section 3.2.4.5, the soil was sampled after 14 or 28 days incubation. The five collected soil samples from each pot were thoroughly mixed, then divided into two parts, one was stored at -80°C prior to DNA extraction (as described in Section 3.2.4.7), and the other was stored at 4°C for determining soil microbial activity (Section 3.2.4.6).

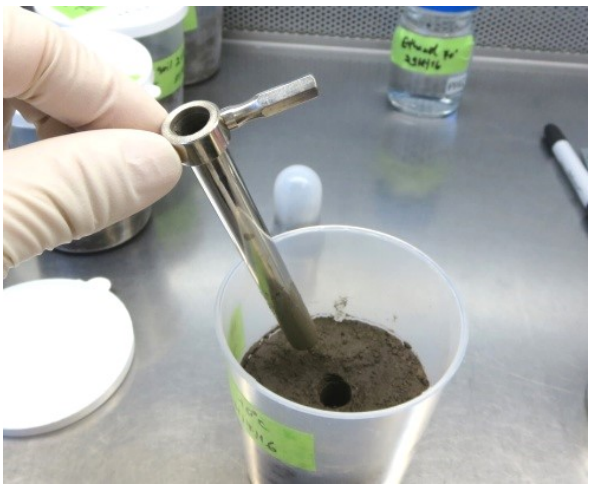


Figure 3.3 Soil sampling after 28 days of incubation.

3.2.4 Assessments

3.2.4.1 Amounts of *Rhizoctonia solani* AG3-PT DNA in soil

The soil samples were prepared for DNA extraction based on the unpublished method of Dr Jana Monk (personal communication). For each replicate pot, 15 g of the composite soil sample was placed into a 150 mL capacity autoclaved glass bottle with 120 mL of sterilised water, and shaken for 10 minutes on a flask shaker (SF1, Stuart, Cole-Parmer, Stafforshire, United Kingdom). A 2 mL aliquot of the soil suspension was then placed into a 2 mL O-ring centrifuge microtube (Axygen™, USA). The microtubes were centrifuged (GyroSpin, Biolab, Canada) at 11,400 *g* for 10 minutes, the supernatant was discarded, and the remaining pellet (≅ 250 mg) used for DNA extraction. Soil DNA from the pellet was extracted using the PowerSoil® DNA isolation kit (MoBio Laboratories, Carlsbad, California, USA) following the manufacturer's instructions. DNA extracted from sclerotia of *R. solani* AG3-PT (LUPP2519) (35 days growth on PDA at 25°C) was used as standards for the qPCR. Sclerotia were frozen in liquid nitrogen for 1 minute, and then were ground into fine powder using a sterilised mortar and pestle. DNA was extracted from the ground sclerotium powder using the PowerSoil® DNA isolation kit.

The amounts of *R. solani* AG3-PT DNA in different treatment soil samples were determined using the qPCR method described by Woodhall *et al.* (2013). The quantitative PCR assay used rDNA (TaqMan™) probe sequences specific to *R. solani* AG3-PT, which were developed from the ribosomal ITS1 region. The specific primers and probe were as described in Table 3.3.

Table 3.3 Primers and TaqMan probe used in qPCRs to determine amounts of *Rhizoctonia solani* AG3-PT DNA in soil samples.

Primer/Probe name	Sequence (5'-3')
AG3-PT_F (Forward primer)	ATGAAGAGTTTGGTTGTAGCTGGTCT
AG3-PT_R (Reverse primer)	TATTACAA**W**AAATAACAAATAAATTCCCCAA
AG3-PT_P (Probe)	FAM-CCCTCTTTCATCCCACACACACCTG-TAMRA

W: ambiguous code for an A and a T; FAM: 6-carboxyfluorescein; TAMRA: tetra-methylrhodamin

Validation of specific primers for *Rhizoctonia solani* AG3-PT

Before setting up the qPCR, the primers (AG3-PT_F and AG3-PT_R) were validated using standard PCR, to confirm they were specific for *R. solani* AG3-PT (LUPP2519), and only. DNA of *R. solani* AG2-1 (LUPP2522) was used as the control.

Each 20 μL reaction included sterile Millipore water, 2.0 μL of 10X PCR buffer (final concentration of 1X), 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 μL of 6 μM each forward/reverse primers (300 nM), 1.25U of Taq DNA polymerase (FastStart, Roche) and 1 μL of DNA template (50 ng/μL). The thermal cycle was 95°C for 4 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute; and a final stage at 72°C for 10 minutes.

The PCR products were separated in 1.5% agarose gels (in 1X TAE, Tris-Acetate-EDTA) at 100 V for 50 minutes. The gels were stained in ethidium bromide solution (0.5 μg/mL) for 20 minutes, and washed by soaking in tap water for 10 minutes to remove unbound ethidium bromide. The gels were visualised in a UV transilluminator (UVITEC Cambridge, Total Lab System Ltd, New Zealand). The expected amplicon was approx. 122 bp (Woodhall *et al.*, 2013).

Quantitative PCR

The reactions were performed in 0.1-mL MicroAmp™ fast optical 96-well reaction plates (Cat. No. 4398021, Applied Biosystems™, USA). The components for each 20 μL reaction contained 10 μL of Taqman™ environmental master mix 2.0 (Cat. No. 4396838, Applied Biosystems™, USA), 300 nM of each primer and 100 nM of Taqman™ probe (Cat. No. 450025, Applied Biosystems™, USA), 4 μL of DNA template, and the appropriate amount of ultrapure™ DNase/RNase-free distilled water (Cat. No. 10977-015, Invitrogen, USA). Each plate was then covered with a MicroAmp™ optical adhesive film (Cat. No: 4311971, Applied Biosystems™, USA) and put in the StepOnePlus™ Real-Time PCR System (Applied Biosystems™, USA). The thermal cycle included 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute (Woodhall *et al.*, 2013). Each sample was replicated twice. The negative control consisted of ultrapure™ DNase/RNase-free distilled water in

place of the DNA template, and a second control of pure DNA of *R. solani* AG2-1 (LUPP2522) (10 ng/μL) was also included.

A standard curve based on cycle threshold (C_T) values and pure DNA concentrations (10-fold dilution series) of *R. solani* AG3-PT extracted from pure cultures was constructed. For samples from the four experiments described in Sections 3.2.4.1, 3.2.4.2, 3.2.4.3, 3.2.4.4, six pure DNA concentrations were prepared as standards at 11.10, 1.11, 1.11 x 10^{-1}, 1.11 x 10^{-2}, 1.11 x 10^{-3}, or 1.11 x 10^{-4} ng/μL, with two replicates for each concentration. For samples from the experiment described in Section 3.2.4.5, seven pure DNA concentrations were used as standards at 11.35, 1.135, 1.135 x 10^{-1}, 1.135 x 10^{-2}, 1.135 x 10^{-3}, 1.135 x 10^{-4}, or 1.135 x 10^{-5} ng/μL, with three replicates for each concentration. The C_T values were calculated by the StepOne™ software (version 2.2) installed in the StepOnePlus™ Real-Time PCR System (Applied Biosystems™, USA) (Woodhall *et al.*, 2013). The C_T was determined as the starting point of the thermal cycle at which fluorescence intensity was exponentially increased (Hussain *et al.*, 2014). Calculated C_T values were then plotted against the log of the serial concentrations of pure *R. solani* AG3-PT DNA to generate a standard curve to determine the relationship between known *R. solani* AG3-PT DNA concentrations and C_T values (Appendices A.3.3, A.3.4, A.3.5, A.3.6, A.3.7). The *R. solani* AG3-PT DNA amounts in the soil samples (pg DNA/g dried soil) at the different sampling times for the different treatments were estimated based on the standard curve (Lees *et al.*, 2002). The qPCR products generated for the standard curves (≅ 122 bp) were examined by 1.5% agarose (Figure 3.5).

3.2.4.2 Soil microbial activity

The soil microbial activity was determined using dehydrogenase activity, as described by Cresswell and Hassall (2015) (Appendix A.3.8) (Figure 3.4).

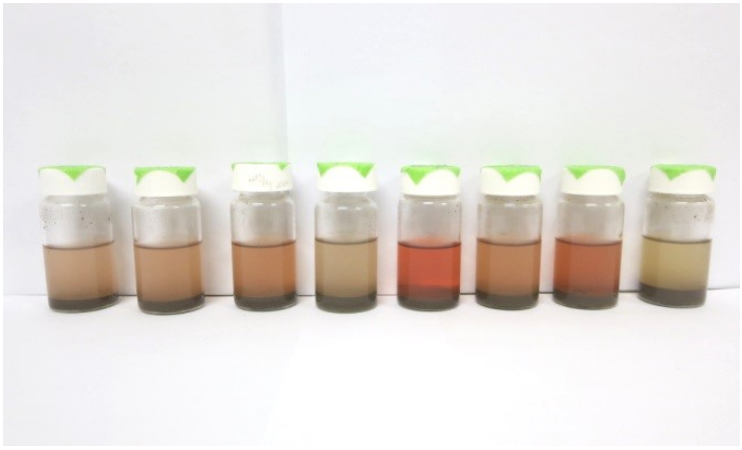


Figure 3.4 Soil samples after treatment with 2,3,5-triphenyltetrazolium chloride and incubated 24 hours at 25°C, then treated with methanol before measurement of dehydrogenase activity.

3.2.5 Data analyses

The raw data were first examined for assumption of normal distributions using GenStat software (Version 18.1.0.17005; VSN International Ltd, Hemel Hempstead, United Kingdom). If the data satisfied normal distributions, they were directly used for statistical analyses; otherwise, they were appropriately transformed before analysis.

For the data from the experiments described in Sections 3.2.4.1, 3.2.4.2, 3.2.4.3 and 3.2.4.4, the mean amounts of *R. solani* AG3-PT DNA in soil (pg DNA per g dried soil) from the different treatments were used to compare treatment differences, using two-way analysis of variance (ANOVA), with means for treatment, soil texture, soil pH, soil temperature, soil water holding capacity, and interactions (treatment x soil texture, treatment x soil pH, treatment x soil temperature, treatment x soil water holding capacity) separated using Tukey's Honestly Significant Difference (HSD) at P=0.05.

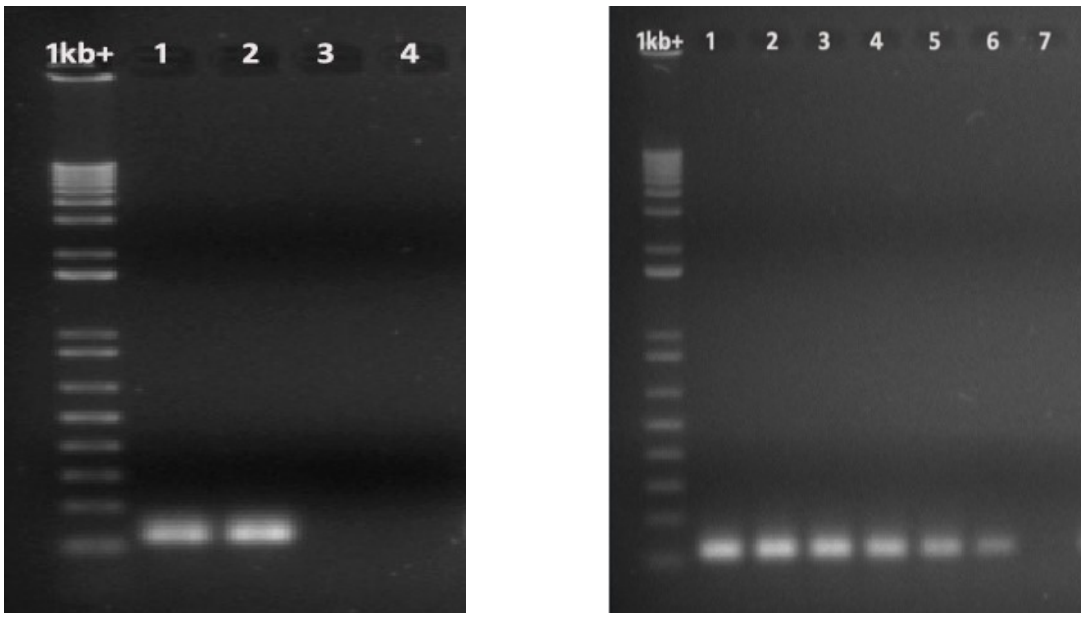
For the data from the experiment described in Section 3.2.4.5, mean amounts of *R. solani* AG3-PT DNA (pg DNA per g dried soil) from different treatments were used to compare differences using three-way analysis of variance (ANOVA), with means for treatment, soil temperature, soil water holding capacity, and interactions (treatment x soil temperature, treatment x soil water holding capacity, soil temperature x soil water holding capacity, treatment x soil temperature x soil water holding capacity) separated using Tukey's HSD test at P=0.05.

Possible correlations between *R. solani* AG3-PT DNA amounts and soil microbial activity were determined using the GenStat software.

3.3 Results

3.3.1 Sensitivity of qPCR used to detect *Rhizoctonia solani* AG3-PT DNA in soil

A PCR product amplicon (≅ 122 bp) was only generated using the AG3-PT_F/AG3-PT_R primers from DNA of two *R. solani* AG3-PT isolates (LUPP2517 and LUPP2519), and no visible amplimers were produced from *R. solani* AG2-1 DNA (LUPP2522) (Figure 3.5A). In addition, the qPCR amplicon from the standard (isolate LUPP2519) was approx. 120 bp (Figure 3.5B).



A B

Figure 3.5 Checking PCR product (≅ 122 bp) (A) generated by AG3-PT_F/AG3-PT_R primers on 1.5% agarose gels: 1kb+, 1 kb plus ladder (Invitrogen); 1, AG3-PT DNA (LUPP2517); 2, AG3-PT DNA (LUPP2519); 3, AG2-1 DNA (LUPP2522); 4, negative control. Checking qPCR product (≅ 122 bp) (B) generated by AG3-PT_F/AG3-PT_R primers on 1.5% agarose gel: 1kb+, 1 kb plus ladder (Invitrogen); 1, 44.4 ng; 2, 4.44 ng; 3, 4.44 x 10^{-1} ng; 4, 4.44 x 10^{-2} ng; 5, 4.44 x 10^{-3} ng; 6, 4.44 x 10^{-4} ng; 7, Negative control. The PCR products were separated at 100 V for 55 minutes.

The standard curves generated from the logarithm of the pure *R. solani* AG3-PT DNA concentrations (x) and cycle threshold (y) were closely correlated (R^2 values ≥ 0.995), with the PCR efficiencies calculated as from 90.0 to 91.5% for the different experiments (Table 3.4).

Table 3.4 Regression equations, correlation coefficients (R^2) and PCR efficiencies generated for the qPCRs for quantification of *Rhizoctonia solani* AG3-PT.

Section	Experiment	Regression equation	Correlation coefficient	PCR efficiency (%)
3.2.4.1	Soil type	y = -3.579x + 20.412	R^2 = 1.000	90.3
3.2.4.2	Soil pH	y = -3.571x + 21.458	R^2 = 0.9950	90.6
3.2.4.3	Soil temperature	y = -3.5889x + 19.598	R^2 = 0.9961	90.0
3.2.4.4	Soil water holding capacity (WHC)	y = -3.579x + 20.412	R^2 = 1.000	91.5
3.2.4.5	Soil temperature and WHC	y = -3.5817 + 16.669	R^2 = 0.9962	90.2

3.3.2 Influence of different soil conditions on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

There was no *R. solani* AG3-PT DNA detected in any of the uninoculated controls in any of the experiments, or and for the heat-treated inoculum in the temperature and water holding capacity experiment (Section 3.2.4.5). These control data were therefore omitted from the statistical analyses. Compared with the amounts of *R. solani* AG3-PT DNA in the soil at the beginning of the experiments (presented in Table 3.5), the amounts of *R. solani* AG3-PT DNA from all treatments reduced over the 14-28 day incubation periods (Sections 3.3.2.1 to 3.3.2.5).

Table 3.5 Initial mean amounts of DNA of *Rhizoctonia solani* AG3-PT in the inoculated control treatment soils used in the different experiments.

Section	Experiment	Initial DNA concentration (pg/g dried soil)
3.2.4.1	Soil type	1,554,560
3.2.4.2	Soil pH	1,592,699
3.2.4.3	Soil temperature	1,530,560
3.2.4.4	Soil water holding capacity	1,644,629
3.2.4.5	Soil water holding capacity and temperature	345,463

3.3.2.1 Influence of soil type on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

Statistical analyses for data from this experiment are shown in Appendix A.3.9, and the results are summarised in Table 3.6. There was a statistically significant interaction (P<0.001) between treatment and soil type for mean amounts of *R. solani* AG3-PT DNA. For all treatments apart from the uninoculated control, where no *R. solani* AG3-PT DNA was detected, the *R. solani* AG3-PT DNA amounts in the clay loam soil were greater than those in the loam or loamy sand soils, with that in the loamy sand soil being greater than in the loam soil. All the biofumigant plant type treatments reduced *R. solani* AG3-PT DNA amounts compared the inoculated control in all of the soils. However, the most effective biofumigant plants for reducing *R. solani* AG3-PT DNA varied in the different soils. In the loam soil, 'Nemat' arugula (mean = 706 pg DNA/g dried soil) reduced *R. solani* AG3-PT DNA more than 'Caliente' mustard or brown mustard, with 'Caliente' mustard (1,099 pg DNA/g dried soil) more effective than brown mustard (1,838 pg DNA/g dried soil). In the loamy sand, 'Caliente' mustard (4,675 pg DNA/g dried soil) reduced *R. solani* AG3-PT DNA more than brown mustard (8,204 pg DNA/g dried soil) or 'Nemat' arugula (5,252 pg DNA/g dried soil), with 'Nemat' arugula more

effective than brown mustard. In contrast, in the clay loam soil, brown mustard (25,002 pg DNA/g dried soil) and 'Nemat' arugula (25,485 pg DNA/g dried soil) both reduced *R. solani* AG3-PT DNA to a greater extent than 'Caliente' mustard (35,559 pg DNA/g dried soil).

Across all soil types, there was a statistically significant effect (P <0.001) of treatment on mean amounts of *R. solani* AG3-PT DNA. The inoculated control treatment gave the greatest amount of *R. solani* AG3-PT DNA (24,704 pg DNA/ g dried soil), and the 'Nemat' arugula treatment had the least (5,561 pg DNA/g dried soil). Across the biofumigant treatments, there was a significant effect (P<0.001) of soil texture on mean amount of *R. solani* AG3-PT DNA, with greatest amounts in the clay loam soil (31,359 pg DNA/ g dried soil), followed by the loamy sand soil (8,419 pg DNA/g dried soil) and the loam soil (2,589 pg DNA/ g dried soil).

Table 3.6 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in three soils with different soil types (loam, loamy sand or clay loam), and amended with three biofumigant crops, after 28 days incubation at 22°C.

Treatment	DNA amount (pg/g dried soil)			Mean of treatment
	Loam	Loamy sand	Clay loam	
Uninoculated control	0[(1)]	0[(1)]	0[(1)]	0[(1)]
Inoculated control	15,331 e[(2)]	23,044 d	42,251 a	24,704 A
'Caliente' mustard	1,099 j	4,675 h	35,559 b	6,586 C
Brown mustard	1,838 i	8,204 f	25,002 c	7,790 B
'Nemat' arugula	706 k	5,252 g	25,485 c	5,561 D
Mean of soil texture	2,589 Z	8,419 Y	31,359 X	
P values: <0.001 (Treatment); <0.001 (Soil texture); <0.001 (Treatment x Soil texture)				

[(1)] Zero data omitted from statistical analyses

[(2)]Means within each column or row followed by the same letter are not significantly different (P=0.05) according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.2.2. Influence of soil pH on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

Statistical analyses for data from this experiment are shown in Appendix A.3.10, and the results are summarised in Table 3.7. There was a significant interaction effect (P<0.001) between treatment and soil pH on the mean amounts of *R. solani* AG3-PT DNA in soil samples. For soil at pH 5.8 or 6.6, the amounts of DNA were significantly less for all three biofumigant plant types compared with the inoculated control treatment. At pH 6.6, brown mustard (mean = 352 pg DNA/g dried soil) and

'Nemat' arugula (371 pg DNA/g dried soil) reduced the amounts of DNA compared with 'Caliente' mustard (611 pg DNA/g dried soil). At pH 5.8, Nemat' arugula (906 pg DNA/g dried soil) reduced the amounts of DNA compared with brown mustard (1,217 pg/g dried soil) and 'Caliente' mustard (1,255 pg/g dried soil). However, at pH 4.8, only brown mustard reduced the amount of *R. solani* AG3-PT DNA (15,932 pg DNA/g dried soil) compared with the inoculated control (24,515 pg DNA/g dried soil), with both 'Caliente' mustard (27,635 pg DNA/g dried soil) and 'Nemat' arugula (81,035 pg DNA/g dried soil) giving DNA levels greater than in the inoculated control. For all three biofumigant plant types, *R. solani* AG3-PT DNA amounts were less at pH 6.6 compared with pH 5.8 or pH 4.8, with the amounts at pH 5.8 less than at pH 4.8. In contrast, in the inoculated control treatment, *R. solani* AG3-PT DNA amounts were less at pH 5.8 than at pH 6.6 or pH 4.8, with the amounts at pH 6.6 being less than that at pH 4.8.

Across the three soil pH treatments, there was a significant effect (P<0.001) of treatment on amounts of *R. solani* AG3-PT DNA. The mean amount of *R. solani* AG3-PT DNA was greater from the inoculated control treatment (17,659 pg DNA/g dried soil) compared with all the other treatments, with the DNA amount from the brown mustard treatment being less (2,703 pg/g dried soil) than from 'Caliente' mustard (3,703 pg DNA/g dried soil) or 'Nemat' arugula (4,985 pg DNA/g dried soil). Across treatments, there was a significant effect (P<0.001) of soil pH on amount of *R. solani* AG3-PT DNA in soil, with more DNA at soil pH 4.8 (30,739 pg DNA/g dried soil) compared with pH 5.8 (2,482 pg DNA/g dried soil) or pH 6.6 (1,650 pg DNA/g dried soil), with the amount at pH 5.8 being greater than at pH 6.6.

Table 3.7 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in soil adjusted to three different pHs (4.8, 5.8 or 6.6) and amended with three different biofumigant crops, after 28 days incubation at 22°C.

Treatment	DNA amount (pg/g dried soil)			Mean of treatment
	pH 4.8	pH 5.8	pH 6.6	
Uninoculated control	0[(1)]	0[(1)]	0[(1)]	0[(1)]
Inoculated control	24,515 c[(2)]	14,428 e	15,501 d	17,659 A
'Caliente' mustard	27,635 b	1,255 f	611 h	3,703 C
Brown mustard	15,932 d	1,217 f	352 i	2,703 D
'Nemat' arugula	81,035 a	906 g	371 i	4,985 B
Mean of soil pH	30,739 X	2,482 Y	1,650 Z	
P values: <0.001 (Treatment); <0.001 (Soil pH); <0.001 (Treatment x Soil pH)				

[(1)]Zero data omitted from statistical analyses

[(2)]Means within each column or row followed by the same letter are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.2.3 Influence of soil temperature on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

Statistical analyses for data from this experiment are shown in Appendix A.3.11, and the results are summarised in Table 3.8. There was a statistically significant interaction (*P*<0.001) between treatment and soil temperature for mean amounts of *R. solani* AG3-PT DNA in soil. For all treatments, apart from the uninoculated controls where no *R. solani* AG3-PT DNA was detected, DNA concentration was greater at 15°C than at 10°C or 20°C. At all soil temperatures, all of the biofumigant plant type treatments reduced the amounts of *R. solani* AG3-PT DNA compared with the inoculated control. However, the relative efficiency of the different biofumigation plants in reducing amounts of *R. solani* AG3-PT DNA differed at the different soil temperatures. At 10°C (mean = 21,699 pg DNA/g dried soil) and at 20°C (16,783 DNA pg/g dried soil), brown mustard reduced DNA more than 'Nemat' arugula (25,062 pg DNA/g dried soil at 10°C, and 32,729 pg DNA/g dried soil at 20°C) and 'Caliente' mustard (37,194 pg DNA/g dried soil at 10°C, and 19,606 pg DNA/g dried soil at 20°C). 'Nemat' arugula was more effective than 'Caliente' mustard at reducing *R. solani* AG3-PT DNA at 10°C, while at 20°C 'Caliente' mustard was more effective than 'Nemat' arugula.

Across the three soil temperatures, there was a significant effect (*P*<0.001) of treatment on amounts of *R. solani* AG3-PT DNA in soils. The amount of *R. solani* AG3-PT DNA in the inoculated control treatment (51,360 pg DNA/g dried soil) was more than in the other treatments, followed by 'Nemat' arugula (32,729 pg DNA/g dried soil), 'Caliente' mustard (31,734 pg DNA/g dried soil), and brown mustard (27,840 pg/g dried soil). Across treatments, there was a significant effect (*P*<0.001) of soil temperature on amounts of *R. solani* AG3-PT DNA. There was more DNA at 15°C (51,481 pg DNA/g dried soil) compared with 10°C or 20°C, with the amount at 10°C (30,117 pg DNA/g dried soil) being greater than at 20°C (27,379 pg DNA/g dried soil).

Table 3.8 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in soil incubated at three different temperatures (10°C, 15°C or 20°C) and amended with three biofumigant crops, after 28 days incubation.

Treatment	DNA amount (pg/g dried soil)			Mean of treatment
	10°C	15°C	20°C	
Uninoculated control	0[(1)]	0[(1)]	0[(1)]	0[(1)]
Inoculated control	40,687 e[(2)]	64,464 a	51,481 c	51,360 A
'Caliente' mustard	37,194 f	43,361 d	19,606 j	31,734 C
Brown mustard	21,699 i	58,566 b	16,783 k	27,840 D
'Nemat' arugula	25,062 h	42,752 d	32,729 g	32,729 B
Mean of soil temperature	30,117 Y	51,481 X	27,379 Z	
P values: <0.001 (Treatment); <0.001 (Soil temperature); <0.001 (Treatment x Soil temperature)				

[(1)]Zero data omitted from statistical analyses

[(2)]Means within each column or row followed by the same letter are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.2.4 Influence of soil water holding capacity on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

Statistical analyses for data from this experiment are shown in Appendix A.3.12, and the results are summarised in Table 3.9. There was a statistically significant interaction effect (*P*<0.001) between treatment and soil water holding capacity (WHC) on mean amounts of *R. solani* AG3-PT DNA in soils. For all three WHCs, all the biofumigant crop treatments reduced *R. solani* AG3-PT DNA compared with the inoculated control treatment. However, the relative efficiency of the different biofumigation plant types for reducing pathogen DNA amounts differed at the different WHCs. At 40% WHC, 'Caliente' mustard (mean = 731 pg DNA/g dried soil) reduced DNA amounts compared with 'Nemat' arugula (1,519 pg DNA/g dried soil) and brown mustard (1,149 pg DNA/g dried soil), with brown mustard less than 'Nemat' arugula. 'Nemat' arugula was more effective at reducing *R. solani* AG3-PT DNA at 70% WHC (1,594 pg DNA/g dried soil) compared with 'Caliente' mustard (1,815 pg DNA/g dried soil) or brown mustard (1,930 pg DNA/g dried soil), with 'Caliente' mustard more effective than brown mustard. At 100% WHC, brown mustard was more effective at reducing the DNA amounts (1,999 pg DNA/g dried soil) compared with 'Caliente' mustard (2,118 pg DNA/g dried soil) or 'Nemat' arugula (2,547 pg DNA/g dried soil), and 'Caliente' mustard reduced the DNA amount more than 'Nemat' arugula. For the inoculated control treatment, the amount of *R. solani* AG3-PT DNA was greater at 40% WHC (31,315 pg DNA/g dried soil) compared with the other two soil WHC treatments, with the DNA amount at 70% WHC (17,871 pg DNA/g dried soil) more than at 100% WHC (7,228 pg

DNA/g dried soil). In contrast, for all the biofumigation treatments the DNA amounts at 100% WHC were greater than at either 40% or 70% WHC, with that at 70% WHC greater than 40% WHC.

Across the three soil WHCs, there was a statistically significant effect (P<0.001) of treatment on amounts of *R. solani* AG3-PT DNA in soil. The inoculated control treatment had the greatest DNA concentration (16,120 pg DNA/g dried soil), followed by the treatments of 'Nemat' arugula (1,851 pg DNA/g dried soil), brown mustard (1,663 pg DNA/g dried soil) and 'Caliente' mustard (1,477 pg DNA/g dried soil). Across treatments, there was a statistically significant effect (P<0.001) of soil WHC on *R. solani* AG3-PT DNA amounts, with more DNA in soil at 70% WHC (3,482 pg DNA/g dried soil), compared with at 40% WHC (3,172 pg DNA/g dried soil and 100% WHC (3,064 pg DNA/g dried soil).

Table 3.9 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in soil adjusted to three different soil water holding capacities (40, 70 or 100% WHC), and amended with three biofumigant crops, after 28 days incubation at 22°C.

Treatment	DNA amount (pg/g dried soil)			Mean of treatment
	40% WHC	70% WHC	100% WHC	
Uninoculated control	0[(1)]	0[(1)]	0[(1)]	0[(1)]
Inoculated control	31,315 a[(2)]	17,871 b	7,228 c	16,120 A
'Caliente' mustard	731 l	1,815 h	2,118 e	1,477 D
Brown mustard	1,149 k	1,930 g	1,999 f	1,663 C
'Nemat' arugula	1,519 j	1,594 i	2,547 d	1,851 B
Mean of soil WHC	3,172 Y	3,482 X	3,064 Z	
P values: <0.001 (Treatment); <0.001 (Soil WHC); <0.001 (Treatment x Soil WHC)				

[(1)]Zero data omitted from statistical analyses

[(2)] Means within column or row followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.2.5 Influence of soil water holding capacity and temperature on biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

For the assessments after 14 days incubation, the statistical analyses of data from this experiment are shown in Appendix A.3.13, and the results are summarised in Table 3.10. No *R. solani* AG3-PT DNA was detected from the uninoculated control or heat treated inoculum treatments, and these were omitted from the statistical analyses. There was a statistically significant interaction effect (P=0.002) between treatment, temperature and soil water holding capacity (WHC) on mean amounts of *R. solani* AG3-PT DNA in soil. For all of the WHC and temperature combinations, 'Caliente' mustard

reduced *R. solani* AG3-PT DNA compared with the inoculated control treatment. For the inoculated control, the amount of *R. solani* AG3-PT DNA was greater in the soil incubated at 15°C and 40% WHC (mean = 164,577 pg DNA/g dried soil) compared with all the other WHC and temperature combinations, with the DNA amount in the soil incubated at 15°C and 70% WHC (54,976 pg DNA/g dried soil) more than for soil incubated at 22°C at either 40% WHC (24,882 pg DNA/g dried soil) or 70% WHC (21,961 pg DNA/g dried soil). For 'Caliente' mustard treatments, the amounts of *R. solani* AG3-PT DNA were greater in soil incubated at 15°C and 40% WHC (25,915 pg DNA/g dried soil) or 70% WHC (31,211 pg DNA/g dried soil) compared with soil incubated at 22°C and 40% WHC (31,211 pg DNA/g dried soil) or 70% WHC (12,032 pg DNA/g dried soil).

There was a statistically significant interaction effect ($P<0.001$) between biofumigant plant type treatment and soil WHC for amounts of *R. solani* AG3-PT DNA. The amount of DNA in the inoculated control treatment at 40% WHC was greater (64,464 pg DNA/g dried soil) than for all the other treatments. Of these treatments, the DNA amount from the inoculated control at 70% WHC (34,810 pg DNA/g dried soil) was greater than from the 'Caliente' amended soil, at either 40 or 70% WHC, with there being no statistically significant difference between these two treatments (means = 16,061 pg DNA/g dried soil for 40% WHC, and 19,464 pg DNA/g dried soil for 70% WHC).

There was a statistically significant interaction effect ($P=0.003$) between biofumigant plant type and temperature for the amounts of *R. solani* AG3-PT DNA. The inoculated control treatment at 15°C gave more *R. solani* AG3-PT DNA (95,161 pg/g dried soil) compared with all the other treatments, with the DNA amounts from the inoculated control at 22°C (23,378 pg DNA/g dried soil) and 'Caliente' mustard at 15°C (28,444 pg DNA/g dried soil) not significantly different from each other, but both being greater compared with 'Caliente' mustard at 22°C (10,858 pg DNA/g dried soil). There was a statistically significant effect ($P<0.001$) of treatment on amount of *R. solani* AG3-PT DNA, with the quantity from the inoculated control treatment (47,417 pg DNA/g dried soil) being greater than from the 'Caliente' mustard treatment (17,664 pg/g dried soil).

There was a statistically significant interaction effect ($P=0.002$) between temperature and water holding capacity on amounts of *R. solani* AG3-PT DNA. Mean amount of DNA was greatest at 15°C and 40% WHC (65,681 pg DNA/g dried soil), followed by treatment at 15°C and 70% WHC (41,462 pg DNA/g dried soil). There was a significant effect ($P<0.001$) of temperature on amount of pathogen DNA, with more DNA in soil at 15°C (52,211 pg DNA/g dried soil) compared with 22°C (16,022 pg DNA/g dried soil). There was also a significant effect ($P=0.006$) of WHC on amount of DNA, with more DNA in soil at 40% WHC (32,420 pg DNA/g dried soil) compared with that at 70% WHC (26,102 pg DNA/g dried soil).

For assessments after 28 days incubation, the statistical analyses are shown in Appendix A.3.14, and the results are summarised in Table 3.11. No *R. solani* AG3-PT DNA was detected in the uninoculated

control or heat treated inoculum treatments, and these were omitted from the statistical analyses. There was no significant interaction effect (P=0.495) between treatment, temperature and soil WHC for mean amounts of *R. solani* AG3-PT DNA in soil (1,401 to 30,010 pg DNA/g dried soil). There was a significant interaction effect (P<0.001) between biofumigant crop treatment and WHC for amount of pathogen DNA. For the inoculated control treatment, soil at 40% WHC had more DNA (mean = 14,653pg DNA/g dried soil) compared with that at 70% WHC (10,609 pg DNA/g dried soil). In contrast, for the 'Caliente' mustard treatment, DNA amount at 40% WHC was 2,565 pg DNA/g dried soil, which was less than that at 70% WHC (3,532 pg DNA/g dried soil).

There was a significant interaction effect (P<0.001) between treatment and temperature. The inoculated control at 22°C had more DNA (23,322 pg DNA/g dried soil) compared with 15°C (6,471 pg DNA/g). Similarly, for 'Caliente' mustard the DNA amount was greater at 22°C (4,243 pg DNA/g) compared with 15°C (2,082 pg DNA/g dried soil).

There was a statistically significant effect (P<0.001) of treatment on amount of pathogen DNA, with the amount in the inoculated control treatment (12,490 pg DNA/g dried soil) being greater than that from the 'Caliente' mustard treatment (3,018 pg DNA/g dried soil).

There was a significant interaction effect (P<0.001) between temperature and WHC. Treatment at 22°C and 40% WHC gave more DNA (11,814 pg DNA/g dried soil) compared with all the other treatments, with the mean amount at 22°C and 70% WHC (8,952 pg DNA/g dried soil) being greater than at 15°C and 40% WHC (3,355 pg DNA/g) or 70% WHC (4,288 pg DNA/g).

There was a significant effect (P<0.001) of temperature on amount of pathogen DNA, with treatment at 22°C (10,298 pg DNA/g) giving more DNA than that at 15°C (3,797pg DNA/g). There was no significant effect (P=0.578) of WHC on mean amounts of *R. solani* AG3-PT DNA (6,261 to 6,464 pg DNA/g dried soil).

Table 3.10 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in unamended soil (non-inoculated control), or soil inoculated with barley grain inoculum of *R. solani* AG3-PT or heat treated inoculum (heat treatment), and amended with 'Caliente' mustard. The soil was adjusted to two soil water holding capacities (40 or 70% WHC), and was incubated at two different temperatures (15 or 22°C) for 14 days.

Treatment	DNA amount (pg/g dried soil)				Mean across temperature[2]		Mean across water holding capacity[3]		Mean[4]
	15°C		22°C						
	40% WHC	70% WHC	40% WHC	70% WHC	40% WHC	70% WHC	15°C	22°C	
Uninoculated control	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]
Heat treated inoculum	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]
Inoculated control	164,577 a[(**)]	54,976 b	24,882 c	21,961 c	64,464 a'	34,810 b'	95,161 A	23,378 B	47,417 A'
'Caliente' mustard	25,915 c	31,211 c	9,789 d	12,032 d	16,061 c'	19,464 c'	28,444 B	10,858 C	17,664 B'
Mean across treatment[5]	65,681 x	41,462 y	15,711 z	16,298 z					
Mean[6]							52,211 M	16,022 N	
Mean[7]					32,420 P	26,102 Q			

P values: <0.001 (Treatment), <0.001 (Temperature), 0.006 (WHC), 0.003 (Treatment x Temperature), <0.001 (Treatment x WHC), 0.002 (Temperature x WHC), 0.002 (Treatment x Tempareture x WHC)

[(*)]Zero data omitted from statistical analyses

[(**)]Means within each column or row followed by the same letter symbol are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

[1]Interaction means between treatment, temperature and water holding capacity (a-e) were different (*P*=0.002).

[2]Interaction means between treatment and water holding capacity (a'-c') were different (*P*<0.001).

[3]Interaction means between treatment and temperature (A-C) were different (*P*=0.003).

[4]Means of overall treatment effect (A'-B') were different (*P*<0.001).

[5]Interaction means of temperature and water holding capacity (x-z) were different (*P*=0.002).

[6]Means of overall temperature effect (M-N) were different (*P*<0.001).

[7]Means of overall water holding capacity effect (P-Q) were different (*P*=0.006).

Table 3.11 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in unamended soil (non-inoculated control) or soil inoculated with barley grain inoculum of *R. solani* AG3-PT or heat treated inoculum (heat treatment), and amended with 'Caliente' mustard. The soil was adjusted to two different soil water holding capacities (40 or 70% WHC) and incubated at two different temperatures (15 or 22°C) for 28 days.

Treatment	DNA amount (pg/g dried soil)				Mean across temperature[2]		Mean across water holding capacity[3]		Mean[4]
	15°C		22°C						
	40% WHC	70% WHC	40% WHC	70% WHC	40% WHC	70% WHC	15°C	22°C	
Uninoculated control	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]
Heat treated inoculum	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]	0[(*)]
Inoculated control	6,900	6,065	30,010	18,072	14,653 a[(**)]	10,609 b	6,471 B	23,322 A	12,490 A'
'Caliente' mustard	1,401	2,957	4,295	4,192	2,565 d	3,532 c	2,082 D	4,243 C	3,018 B'
Mean across treatment[5]	3,355 z	4,288 z	11,814 x	8,952 y					
Mean[6]							3,797 N	10,298 M	
Mean[7]					6,464	6,261			

P values: <0.001 (Treatment), <0.001 (Temperature), 0.578 (WHC), <0.001 (Treatment x Temperature), <0.001 (Treatment x WHC), <0.001 (Temperature x WHC), 0.495 (Treatment x Tempareture x WHC)

[(*)]Zero data was omitted from statistical analysis.

[(**)]Means within each column or row followed by the same letter symbol are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analysis, and are presented as back-transformed means.

[1]Interaction means between treatment, temperature and water holding capacity were not different (*P*=0.495)

[2]Interaction means between treatment and water holding capacity (a-e) were different (*P*<0.001).

[3]Interaction means between treatment and temperature (A-D) were different (*P*<0.001).

[4]Means of overall treatment effect (A'-B') were different (*P*<0.001).

[5]Interaction means of temperature and water holding capacity (x-z) were different (*P*< 0.001).

[6]Means of overall temperature effect (M-N) were different (*P*<0.001).

[7]Means of overall water holding capacity effect were not different (*P*=0.578).

3.3.3 Influence of soil conditions on biofumigation effects on soil microbial activity

3.3.3.1 Influence of soil type on biofumigation effects on soil microbial activity

The statistical analyses for data from this experiment are shown in Appendix A.3.15, and the results are summarised in Table 3.12. There was a statistically significant interaction effect (P<0.001) between biofumigant plant types treatments and soil type on mean soil microbial activity (measured by soil dehydrogenase activity; DHA). For the loam and loamy sand soils, the DHA levels from the biofumigant plant type treatments and the inoculated control were greater than that from the uninoculated control. In the loam soil the DHA level was greater from the 'Nemat' arugula treatment (mean = 3.91 µg/g dried soil.hr^{-1}) compared with all the other treatments (1.08 to 2.17 µg/g dried soil.hr^{-1}), and for the loam soil the DHA level from the 'Caliente' mustard treatment (3.59 µg/g dried soil.hr^{-1}) was greater than from all the other treatments (0.25 to 2.80 µg/g dried soil.hr^{-1}). In contrast, for the clay soil, the uninoculated control (0.38 µg/g dried soil.hr^{-1}) gave more DHA compared with all the other treatments (0.01 to 0.30 µg/g dried soil.hr^{-1}). For all the biofumigant plant treatments and the inoculated control, the DHA levels were less in the clay loam soil compared with the other two soil texture types. For the uninoculated control, the DHA level in the loam soil was less than the loam or clay loam soils. For both the uninoculated control and the 'Nemat' arugula treatments, the DHA levels in the loam soil were more than in the loamy sand or clay loam soils. For all the other treatments, the DHA levels were greater in the loamy sand soil compared with those in the loam or clay loam soils.

There were statistically significant effects (P<0.001) of treatment or soil type on DHA. Across the biofumigant treatments, the DHA level was greater in the loam soil (1.94 µg/g dried soil.hr^{-1}) compared with the other two soil texture types, with that in the loamy sand (1.88 µg/g dried soil.hr^{-1}) more than in the clay loam (0.09 µg/g dried soil.hr^{-1}). Across the soil texture treatments, DHA from the 'Nemat' arugula treatment (1.96 µg/g dried soil.hr^{-1}) was greater compared with all other treatments, followed by that from the 'Caliente' mustard treatment. Inoculating the soil with *R. solani* barley grain fragments increased DHA (0.59 µg/g dried soil.hr^{-1}) compared with the uninoculated control treatment (0.51 µg/g dried soil.hr^{-1}).

Table 3.12 Mean soil dehydrogenase activities (µg/g dried soil.hr^{-1}) in three soils of different soil types (loam, loamy sand or clay loam), that were amended with three biofumigant plant types, after 28 days incubation at 22°C.

Treatment	Soil texture			Mean of treatment
	Loam	Loamy sand	Clay loam	
Uninoculated control	1.08 i	0.25 l	0.38 j	0.51 E
Inoculated control	1.18 h	1.31 g	0.006 n	0.59 D
'Caliente' mustard	2.17 e	3.59 b	0.015 m	1.36 B
Brown mustard	1.91 f	2.69 d	0.012 m	1.09 C
'Nemat' arugula	3.91 a	2.80 c	0.30 k	1.96 A
Mean of soil texture	1.94 X	1.88 Y	0.09 Z	
P values: <0.001 (Treatment); <0.001 (Soil texture); <0.001 (Treatment x Soil texture)				

Means within each column or row followed by the same letter are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.3.2. Influence of soil pH on biofumigation effects on soil microbial activity

The statistical analyses for data from this experiment are shown in Appendix A.3.16, and the results are summarised in Table 3.13. There was a statistically significant interaction effect (*P*<0.001) between biofumigant treatment and soil pH on DHA. For all the biofumigant treatments, mean soil microbial activity was less in the soil adjusted to pH 4.8 compared with pH 5.8 or 6.6, and activity at pH 5.8 was less than that at pH 6.6. At pH 6.6 or pH 5.8, DHA activity was significantly greater in the soil amended with the biofumigant plants compared with the non-inoculated and inoculated controls, with brown mustard increasing DHA compared with 'Caliente' mustard or 'Nemat' arugula. However, at pH 4.8, only the treatment with 'Nemat' arugula increased DHA compared with the non-inoculated control, with all the biofumigant treatments increasing DHA compared with the inoculated control treatment.

There were statistically significant effects (*P*<0.001) of biofumigant treatment and soil pH on mean DHA. Across treatments, the DHA was greatest in soil at pH 6.6 (mean = 0.93 µg/g dried soil.hr^{-1}), followed by that in soil at pH 5.8 (0.71 µg/g dried soil.hr^{-1}). Across the pH treatments, soil microbial activity was greater in the soil amended with 'Nemat' arugula (0.87 µg/g dried soil.hr^{-1}) compared with all the other treatment, with activity from the 'Caliente' and brown mustard treatments (0.71 to 0.73 µg/g dried soil.hr^{-1}) more than both the uninoculated and inoculated controls.

Table 3.13 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) in soil adjusted to three soil pHs (4.8, 5.8 or 6.6) and amended with three biofumigant crops, after 28 days incubation at 22°C.

Treatment	Soil pH			Mean of treatment
	pH 4.8	pH 5.8	pH 6.6	
Uninoculated control	0.16 j	0.24 h	0.45 f	0.27 C
Inoculated control	0.09 k	0.28 g	0.53 e	0.27 C
'Caliente' mustard	0.17 ij	1.12 c	1.20 bc	0.73 B
Brown mustard	0.19 ij	1.28 b	1.53 a	0.71 B
'Nemat' arugula	0.21 hi	1.00 d	1.17 c	0.87 A
Mean of soil pH	0.16 Z	0.71 Y	0.93 X	
P values: <0.001 (Treatment); <0.001 (Soil pH); <0.001 (Treatment x Soil pH)				

Means within each column or row followed by the same letter are not significantly different (*P* = 0.05) according to Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.3.3 Influence of soil temperature on biofumigation effects on soil microbial activity

The statistical analyses for data from this experiment are shown in Appendix A.3.17, and the results are summarised in Table 3.14. There was a statically significant interaction effect (*P*<0.001) between treatment and soil temperature on soil DHA. All the biofumigant crop treatments increased mean DHA at all the soil temperatures compared with the uninoculated or inoculated treatments. The greatest increase in microbial activity due to individual biofumigant treatments differed at the different soil temperatures. At 10°C, mean DHA was greater from the brown mustard treatment compared with 'Caliente' mustard or 'Nemat' arugula. However at 15°C DHA was greatest from 'Caliente' mustard, while at 20°C the DHA was greatest from the 'Nemat' arugula treatment. For the uninoculated or inoculated controls, soil temperature had no statistically significant effect on soil microbial activity. However, the 'Nemat' arugula treatment produced greater DHA in soil incubated at 15°C and 20°C than at 10°C. For the 'Caliente' mustard and brown mustard treatments, DHA was greater in the soil incubated at 15°C than at 10°C or 20°C.

There were significant effects (*P*<0.001) of the biofumigant treatments and soil temperatures on mean soil DHA. Across the biofumigant treatments, soil microbial activity was greatest at 15°C (mean = 2.45 µg/g dried soil.hr^{-1}) followed by that at 20°C (1.61 µg/g dried soil.hr^{-1}), and least at 10°C (0.96 µg/g dried soil.hr^{-1}). Across the temperature treatments, DHA was greatest from the brown mustard treatment (3.16 µg/g dried soil.hr^{-1}), followed by 'Caliente 'mustard (2.73 µg/g dried soil.hr^{-1}), and 'Nemat' arugula (2.50 µg/g dried soil.hr^{-1}).

Table 3.14 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) in soil incubated at three different temperatures (10°C, 15°C or 20°C) amended with three biofumigant crops, after 28 days incubation.

Treatment	Soil temperature			Mean of treatment
	10°C	15°C	20°C	
Uninoculated control	0.38 h	0.37 h	0.39 gh	0.38 E
Inoculated control	0.45 fgh	0.60 f	0.55 fg	0.53 D
'Caliente' mustard	1.33 d	5.17 a	2.33 c	2.73 B
Brown mustard	2.40 c	4.89 a	2.48 c	3.16 A
'Nemat' arugula	0.83 e	3.83 b	3.50 b	2.50 C
Mean of soil temperature	0.96 c	2.45 a	1.61 b	
P values: <0.001 (Treatment); <0.001 (Soil temperature); <0.001 (Treatment x Soil temperature)				

Means within each column or row followed by the same letter are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.3.4 Influence of soil water holding capacity on biofumigation effects on soil microbial activity

The statistical analyses for data from this experiment are shown in Appendix A.3.18, and the results are summarised in Table 3.15. There was a statistically significant interaction effect (*P*<0.001) between biofumigant treatment and soil WHC on mean DHA. For all treatments, soil DHA was greater in soils at 40% or 70% WHC compared with at 100% WHC. At 40% and 70% WHC, DHA was greater from the three biofumigant treatments ('Caliente' mustard, brown mustard or 'Nemat' arugula) compared with the uninoculated or inoculated controls, with that from the inoculated control being greater than that from the non-inoculated control. In contrast, at 100% WHC, the soil DHA was greatest from the uninoculated control, compared with the other treatments, with the activity from the brown mustard and 'Nemat' arugula treatments being greater than from 'Caliente' mustard and the inoculated control treatments. At 40% WHC there were no significant differences in DHA in the soil amended with the three biofumigant plant types, while at 70% WHC, the DHA in the soil amended with 'Caliente' mustard or 'Nemat arugula was greater compared with that from the brown mustard treatment.

There were statistically significant effects (*P*<0.001) of treatment and soil WHC on soil DHA. Across treatments, soil DHA at 40% or 70% WHC was not significantly different (mean = 0.54 µg/g dried soil.hr^{-1}), but was greater than that at 100% WHC (0.14 µg/g dried soil.hr^{-1}). Across the soil WHCs, DHA in the soil treated with 'Nemat' arugula (0.45 µg/g dried soil.hr^{-1}) was greater than for all the

other treatments, followed by 'Caliente' mustard (0.42 µg/g dried soil.hr^{-1}) and brown mustard (0.43 µg/g dried soil.hr^{-1}).

Table 3.15 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) in soil adjusted to three soil water holding capacities (40, 70 or 100% WHC) and amended with three biofumigant crops, after 28 days incubation at 22°C.

Treatment	Soil water holding capacity			Mean of treatment
	40% WHC	70% WHC	100% WHC	
Uninoculated control	0.35 e	0.35 e	0.23 f	0.31 C
Inoculated control	0.43 d	0.52 c	0.05 i	0.29 D
'Caliente' mustard	0.66 a	0.64 a	0.11 h	0.42 B
Brown mustard	0.66 a	0.57 b	0.16 g	0.43 B
'Nemat' arugula	0.65 a	0.66 a	0.16 g	0.45 A
Mean of soil WHC	0.54 X	0.54 X	0.14 B	
P values: <0.001 (Treatment); <0.001 (Soil WHC); <0.001 (Treatment x Soil WHC)				

Means within each column or row followed by the same letter are not significantly different (*P*=0.05) according to Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.3.5 Influence of soil water holding capacity and temperature on biofumigation effects on soil microbial activity

For the assessments for this experiment after 14 days incubation, the statistical analyses are shown in Appendix A.3.19 and the results are summarised in Tables 3.16. There was a statistically significant interaction effect (*P*<0.001) between treatment, temperature and water holding capacity (WHC) on soil microbial activity (DHA). For all treatments, mean DHA was greatest at 40% WHC and 15°C compared with all the other WHC and temperature combinations. For soil adjusted to 40% WHC and incubated at 15°C, DHA was greatest from the heat treated inoculum treatment (mean = 2.76 µg/g dried soil.hr^{-1}), followed by that from 'Caliente' mustard (1.91 µg/g dried soil.hr^{-1}), whereas for soil at 70% WHC and 22°C, the DHA was greatest from the 'Caliente' mustard treatment (1.55 µg/g dried soil.hr^{-1}). In contrast, for soil at 40% WHC incubated at 22°C there was no significant difference in the DHA, while at 70% WHC DHA incubated at 15°C was greatest in the soil amended with 'Caliente' mustard (1.73 µg/g dried soil.hr^{-1}) compared with all the other treatments.

There was a statistically significant interaction effect (*P*<0.001) between biofumigant treatment and WHC on soil DHA. All treatments at 40% WHC resulted in greater DHA compared with 70% WHC. At 40% WHC, the DHA from heat treated inoculum (mean = 1.78 µg/g dried soil.hr^{-1}) treatment was

greater than for all the other treatments, followed by that from the 'Caliente' mustard treatment (1.64 µg/g dried soil.hr^{-1}). At 70% WHC, the DHA in the 'Caliente' mustard treated soil was greater than from all the other treatments, followed by that for the heat treated inoculum treatment.

There was a statistically significant interaction effect (P<0.001) between biofumigant treatment and temperature on soil DHA. For all the treatments, DHAs were greater at 15°C compared with at 22°C. At 15°C the soil microbial activity was greater from the 'Caliente' mustard and the heat treated inoculum treatments (mean = 1.82-1.85 µg/g dried soil.hr^{-1}) compared with all other treatments. At 22°C, DHA was greater from the' Caliente' mustard treatment (1.14 µg/g dried soil.hr^{-1}) compared with all the other treatments, with the activity from the heat treated inoculum treatment being greater than both the non-inoculated and inoculated control treatments.

There was a significant effect (P<0.001) of biofumigant treatments on soil DHA. Soil incorporated with 'Caliente' mustard resulted in greater DHA (mean = 1.47 µg/g dried soil.hr^{-1}) compared with the heat treated inoculum treatment (1.35 µg/g dried soil.hr^{-1}), which gave greater activity than both the uninoculated or inoculated control treatments (respectively, 0.96 and 0.93 µg/g dried soil.hr^{-1}). There was a significant interaction effect (P<0.001) between temperature and WHC on soil DHA. Soil at 40% WHC and incubated at 15°C had greater DHA (1.84 µg/g dried soil.hr^{-1}) compared with soil at 15°C and 70%WHC (1.12 µg/g dried soil.hr^{-1}), which gave greater DHA than soil incubated at 22°C at either 40% or 70% WHC (0.87-0.92 µg/g dried soil.hr^{-1}).

There was a significant effect (P<0.001) of temperature on soil DHA, with greater DHA recorded at 15°C (mean = 1.47 µg/g dried soil.hr^{-1}) compared with 20°C (0.89 µg/g dried soil.hr^{-1}). There was also a significant effect (P<0.001) of WHC on soil DHA, with greater DHA in soil at 40% WHC (1.33 µg/g dried soil.hr^{-1}) than that at 70% WHC (1.02 µg/g dried soil.hr^{-1}).

For assessments after 28 days of incubation, the statistical analyses are shown in Appendix A.3.20, and the results are summarised in Table 3.17. There was a significant interaction effect (P<0.001) between biofumigation treatment, temperature and WHC on soil DHA. For all treatments, apart from 'Caliente' mustard, DHA was greater at 40% WHC and 15°C compared with all the other temperature and WHC combinations. For 'Caliente' mustard there was no significant difference in DHA in soil incubated at 15°C and 40% or 70% WHC (4.42-4.54 µg/g dried soil.hr^{-1}), with greater activity than at 22°C at both 40% and 70% WHC (0.65-0.82 µg/g dried soil.hr^{-1}). The DHA in soils incubated at 15°C and adjusted to 40% or 70% WHC (4.42-4.54 µg/g dried soil.hr^{-1}) was greater from the 'Caliente' mustard treatment compared with the other biofumigant treatments, with activity from the heat treated inoculum treatment (3.72 µg/g dried soil.hr^{-1}) being greater than from the uninoculated (1.42 µg/g dried soil.hr^{-1}) or inoculated control (3.27 µg/g dried soil.hr^{-1}) treatments. In contrast, for soil incubated 22°C and 40% WHC, the DHA was greater from the uninoculated (1.03 µg/g dried soil.hr^{-1}) or inoculated control (0.97 µg/g dried soil.hr^{-1}) treatments compared with the 'Caliente' mustard

(0.65 µg/g dried soil.hr^{-1}) or the heat treated inoculum (0.70 µg/g dried soil.hr^{-1}) treatments, while at 70% WHC, DHA was greatest from the inoculated control treatment(1.08 µg/g dried soil.hr^{-1}) compared with all the other treatments.

There was a significant interaction effect (*P*<0.001) between biofumigant treatment and WHC on soil DHA. For all treatments apart from 'Caliente' mustard there was greater DHA at 40% WHC compared with at 70% WHC. There was no significant difference in the DHA for the 'Caliente' mustard treatment between 40% or 70% WHC. At both 40% and 70% WHC, the DHA was greater from the 'Caliente' mustard treatment compared with all the other treatments. There was a significant interaction effect (*P*<0.001) between biofumigant treatment and temperature on soil DHA. DHA was greatest for all treatments incubated at 15°C compared with those at 22°C. At 15°C, DHA from 'Caliente' mustard (mean = 4.48 µg/g dried soil.hr^{-1}) was greater than that from the other treatments, followed by the heat treated inoculum treatment (3.22 µg/g dried soil.hr^{-1}), which gave greater DHA than the inoculated control (2.43 µg/g dried soil.hr^{-1}) and the uninoculated control (1.23 µg/g dried soil.hr^{-1}). At 22°C, the DHA from the inoculated control (1.02 µg/g dried soil.hr^{-1}) was greater than from the other treatments.

There was a significant effect (*P*<0.001) of treatment on soil DHA. 'Caliente' mustard gave greater DHA (2.35 µg/g dried soil.hr^{-1}) than the other biofumigant treatments, followed by the heat treated inoculum treatment (1.82 µg/g dried soil.hr^{-1}). There was a significant interaction effect (*P*<0.001) between temperature and WHC on soil DHA. Soil incubated at 15°C and 40% WHC had greater DHA (3.15 µg/g dried soil.hr^{-1}) compared with the other treatments, followed by that at 15°C and 70% WHC (2.36 µg/g dried soil.hr^{-1}). There was significant effect (*P*<0.001) of temperature on soil DHA. Mean DHA in soil at 15°C was 2.74 µg/g dried soil.hr^{-1} which was greater than that at 22°C (0.82 µg/g dried soil.hr^{-1}). There was also a significant effect (*P*<0.001) of WHC on soil DHA, with greater DHA in soil at 40% WHC (1.87 µg/g dried soil.hr^{-1}) than that at 70% WHC (1.52 µg/g dried soil.hr^{-1}).

Table 3.16 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) in unamended soil (uninoculated control) or soil inoculated with *R. solani* AG3-PT colonised grain inoculum (inoculated control) or heat treated inoculum (heat treatment), amended with 'Caliente' mustard, and adjusted to two different soil water holding capacities (40% or 70% WHC) and incubated at two different temperatures (15 or 22°C), after 14 days incubation.

Treatment	Soil dehydrogenase activity (µg/g dried soil.hr^{-1})[1]				Mean across temperature[2]		Mean across water holding capacity[3]		Mean[4]
	15°C		22°C						
	40% WHC	70% WHC	40% WHC	70% WHC	40% WHC	70% WHC	15°C	22°C	
Uninoculated control	1.28 d	0.89 fg	0.96 ef	0.74 gh	1.11 d'	0.81 f'	1.08 B	0.85 C	0.96 C'
Heat treated inoculum	2.76 a	1.07 e	0.95 ef	0.85 fg	1.78 a'	0.96 e'	1.85 A	0.90 C	1.35 B'
Inoculated control	1.54 c	0.84 fg	0.80 fg	0.60 h	1.16 d'	0.72 f'	1.18 B	0.70 D	0.93 C'
'Caliente' mustard	1.91 b	1.73 bc	0.77 g	1.55 c	1.64 b'	1.30 c'	1.82 A	1.14 B	1.47 A'
Mean across treatment[5]	1.84 x	1.12 y	0.87 z	0.92 z					
Mean[6]							1.47 M	0.89 N	
Mean[7]					1.33 P	1.02 Q			

P values: <0.001 (Treatment), <0.001 (Temperature), <0.001 (WHC), <0.001 (Treatment x Temperature), <0.001 (Treatment x WHC), <0.001 (Temperature x WHC), <0.001 (Treatment x Tempareture x WHC)

Means within each columns or row followed by the same letter symbol are not significantly different (*P* = 0.05) according to Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

[1]Interaction means between treatment, temperature and water holding capacity (a-h) were different (*P*<0.001)

[2]Interaction means between treatment and water holding capacity (a'-f') were different (*P*<0.001).

[3]Interaction means between treatment and temperature (A-D) were different (*P*<0.001).

[4]Means of overall treatment effect (A'-C') were different (*P*<0.001).

[5]Interaction means between temperature and water holding capacity (x-z) were different (*P*<0.001).

[6]Means of overall temperature effect (M-N) were different (*P*<0.001).

[7]Means of overall water holding capacity effect (P-Q) were different (*P*<0.001).

Table 3.17 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) in unamended soil (uninoculated control) and soil inoculated with *R. solani* AG3-PT grain inoculum (positive control) or heat treated inoculum (heat treatment), amended with 'Caliente' mustard and adjusted to two different soil water holding capacity (40% or 70% WHC) and incubated at two different temperatures (15 or 22°C) after 28 days incubation.

Treatment	Soil dehydrogenase activity (µg/g dried soil.hr^{-1})[1]				Mean across temperature[2]		Mean across water holding capacity[3]		Mean[4]
	15°C		22°C						
	40% WHC	70% WHC	40% WHC	70% WHC	40% WHC	70% WHC	15°C	22°C	
Uninoculated control	1.42 f	1.05 g	1.03 g	0.63 j	1.22 e'	0.84 f'	1.23 D	0.83 F	1.02 D'
Heat treatment	3.72 b	2.74 d	0.70 ij	0.71 ij	2.02 b'	1.63 c'	3.22 B	0.71 G	1.82 B'
Positive control	3.27 c	1.69 e	0.97 gh	1.08 g	2.01 b'	1.37 d'	2.43 C	1.02 E	1.68 C'
'Caliente' mustard	4.54 a	4.42 a	0.65 ij	0.82 hi	2.31 a'	2.39 a'	4.48 A	0.73 FG	2.35 A'
Mean across treatment[5]	3.15 x	2.36 y	0.83 z	0.81 z					
Mean[6]							2.74 M	0.82 N	
Mean[7]					1.87 P	1.52 Q			

P values: <0.001 (Treatment), <0.001 (Temperature), <0.001 (WHC), <0.001 (Treatment x Temperature), <0.001 (Treatment x WHC), <0.001 (Temperature x WHC), <0.001 (Treatment x Tempareture x WHC)

Means within each column or row followed by the same letter symbol are not significantly different according to Tukey's HSD test at *P*=0.05. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

[1]Interaction means between treatment, temperature and water holding capacity (a-j) were different (*P*<0.001)

[2]Interaction means between treatment and water holding capacity (a'-f') were different (*P*<0.001).

[3]Interaction means between treatment and temperature (A-G) were different (*P*<0.001).

[4]Means of overall treatment effect (A'-D') were different (*P*<0.001).

[5]Interaction means between temperature and water holding capacity (x-z) were different (*P*<0.001).

[6]Means of overall temperature effect (M-N) were different (*P*<0.001).

[7]Means of overall water holding capacity effect (P-Q) were different (*P*<0.001).

3.3.4 Potato stem canker and plant growth assessments

In this experiment, potato tubers were planted in soil amended with different biofumigant plant type treatments in combination with different soil water holding capacity and temperature treatments, and were grown for 28 days. The resulting potato plants were harvested and assessed 35 days after planting.

3.3.4.1 Stem canker severity

The statistical analyses of stem canker severity score data are shown in Appendix A.3.21. No stem canker symptoms were observed on any of the plants from the uninoculated control or heat treated inoculum treatments, so these data were omitted from the analyses. There were no significant interaction effects on mean stem canker severity score between biofumigant treatment, temperature and WHC (P=0.134), treatment and WHC (P=0.283), or temperature and WHC (P=0.595). There was no significant effect (P=0.411) of WHC on stem canker severity, with mean severity from 40% WHC equivalent to 13.6% of stem surface affected, and from 70% WHC equivalent to 15.0% affected.

There was a significant interaction effect (P=0.022) between treatment and temperature on stem canker severity (Figure 3.6). There was no significant difference in mean severity between the inoculated control (19.0% stem surface affected) or the ‘Caliente’ mustard treatment (15.8%) at 22°C (Table 3.18). In contrast, at 15°C, stem canker severity was greater from the inoculated control (17.6% stem surface affected) than from the ‘Caliente’ mustard treatment (6.5%).

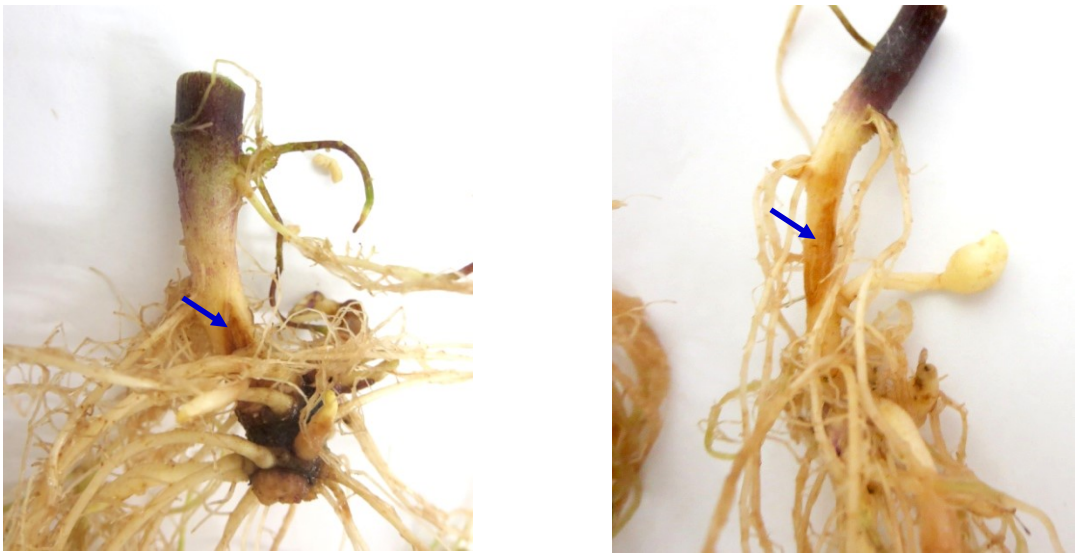
There was a significant effect (P<0.001) of treatment on stem canker severity, with the inoculated control resulting in greater mean severity (18.3% stem surface affected) than for plants from the ‘Caliente' mustard treatment (10.7%). There was a significant effect (P=0.005) of temperature on stem canker severity. Plants grown in soil incubated at 22°C had greater severity (17.4% stem surface affected) than for those in the 15°C treatment (11.4%).

Table 3.18 Mean potato stem canker severity (% stem surface area affected) assessed on potato plants at 35 days after planting in soil previously inoculated with grain inoculum of *Rhizoctonia solani* AG3-PT (inoculated control), or heat treated grain inoculum, (heat treated inoculum), amended with 'Caliente' mustard and incubated at two different temperatures (15 or 22°C) in shadehouse conditions. Data combined for different soil water holding capacities.

Treatment	Temperature		Mean of treatment
	15°C	22°C	
Uninoculated control	0[(1)]	0[(1)]	0[(1)]
Heat treated inoculum	0[(1)]	0[(1)]	0[(1)]
Inoculated control	17.6 a[(2)]	19.0 a	18.3 X
'Caliente' mustard	6.5 b	15.8 a	10.7 Y
Mean of temperature	11.4 B	17.4 A	
P values: <0.001 (Treatment); 0.005 (Temperature), 0.022 (Treatment x Temperature)			

[(1)]Zero data were omitted from analyses

[(2)]Means within each row or column followed by the same letters are not significantly different (*P*=0.05) based on Tukey's HSD test. Data were arcsine (sqrt(X/100)) transformed prior to statistical analysis, and are presented as back-transformed means.



A B

Figure 3.6 Stem cankers (arrows) caused by *Rhizoctonia solani*, observed on potato plants 35 days after planting in *R. solani* inoculated soils, (A) amended with macerated 'Caliente' mustard tissues and incubated at 22°C and 40% water holding capacity (WHC), and (B) inoculated control treatment, incubated at 22°C and 40% WHC.

3.3.4.2 Potato plant height measurements

The statistical analyses of plant height data are shown in Appendix A.3.22. There was no significant interaction effect on mean plant height between biofumigant treatment, temperature and WHC (P=0.512), or treatment and WHC (P=0.083). There was also no significant effect (P=0.993) of WHC on plant height, with mean plant height at 40% WHC and 70% WHC both being 25.4 cm. There was a significant interaction effect on plant height (P<0.001) between treatment and temperature (Table 3.19). For the 15°C treatment, plants in soil incorporated with 'Caliente' mustard (mean height = 39.8 cm) were taller than from all the other treatments, apart from plants in the inoculated controls. In contrast, at 22°C, the plants were taller from the non-inoculated and heat treated inoculum treatments (means, respectively, = 27.2 cm and 26.6 cm) compared with the inoculated control (16.2 cm), while plants from the 'Caliente' mustard treatment (20.0 cm) were not different from those in any of the other treatments. Apart from the 'Caliente' mustard treatment, there were no significant differences in the plant heights between the two temperature treatments.

Across both temperatures, there was a significant effect (P<0.001) of treatment on plant height. Plants in the inoculated control treatment (mean height = 18.4 cm) were significantly shorter than in all other treatments (26.4 to 29.0 cm) (Figure 3.7). Across the treatments, there was a significant effect (P<0.001) of temperature on plant height. Plants grown in soil incubated at 15°C (mean height = 28.7 cm) were taller than those in the 22°C treatment (22.3 cm). There was a significant interaction effect (P=0.007) between temperature and WHC (Table 3.20). Plants in soil incubated at 22°C and 70% WHC (mean height = 20.3cm) were shorter than those at 15°C and 40% WHC (26.5 cm) or 70% WHC (31.1 cm), but mean plant height was not significantly different for plants grown in soil incubated at 22°C and 40% WHC (24.3 cm).

Table 3.19 Mean potato plant height (cm) assessed 35 days after planting in soil previously inoculated with *Rhizoctonia solani* AG3-PT grain inoculum (inoculated control) or heat treated grain inoculum (heat treated inoculum), amended with 'Caliente' mustard and incubated at two temperatures (15 or 22°C) in shadehouse conditions. Data combined for different soil water holding capacities.

Treatment	Temperature		Mean of treatment
	15°C	22°C	
Uninoculated control	25.6 bc[(1)]	27.2 bc	26.4 X
Heat treated inoculum	30.5 ab	26.6 bc	28.5 X
Inoculated control	20.8 bcd	16.2 d	18.4 Y
'Caliente' mustard	39.8 a	20.0 cd	29.0 X
Mean of temperature	28.7 A	22.3 B	
P values: <0.001 (Treatment); <0.001 (Temperature), <0.001 (Treatment x Temperature)			

[(1)]Mean values within each row or column followed by the same letters are not significantly different (*P*=0.05) based on Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

Table 3.20 Mean height (cm) of potato plants assessed 35 days after planting in soil previously incubated at two temperatures (15 or 22°C) and two water holding capacities (40 or 70% WHC), in shadehouse conditions.

Temperature	Water holding capacity	
	40%	70%
15°C	26.5 ab[(1)]	31.1 a
22°C	24.3 bc	20.3 c
P (Temperature x WHC): 0.007		

[(1)]Mean values within each row or column followed by the same letters are not significantly different based on Tukey's HSD test (*P*=0.05). Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

A B

Figure 3.7 Potato plants (of **'**Jersey Benne') in *Rhizoctonia solani* inoculated soil incubated at 15°C and 70% water holding capacity (WHC) (A) and from the 'Caliente' mustard treatment incubated at 15°C and 40% WHC, at 35 days after planting.

3.3.4.3 Total plant dry biomass

The statistical analyses for plant dry matter data are shown in Appendix A.3.23. There was no significant interaction effect on plant biomass between treatment, temperature and WHC (P=0.468), treatment and WHC (P=0.141) or temperature and WHC (P=0.484). There was a significant effect (P=0.006) of soil WHC on plant biomass with plants at 40% WHC (mean = 7.3 g) weighing more than those at 70% WHC (6.6 g).

There was a significant interaction effect (P<0.001) on plant weight between biofumigant treatment and temperature (Table 3.21). For the 15°C treatment, the mean biomass of the potato plants planted in the positive control treatment (5.8 g) was significantly less than from all other treatments, with the plants in the 'Caliente' mustard treatment (11.2 g) having significantly greater biomass compared with all other treatments. In contrast, at 22°C the plants in the positive control treatment (2.3 g) were significantly lighter compared with all other treatments (7.3 to 7.6 g). For both 'Caliente' mustard and the inoculated control treatments, plant biomass was significantly greater at 15°C (respectively, 11.2 g and 5.8 g) compared with biomass at 22°C (respectively, 7.3 g and 2.3 g). Plant biomass from the uninoculated control and heat-treated inoculum treatments at both temperatures were not significantly different (7.4 to 8.4 g).

Across temperature, there was a significant effect (P<0.001) of treatment on plant weight, with plants in the inoculated control treatment soil (3.9 g) being significantly lighter compared with all other treatments (7.5-9.1 g), with the 'Caliente mustard treatment giving significantly heavier plants than all the other treatments. Across treatments, there was a significant effect (P < 0.001) of

temperature on plant biomass. Potatoes planted in soil incubated at 15°C (8.1 g) were significantly heavier compared with those in the 22°C treatment (5.9 g).

Table 3.21 Mean potato plant dry biomass (g) assessed 35 days after planting in soil previously inoculated with *Rhizoctonia solani* and incubated at two different temperatures (15 or 22°C) under shadehouse conditions. Data combined for different soil water holding capacities.

Treatment	Temperature		Mean of treatment
	15°C	22°C	
Uninoculated control	8.4 b	7.6 b	8.0 Y
Heat treated inoculum	7.5 b	7.4 b	7.5 Y
Inoculated control	5.8 c	2.3 d	3.9 Z
'Caliente' mustard	11.2 a	7.3 bc	9.1 X
Mean of temperature	8.1 A	5.9 B	
P values: <0.001 (Treatment); <0.001 (Temperature), <0.001 (Treatment x Temperature)			

Mean values within each row or column followed by the same letters are not significantly different (*P*=0.05) based on Tukey's HSD test. Data were sqrt(X) transformed prior to statistical analysis, and are presented as back-transformed means.

3.3.5 Correlations

Amounts of *R. solani* AG3-PT DNA in soil were negatively correlated with soil microbial activity (DHA) in the soil texture (Section 3.2.1) and soil pH experiments (Section 3.2.4.2), and positively correlated with DHA in the soil temperature experiment (Section 3.2.4.3) (Table 3.22). There were no correlations between amounts of *R. solani* AG3-PT DNA and DHA in the soil water holding capacity experiment (Section 3.2.4.4) (Table 3.22), or in the soil temperature/water holding capacity experiment (Section 3.2.4.5) (Table 3.23). Stem canker severity was negatively correlated with plant height and dry biomass (Table 3.23).

Table 3.22 Correlations between amounts of *Rhizoctonia solani* AG3-PT DNA in soil and soil microbial activity after 28 days incubation, for data from experiments to determine the effects of different soil edaphic factors on biofumigation efficacy (Sections 3.2.4.1, 3.2.4.2, 3.2.4.3 and 3.2.4.4).

Section	Correlation coefficient (R)
3.2.4.1 (Soil type)	**-0.42*** **
3.2.4.2 (Soil pH)	**-0.57*** **
3.2.4.3 (Soil temperature)	**0.52*** **

3.2.4.4 (Soil water holding capacity)	-0.07^{ns}

ns: not statistically significant; ***: significant at $P \leq 0.001$.

Table 3.23 Correlations between amounts of *Rhizoctonia solani* AG3-PT DNA in soil and soil microbial activity after 14 or 28 days incubation, and between potato plant stem canker severity and plant height and dry biomass 35 days after planting, in an experiment to determine effects of temperature and soil water holding capacity on biofumigation efficacy (Section 3.2.4.5).

Variable	Correlation coefficient (R)						
	1	2	3	4	5	6	7
1. DNA concentration (2 WI)	-						
2. Soil microbial activity (2 WI)	0.08^{ns}	-					
3. DNA concentration (4 WI)			-				
4. Soil microbial activity (4 WI)			-0.01^{ns}	-			
5. Potato stem canker (5WPP)					-		
6. Potato plant height (5WPP)					**-0.47*** **	-	
7. Potato dry biomass						**-0.49*** **	-

ns: not significant; ***: significantly at $P \leq 0.001$

3.4 Discussion

This is the first study to evaluate the effects of soil conditions on biofumigation efficacy to reduce *R. solani* AG3-PT inoculum, and the influence on soil microbial activity. The qPCR method of Woodhall *et al.* (2013) was sensitive and specific for detecting and quantifying DNA of *R. solani* AG3-PT. Overall, the levels of *R. solani* AG3-PT DNA and soil dehydrogenase activity (DHA) were greatly influenced both by the soil edaphic conditions, and the interaction between biofumigant treatments and the edaphic conditions. After 28 days incubation, the levels of *R. solani* AG3-PT DNA in biofumigant treatments (apart from 'Nemat' arugula) added to loam soil at pH 6.6, 20°C and 40% water holding capacity (WHC), and in the combination of soil temperature at 15°C and soil WHC of 40%, were significantly reduced compared with those in positive or other biofumigant treatments at same or different soil edaphic conditions. In addition, incorporation of *R. solani* AG3-PT inoculum and biofumigant plant tissues increased soil microbial activity as indicated by soil DHA levels. DHA levels were the greatest in the loam soil, at pH 6.6, 15°C, and 40 or 70% WHC, and the interaction of soil temperature (15°C) and soil WHC (40%). Potato plants grown in *R. solani*-inoculated soils amended with macerated 'Caliente' mustard tissue (5%, w:w) and after incubation at 15°C had the least severe stem canker, and greatest plant height and plant dry biomass compared with plants grown in soils from the inoculated control treatments.

There was a reduction in *R. solani* AG3-PT DNA from the inoculated control treatments after 28 days incubation compared with the initial levels. In addition, soil DHA from the inoculated control treatments was greater than in uninoculated treatment. Nutrients in the colonised barley grain fragments could have stimulated growth of soil microorganisms which in turn may have competed with, and displaced, *R. solani* AG3-PT either from the grain inoculum or from the soil, resulting in decreases in *R. solani* AG3-PT inoculum. All treatments amended with biofumigant plant tissues resulted in greater soil DHA levels compared with the inoculated control treatments with no added biofumigant tissues. The current study partially agreed with Potgieter *et al.* (2013) that incorporation of canola (1.5%, w:w) increased DHA in 3-5 weeks, then were not significantly different from the untreated control. However, the authors (Potgieter *et al.*, 2013) did not find the increase of DHA in the positive treatment. This is likely to be that the authors incorporation of *Sclerotinia sclerotiorum* inoculated oat at 1.2% (w:w) which was lower than that used in this present study (5%, w:w, *R. solani* colonised barley grains). In addition, biofumigant treatments were amended with *R. solani* colonised barley grain inoculum (5%, w:w) and with macerated *Brassica* tissue (5%, w:w), and these organic materials were likely to have stimulated soil microbial activity to a greater extent compared with the inoculated control treatments, which only incorporated *R. solani* colonised barley grain inoculum. This could be that the greater carbon sources or more accessible C sources in biofumigant treatments than in the positive control, thus resulted in increasing soil microbial activity by assessing DHA (Garcia *et al.*, 1997; Paudel *et al.*, 2016).

In the present study, where the biofumigant treatments were applied, *R. solani* AG3-PT DNA was detected at greater amounts in the clay loam soil compared with the loamy sand and loam soils. In contrast, soil DHA (microbial activity) was greatest in the loam soil compared with in the loamy sand and clay loam soils. The variation in the results between the soil types could be due to differences in soil physical and/or chemical characteristics (Table 3.2), as well as natural variation in the soil microbial activity in each soil type. The three soils had different textures, pHs, water holding capacities and organic matter (OM) contents. The measured pH of the three soils was 5.7 to 6.1 which is in the pH range (5 to 7) for ITC formation (Brown and Morra, 1997; Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006). Allyl ITC concentration released from freeze-dried homogenised material from whole Indian mustard plants or fresh Indian mustard leaf discs in a clay loam soil was reported to be less than in a sandy loam soil (Bending and Lincoln, 1999; Price *et al.*, 2005). The small pore space in clay soils could also reduce the diffusion of allyl ITC compared with in sandy loam soils (Price *et al.*, 2005), so the clay loam soil used in this study could have reduced biofumigation efficiency. Kyritsis (2003) demonstrated that *R. solani* AG3-PT caused more severe stem canker on potato plants in sandy clay loam soil than in fine sand soil, so *R. solani* AG3-PT is reported to favour soils with heavy textures compared with lighter soils. ITCs are also reported to be strongly absorbed and have reduced bioavailability in organic-rich soils (Gimsing *et al.*, 2006; Poulsen *et al.*, 2008). Thus, the

high OM content (7.3%) in the loam soil used in the present study could have resulted in decreased ITC availability, due to greater absorption by the OM, compared with the loamy sand and clay loam soils. However, soil DHA has been shown to be positively correlated with soil OM (Wolińska and Stępniewska, 2012), meaning that greater OM gives increased DHA. Studies have shown that incorporation of biofumigant tissues into soil enhances soil microbial activities, especially beneficial groups, which in turn could act to effectively suppress pathogens (Cohen *et al.*, 2005; Cohen and Mazzola, 2006; Ascencion *et al.*, 2015). In the present study, the negative correlation between amount of *R. solani* AG3-PT DNA and soil DHA level indicated that high microbial activity decreased the amounts of *R. solani* AG3-PT DNA in soil, and that low microbial activity increased *R. solani* amounts. Thus, the lower level of microbial activity could explain, at least in part, the higher *R. solani* AG3-PT DNA level in the clay soil after 28 days incubation than in the other soils. Due to these issues, the effects of soil pH, temperature and WHC on biofumigation potential for suppression of *R. solani* AG3-PT inoculum was only assessed in one soil in the remaining experiments. There is also a need for further experiments to determine the interaction effects between different soil factors on biofumigation potential for inhibiting *R. solani*.

Rhizoctonia solani DNA from the biofumigant treatments remained at high levels after 28 days incubation in soil at pH 4.8, compared with that in soil at pH 5.8 or 6.6. However, soil DHA from the biofumigant treatments was greatest in soil at pH 6.6. At a soil pH of 4.8, nitriles, compounds that are less bioactive than ITCs or thiocyanates, were reported to be the dominant types formed from the hydrolysis of glucosinolates (Borek *et al.*, 1994; Mithen, 2001; Bennett *et al.*, 2004; Grubb and Abel, 2006). Thus, the effects of the biofumigation plants for suppressing *R. solani* inoculum in treatments at pH 4.8 are likely to be less than in treatments at pH 5.8 or 6.6. *Rhizoctonia solani* mycelium is reported to grow at a pH range of 4 to 9, with optimum growth at pH 5.6 (Ritchie *et al.*, 2009). Furthermore, Wolińska and Stępniewska (2012) stated that the optimum pH range for DHA (microbial activity) was variable from 5.2 to 7.8, and that low or acidic pH (<5) tended to weaken enzyme activities of soil microorganisms, and consequently reduced soil DHA. Although pH 5.8 and 6.6 would allow growth of *R. solani* AG3-PT, biofumigation was more effective at soil pH 5.8 and 6.6, due to ITCs release. Due to the high biofumigation efficiency and high soil microbial activity at pH 5.8 and 6.6, *R. solani* AG3-PT inoculum was effectively suppressed compared with pH of 4.8.

In the present study, the maximum biofumigation efficiency through the greatest reduction of *R. solani* inoculum (amounts of DNA) was recorded at 20°C from the biofumigant treatments (other than from 'Nemat' arugula). This is likely to be due to the high activity of myrosinase enzymes which covert the GLSs into ITCs (Springett and Adams, 1989; Van Eylen *et al.*, 2006), and the high mobility of the ITCs released by these enzymatic activities (Price *et al.*, 2005). Wolińska and Stępniewska (2012) noted that the optimum temperature for soil DHA, which is only found in viable soil microbial

cells, was 28-30°C. However, in the present experiments the levels of soil DHA in the biofumigant treatments were greater at 15°C than at 20°C. These results may be due to a time lag during the process at 15°C compared with at the warmer temperature (20°C). At 20°C there could be more rapid utilisation by soil microorganisms of the soil organic matter from biofumigant crop tissue and barley grain inoculum, so by 28 days these organic materials would have been utilised resulting in a reduced soil DHA. In contrast, at 15°C, there may still be sufficient organic matter left after 28 days to support microbial activity. The present results are supported by the positive relationship between soil temperature and organic matter content which was demonstrated by Kirschbaum (1995) and Conant *et al.* (2011). Further research to sample at different time intervals would be useful to determine the dynamics of DHA as well as *R. solani* DNA amounts. There is also a possibility that ITCs could have a non-target effect on other soil microbes resulting in lower DHA at 20°C in comparison with 15°C. Lacey (2000) reported that incorporation of canola green manure (*B. napus*, unknown cultivar) reduced soil microbial activity (by assessing FDA) compared with a fallow (untreated) treatment in a field trial. Thus, further research is required to determine the impacts of biofumigation on non-target soil microbes.

The amounts of *R. solani* AG3-PT DNA were least from the biofumigant treatments at 40% WHC, and the DHA levels were greatest from the biofumigant treatments at 40 or 70% WHC. These results contrast with previous studies indicating that 100% WHC was optimum for biofumigation efficiency and DHA reaction (Matthiessen *et al.*, 2004; Gimsing and Kirkegaard, 2006; Wolińska and Stępniewska, 2012). High biofumigation efficiency at 100% WHC may reduce *R. solani* inoculum and other non-target microorganisms, thus decreasing measured DHA levels. In contrast, incorporation of biofumigant tissues at lower WHC (40% or 70%) could release less ITCs than at 100% WHC, reducing on inoculum and other soil microbes. Since biofumigation efficiency at 40% or 70% WHC is likely to be reduced, non-target soil microbes could suppress *R. solani* at low soil moisture levels.

In the experiment investigating the combined effects of soil temperature and WHC, the amounts of *R. solani* DNA were greatest after 14 days incubation, and least after 28 days incubation in soil amended with biofumigant tissue and at 15°C and 40% WHC. The DHA level was greatest after 14 days and 28 days incubation in soil amended with biofumigant tissue also at 15°C and 40% WHC. Temperatures of 20-25°C are reported to be the optimum range for *R. solani* mycelium growth (Ritchie *et al.*, 2009), with 28-30°C being the optimum range for DHA (Wolińska and Stępniewska, 2012). However, biofumigation efficiency was reported to be greater at high soil temperatures (30 or 45°C) than at 15°C (Price *et al.*, 2005), but the activity is short-lived (14 days) as ITCs are rapidly volatilised at high temperatures (Price *et al.*, 2005; Gimsing and Kirkegaard, 2006, 2009). Therefore, the present results could be due to the biofumigation treatments giving greater efficiency of ITC release at high temperature (22°C) than at 15°C, and subsequently causing greater suppression of *R.*

solani inoculum (low amount of DNA) and soil microbial activity (low DHA) after 14 days incubation. After 28 days, since reduction of biofumigation effect (Gimsing and Kirkegaard, 2006, 2009), any *R. solani* inoculum which survived after the biofumigation process could utilise the organic matter from the incorporated biofumigant tissues resulting in increases in inoculum at an optimum mycelium growth temperature (22°C). In addition, the organic matter may have been utilised in the soils at high temperatures (22°C) resulting in a lower microbial activity than at 15°C after 28 days. Biofumigation treatment at 15°C and 40% WHC could have less effect on *R. solani* and other soil microbes due to low concentration of toxic volatiles, resulting in the greatest *R. solani* DNA amounts and the highest levels of DHA after 14 days incubation. Soil microbial activity (DHA) was the greatest, and *R. solani* amounts were least in biofumigant treatments at 15°C and 40% WHC after 28 days incubation. These results potentially mean that high microbial activity could contribute to suppression of *R. solani*. Moreover, more soil organic matter could remain at 15°C than at 22°C (Kirschbaum, 1995; Conant *et al.*, 2011), and DHA is positively correlated to soil organic matter (Wolińska and Stępniewska, 2012). These factors could result in high DHA levels measured in biofumigant treatment at 15°C and 40% WHC after 14 and 28 days incubation.

Potato plants grown in *R. solani* infested soil previously amended with 'Caliente' mustard at 5% (w:w) and incubated at 15°C (across soils at two WHC levels) had less severe stem canker, and greater plant height and plant biomass, compared with the plants from the inoculated control treatment, at both temperatures and from the 'Caliente' treatment at 22°C. ITCs and other volatiles are likely to be released at greater rates at 22°C, resulting in a greater reduction in soil microbial activity and amounts of *R. solani* DNA at 15°C. However, after the decline in the toxic effects of ITCs and other volatiles through volatilisation (Price *et al.*, 2005) or breakdown in soil environment through soil texture (clay) or organic matter adsorption, or microbial degradation (Bending and Lincoln, 1999; Price *et al.*, 2005; Poulsen *et al.*, 2008; Gimsing and Kirkegaard, 2006, 2009), any *R. solani* inoculum remaining viable is likely to grow more rapidly at 22°C than at 15°C. This would increases the inoculum potential and hence disease pressure. In addition, due to the lower likely non-target impacts of toxic volatiles on overall soil microbial activity at 15°C than at 22°C, *R. solani* inoculum levels could also be affected through increased suppressive activity by the soil microbial community. This could be through competition for and displacement of *R. solani* from soil organic matter.

The present study used the qPCR techniques developed by Woodhall *et al.* (2013) to detect and quantify *R. solani* DNA in soils. The AG3-PT isolate of *R. solani* was chosen for these experiments because of its predominant association with black scurf of potato tubers in New Zealand (Das *et al.*, 2014). The present results showed that the designed primers AG3-PT_F/ AG3-PT_R were specific and sensitive for detection of *R. solani* AG3-PT isolates. Woodhall *et al.* (2013) confirmed that the primers did not cross-react with other *R. solani* isolates (of AG1-IB, AG2-1, AG2-2, AG2-3, AG3-TB (tobacco),

AG3-TM (tomato), AG4, AG5, AG6, AG7, AG8, AG9, AG10, or AG11). The PCR amplicon was approx. 122 bp, which was similar to that described by Woodhall *et al.* (2013).

In the experiments described here, *R. solani* AG3-PT colonised barley grain fragments was mixed thoroughly with air-dried soil, so there was even distribution of the inoculum in soil at the start of the experiments. Further, DNA extractions were carried out from soil subsamples. Although this was feasible for artificially inoculated soils used in the *in vitro* experiments, this sampling regime would not be suitable for determination of *R. solani* inoculum levels in field experimentation. *Rhizoctonia solani* AG3-PT has been reported to be at low levels in field soils (Lees *et al.*, 2002; Ophel-Keller *et al.*, 2008), and have a patchy distribution (Lees *et al.*, 2002). Small samples, as used in the present study for pathogen DNA extraction and qPCR detection, could result in low accuracy for field assessments (Ophel-Keller *et al.*, 2008; Brierley *et al.*, 2013; Woodhall *et al.*, 2013). For any further field studies, the strategy outlined by Ophel-Keller *et al.* (2008) should be used, whereby DNA is extracted from 500 g soil samples following a comprehensive field sampling protocol, which would increase *R. solani* detection sensitivity. However, in the present study, the levels detected in the replicate soil samples taken from each treatment were similar, indicating that the method provided accurate quantification of the *R. solani* DNA levels in the soil after different treatments.

This present study used the PowerSoil® DNA isolation kit to extract DNA from soil samples. Although the kit provided high quality extracted DNA (Mahmoudi *et al.*, 2011; Baker and Kellogg, 2014; Leite *et al.*, 2014), it could have resulted in lower total DNA yields compared with other kits such as the FastDNA SPIN kit (Mahmoudi *et al.*, 2011) or the PowerPlant Pro kit (Baker and Kellogg, 2014). Mahmoudi *et al.* (2011) also reported that there were no effects of soil texture and soil organic matter on DNA yields when using commercial DNA extraction kits (UltraClean Soil DNA Isolation kit, PowerSoil DNA Isolation kit, PowerMax Soil DNA Isolation kit, and FastDNA SPIN kit). The evidence demonstrated that the chosen DNA extraction kit was suitable and that differences in the *R solani* AG3-PT DNA amounts were not due to extraction efficiency in the different soils and treatments, but represent actual differences in inoculum levels due to treatments.

It has been reported that relic DNA from bacteria and plants could remain in soil for hours to years, although persistence of relic fungal DNA has not been studied (Nielsen *et al.*, 2007). This relic DNA could result in overestimation of DNA amounts of target species when used in subsequent qPCR (Paul *et al.*, 2016). In the present study, no DNA was detected from soil 28 days after inoculation with heat treated *R. solani* AG3-PT colonised barley grain fragments, demonstrating that the long heat treatment (8 hours) of the colonised barley grain fragments used in this study resulted in non-detection of DNA from dead fungal inoculum. This indicates that any treatment which reduced the viability of *R solani* inoculum in soil would not result in false positive DNA detections. In addition, it is possible that the biofumigation treatments may not have affected the propagules as much, and that

some relic DNA would still be detected. Further research could consider relic DNA of *R. solani* AG3-PT by treating soil samples with propidium monoazide (PMA) prior DNA extraction (Paul *et al.*, 2016).

The use of sulphuric acid to adjust the soil pH from 5.9 to pH 4.8 could have affected the soil microbial communities. Similarly, adjustment of the pH from 5.9 to 5.8 using sulphuric acid could have also adversely affected soil microbial activity, but probably to a lesser degree than for soils adjusted to pH 4.8. Babich *et al.* (1980) reported that toxicity of H_2SO_4 in soil was mainly caused by H^+ rather than SO_4^{2-}. Soil acidification with sulphuric acid resulted in soil pHs less than 3.5, and has been shown to decrease mycelium growth of soil fungi, such as *Aspergillus niger, A. flavipes, Trichoderma viride* and *Penicillium brefeldiunum* (Bewley and Stotzky, 1983). Furthermore, Liu *et al.* (2017) reported that sulphuric acid rain reduced litter decomposition rates and soil microbial community diversity (measured by phospholipid fatty acid). Shin *et al.* (2017) found that Gram-negative β-Proteobacteria were the most acid-sensitive, while spore-forming Gram-positive *Bacilli* were the most acid-tolerant. Davet (2004) reported that soil bacteria were more sensitive to low pH compared to soil fungi. Similarly, Wakelin (2018) indicated that soil bacteria were less tolerant to soil pH changes than soil fungi. Thus, the pH adjustment could affect to bacterial activity (through DHA measurement) more than fungal activity. In addition, the use of $Ca(OH)_2$ to adjust the pH of soil from 5.9 to 6.6 is also likely to influence soil microbial communities. Mühlbachová and Tlustoš (2011) reported that CaO (dissolved in water to become $Ca(OH)_2$) negatively affected soil microbial biomass C and microbial respiration. CaO and other calcium salts were reported to effectively suppress propagules and diseases caused by *Phytophthora* spp. such as *P. nicotianae* (causing citrus root rot) and *P. pistaciae* (pistachio gummosis) (Campanella *et al.*, 2002; Mostowfizadeh-Ghalamfarsa *et al.*, 2018). Calcium salts inhibiting the growth of *Phytophthora* spp. could be indirectly influenced by increasing pH (Serrano *et al.*, 2012) or direct effects of Ca^{2+} (Sugimoto *et al.*, 2007).

Results from the present study showed that incorporation of biofumigant crops into soil reduced the inoculum of *R. solani* AG3-PT compared with that from the positive (inoculated) control treatments. However, the inoculum levels remaining after the biofumigation treatments was still sufficient to cause stem canker in subsequently grown potato plants. This could be due to the high *R. solani* AG3-PT inoculum level (345,463-1,644,629 pg/g dried soil) initially incorporated into the soil compared with those that naturally occur in potato fields [2.4-21.9 pg/g dried soil in Tasmania and 1.9-27.5 pg/g dried soil in South Australia, Australia (Sparrow *et al.*, 2015); 0-46 pg/g dried soil in New Zealand (Sinton *et al.*, 2016)]. The biofumigation treatments did not completely eliminate the pathogen inoculum in the pot experiments. Agrios (2005) stated that the amount of pathogen inoculum near plant hosts was one of the important components in plant disease epidemiology: the greater the pathogen inoculum levels in contact with the host plants, the quicker will be the development of disease epidemics. After the biofumigation treatment with 'Caliente' mustard (28 days incubation),

R. solani AG3-PT DNA was detected at 1,401-4,295 pg/g dried soil, which is considered a high inoculum concentration (Sparrow *et al.*, 2015; Sinton *et al.*, 2016), and likely to result in stem canker in subsequent potato crops. Further research is required to evaluate the efficacy of biofumigation with 'Caliente' mustard and other biofumigant crops to reduce disease in field soils containing different *R. solani* inoculum concentrations. This would provide valuable information to the potato industry as to the inoculum concentration range at which biofumigation treatments are likely to provide effective control of *Rhizoctonia solani* diseases in potato crops.

In summary, soil edaphic conditions, including soil type, pH, temperature, and WHC, had different effects on the biofumigation potential of 'Caliente' mustard, brown mustard and 'Nemat' arugula for to suppression of *R. solani* inoculum and soil microbial activity. Biofumigation was most effective in reducing *R. solani* AG3-PT inoculum at loam soil, at pH 6.6, 20°C and 40% soil WHC, and combinations of 15°C and 40% WHC. Moreover, biofumigation resulted in the greatest levels of soil microbial activity (DHA), which in turn could suppress *R. solani* AG3-PT inoculum, in the loam soil, at pH 6.6, 15°C and 40 or 70% WHC. Therefore, effects of soil factors should be considered to maximise the efficacy of biofumigation to provide disease control for field applications. Repeated studies are required to confirm those findings.

The purpose of the experiments described in this chapter was to determine the effects of soil edaphic factors on the biofumigation processes in soil, whereby the biofumigant plants were grown in potting mix and then incorporated into the soils adjusted to, or incubated at, different conditions. However, under field situations, the biofumigant crops would be grown under conditions which are likely to affect the production of glucosinolates in the plants, either directly or due to differences in crop biomass. Falk *et al.* (2007) noted that sulfur fertilisers could increase total glucosinolates in *Brassica* plants by 25-50%. Moreover, application of appropriate amounts of nitrogen (N) and sulfur (S) fertilisers with N : S ratio less than 10:1, resulted in greater content of aliphatic GLSs (glucoraphanin and glucoibein) in broccoli (*B. oleracea* var. *italica* 'Monaco') (Schonhof *et al.*, 2007). In addition, during the growth of crops, the plants can release toxic ITC and other metabolites into the surrounding soil as root exudates, with sloughing of root cells/tissues and senescing plant tissues. This may influence pathogen growth, either directly or indirectly, due to effects on overall soil microbial activity. Kirkegaard *et al.* (2000) reported that 2-phenylethyl ITC (2-PEITC) released in field trials from active or decayed roots of canola (*B. napus*) effectively suppressed inoculum of *Gaeumannomyces graminis* var. *tritici* (the cause of take-all of wheat). In addition, 2-PEITC released from living roots of canola can change rhizosphere bacterial communities (Rumberger and Marschner, 2003, 2004).

Research on effects of biofumigation on Rhizoctonia diseases of potato under shadehouse conditions is described in the Chapter 4 of this thesis.

Chapter 4

Biofumigation potential of selected *Brassica* plants for suppression of *Rhizoctonia solani* infection of potato plants in a shadehouse experiment

4.1 Introduction

Although biofumigation have shown to effectively control the diseases caused by *Rhizoctonia solani* in a wide range of crops (Specht and Leach, 1987; McGuire, 2003; Little *et al.*, 2004; Larkin and Griffin, 2007; Sexton *et al.*, 2007; Tsror (Lahkim) *et al.*, 2007; Yulianti *et al.*, 2007; Snapp *et al.*, 2007; Halloran *et al.*, 2008; Motisi *et al.*, 2009; Mazzola and Zhao, 2010; Larkin, 2013; Larkin and Halloran, 2014; Ascencion *et al.*, 2015; El-Sharouny, 2015; Larkin *et al.*, 2010, 2011, 2012, 2017), the disease suppression efficiency achieved has often been variable, both within and between studies with inconsistent results between *in vitro* studies and those obtained under greenhouse or field conditions. For example, Indian mustard (*Brassica juncea*) provided the greatest *in vitro* suppression of the mycelium growth of *R. solani* isolates AG3 in comparison with canola and rapeseed (*B. napus* species), but gave inconsistent disease control under field conditions (Larkin and Griffin, 2007). In field trials, Indian mustard was the most effective at reducing powdery scab (caused by *Spongospora subterranea*) and common scab (caused by *Streptomyces scabies*) on potato tubers, while canola and rapeseed were the most effective at suppressing black scurf (caused by *R. solani*) (Larkin and Griffin, 2007). Similarly, Ascencion *et al.* (2015) reported that Indian mustard gave greater *in vitro* inhibition of mycelium growth of *R. solani* AG4 compared with that from *B. rapa* and *B. napus*, but was less effective than the two *Brassica* species for reducing incidence of damping off (caused by *R. solani* AG4) on cabbage (*Brassica oleracea* var. *capitata*) in a greenhouse experiment. Thus, biofumigation results obtained from *in vitro* studies have sometimes not been confirmed in greenhouse or field trials, with plants or crops.

Previous research reported in this thesis showed that incorporation of macerated biofumigant plant tissues into soil suppressed *R. solani* AG3-PT and AG2-1 isolates, reduced *R. solani* AG3-PT inoculum in soils and subsequently potato stem canker, and also promoted total soil microbial activity. In addition, biofumigation effects were influenced by soil edaphic conditions, biofumigant amount and

biofumigant plant types. However, if biofumigants are grown as cover crops to reach the maximum biomass (at flowering stage), in field soil naturally infested with *R. solani* or other soilborne pathogens, then incorporated into soil may change the biofumigation efficacy. In these cases, the biofumigation process will include release of toxic volatile compounds from living roots during biofumigant growth, as well as the incorporation of biofumigant residues into the soil (Rumberger and Marschner, 2003). The releases of 2-phenylethyl ITC and other volatile compounds from living roots of canola (*B. napus*) during plant growth reduced the inoculum of *Gaeumannomyces graminis* var. *tritici* (the cause of take-all in wheat) in field trials, and reduced the disease on wheat roots in subsequent seasons (Kirkegaard *et al.*, 2000). Biofumigant incorporation also changed rhizosphere bacterial community structure (Rumberger and Marschner, 2003, 2004).

The experiments reported here were carried to evaluate if selected promising *Brassica* plants from *in vitro* experiments, including 'Caliente' mustard, brown mustard and 'Nemat' arugula, gave control against *R. solani* causing potato diseases under shadehouse conditions. The experiments also evaluated effects the selected *Brassica* plants soil microbial activity, and on potato plant growth.

4.2 Materials and methods

4.2.1 *Rhizoctonia solani* inoculum preparation

The inoculum of a *R. solani* AG3-PT isolate (LUPP2519) was prepared on barley grains as described in Section 3.2.1, except that whole dried colonised barley grains were used to inoculate soil.

4.2.2 Soil preparation

Soil was collected from 0-20 cm depth from a potato-cropped field in Pendarves, Ashburton, Canterbury (S 43°52'1.3332'', E 172°2'18.3804'') (Appendix A.4.1). The field was chosen due to high severity of stem canker occurring on potato 'Innovator' in the 2015/16 growing season. At the sampling time (October 2016), the field was being sown with winter wheat. The soil was passed through a 4-mm mesh sieve, and mixed thoroughly until homogenous. An air-dried and sieved subsample (500 g) was provided to Hill Laboratories (Hamilton, New Zealand) for determination of general chemical characteristics, including pH, contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na); cation exchange capacity (CEC), total base saturation, and organic matter and total carbon contents (Appendix A.4.30).

4.2.3 Experimental design

Sieved field soil (31 kg) was placed into each 35 L capacity Easi-Grip poly woven planter bag (37 cm diameter x 33 cm height; EG25, Egmont, New Zealand). The soil in each bag was inoculated with *R. solani* AG3-PT colonised barley grains (210 g/bag, 0.7% w:w), and thoroughly mixed by hand. The

inoculated bags were left for 24 hours to equilibrate before sowing the *Brassica* crops (Larkin and Griffin, 2007). The experiment was carried out in a completely randomised block design with eight replicates for each treatment, under shadehouse conditions (Table 4.1). The treatments comprised three *Brassica* biofumigants plant types ('Caliente' mustard, brown mustard, 'Nemat' arugula; selected based on the results obtained from experiments described in Chapter 2), and allyl ITC, recommended fungicides (soil treated with azoxystrobin or potato seed tubers treated with pencycuron), and bare soil (with or without *R. solani* inoculum) as experimental controls (Table 4.2).

Seeds of the three biofumigant plant types were sown at a rate equivalent to 10 kg/ha, with the amount for each potting bag calculated based on the surface area of the bag and respective seed germination rate (0.113 g seeds/bag for 'Caliente' mustard, 0.259 g seeds/bag for brown mustard, 0.112 g seeds/bag for 'Nemat' arugula) (Figure 4.1A). The sown bags were watered daily for the first week, and then at 2 day intervals. Four extra bags of each *Brassica* crop were also set up for determination of dry biomass production.

Table 4.1 Temperature (°C), relative humidity (%), and light intensity (lux) (mean (minimum-maximum)) in the shadehouse during growth of biofumigant and potato plants, recorded with a HOBO data logger (Model U12-012, Onset Computer Corporation).

Crop	Temperature (°C)	Relative humidity (%)	Light intensity (lux)
Biofumigants	22.3 (13.8-39.5)	56.3 (13.3-86.8)	7,831 (4-32,280)
Potato	18.5 (10.7-35.8)	68.6 (20.3-94.7)	5,579 (4-32,280)

A **B** **C**

Figure 4.1 Biofumigant plants at 55 days after sowing (A); soil bags were double black plastic bag covers, after incorporation of macerated biofumigant plants, or irrigation of allyl isothiocyanate (B); potato plants at 35 days after planting (C).

Biofumigant plants were harvested 55 days (Figure 4.1A) after sowing. However, since roots of all the three plant types were affected by club root (caused by *Plasmodiophora brassicae*), the roots were removed, and only the shoots (above ground parts) were incorporated. Since *P. brassicae* is specific pathogen to *Brassica* plants (Ludwig-Müller *et al.*, 1999; Hwang *et al.*, 2012) although there was *P. brassicae* inoculum in the soil it was not expected to have any effect on potato plant growth. The shoots from each bag (average fresh weight per bag of 131 g of 'Caliente' mustard, 244 g of brown mustard, or 104 g of 'Nemat' arugula) were finely chopped, macerated using a food blender, and thoroughly incorporated into the soil in the respective bag by hand. The soil bags were then watered to 70% soil water holding capacity (WHC). Each biofumigant-treated soil bag was covered with two black plastic bags (Figure 4.1B) for 2 weeks to limit escape of volatiles. The treated soil bags were then allowed to aerate for an additional 15 days prior to planting of potato seed tubers.

The allyl ITC treatment (Sigma-Aldrich, New Zealand; 95% purity) was used at 10 mg/kg soil (Omirou *et al.*, 2011), and was also set up at this time. The appropriate volume of pure allyl ITC (326 μL) was pipetted into a 500 mL capacity bottle containing 250 mL tap water. The bottle was shaken for 30 minutes on a flask shaker (SF1, Stuart, Cole-Parmer, UK), then the solution was immediately irrigated onto the surfaces of the treated soil bags (Dhingra *et al.*, 2004), followed by sufficient tap water to achieve 70% WHC. The soil bags were also covered with two black plastic bags (Figure 4.1B) for 2 weeks, and then allowed to aerate for a further 15 days before planting of potato seed tubers.

The fungicide treatments of azoxystrobin soil treatment or pencycuron seed tuber treatment (at the recommended dose rates) were applied before seed tuber planting. For the soil treatment, Amistar® 250 SC (250 g azoxystrobin/L) was applied at the equivalent of 10 mL/100 m row (37 μL/planting bag). The required amount of Amistar® 250 SC was diluted with 50 mL tap water, and the total solution (50 mL) was sprayed onto the soil surface in each treated bag 1 day before planting. The fungicide Monceren® DS (125 g pencycuron/kg) was used at the equivalent of 2 kg/tonne of seed tubers (New Zealand Novachem Agrichemical Manual, 2015), and was applied to treated tubers on the day of planting. The appropriate weight of Monceren® DS (0.208 g) was used to thoroughly coat each seed tuber (averaged tuber weight = 104 g) before planting.

Before planting, the certified seed tubers of 'Russet Burbank' (provided by Sarah Sinton, Plant & Food Research, Lincoln) were treated by dipping in 0.5% formaldehyde solution for 5 minutes (Falloon, 2008). This method is used by New Zealand potato growers to ensure the potato seed tubers are free of powdery scab (*Sp. subterranea*) before planting (Falloon, 2008). The tubers were then washed 2-3 times with tap water, then left to completely dry. One formaldehyde-treated seed tuber was then placed in the centre of each planter bag at a depth of 5 cm, and covered with soil. The bags were watered 2-3 times per week until harvest (134 days after planting).

Table 4.2 Details of the treatment set up in the shadehouse experiment to test the effects of biofumigation plant treatments on *Rhizoctonia solani* soil inoculum and subsequent potato infections.

Treatment	Step 1 (Soil treatment)	Step 2 (Soil treatment)	Step 3 (Potato tuber planting)
1	Potato field soil (untreated control)	No treatment	Potato planting
2	Potato field soil artificially inoculated with *R. solani* AG3-PT (positive control)	No treatment	Potato planting
3	'Caliente' mustard growing in potato field soil artificially inoculated with *R. solani* AG3-PT	Maceration and incorporation of shoots into soil bags for 29 days	Potato planting
4	Brown mustard growing in potato field soil artificially inoculated with *R. solani* AG3-PT	Maceration and incorporation of shoots into soil bags for 29 days	Potato planting
5	'Nemat' arugula growing in potato field soil artificially inoculated with *R. solani* AG3-PT	Maceration and incorporation of shoots into soil bags for 29 days	Potato planting
6	Potato field soil artificially inoculated with *R. solani* AG3-PT	Soil treated with allyl ITC (10 mg/kg soil) for 29 days	Potato planting
7	Potato field soil artificially inoculated with *R. solani* AG3-PT (fungicide control)	Fungicide – soil treated with azoxystrobin 1 day before planting	Potato seed tubers treated with pencycuron before planting

4.2.4 Soil sampling process and potato plant assessments

4.2.4.1 Soil sampling

Soil samples (50 g from each bag) from all treatments were collected, either immediately after inoculating the soil with barley grains colonised *R. solani* AG3-PT (T0), or before potato planting (29 days after biofumigant incorporation) (T1), at 35 days after potato planting (T2), or at harvest (134 days after planting) (T3). Soil in each bag was randomly collected by taking six cores (8 cm depth x 2 cm diameter). The six samples per bag were combined, mixed thoroughly, and a 15 g subsample was taken stored at -80°C for DNA extraction. The remaining samples were stored at 4°C and used for determining soil microbial activity, by measuring dehydrogenase activity (DHA). The soil samples in each replicate (block) of six inoculated treatments at T0 were pooled together, and thoroughly mixed and subsamples of 15 g were used for DNA extraction, and the rest of each sample was used for DHA measurement.

Soil DNA extraction

Soil samples were prepared as pellets, as described in Section 3.2.4.1 (Chapter 3). Total DNA was extracted from each pellet using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, USA) following the manufacturer's instructions. Extracted DNA samples were stored at -20°C until used.

Biofumigation effects on *Rhizoctonia solani* AG3-PT inoculum

Changes in the amounts of *R. solani* AG3-PT DNA in soil (primer/probe sequences shown in Table 3.3, Chapter 3) were determined using the qPCR method described in Section 3.2.4.1 (Chapter 3). Pure DNA concentrations of *R. solani* AG3-PT (LUPP2519) were used for constructing a standard curve for 45.40, 4.54, 4.54 x 10^{-1}, 4.54 x 10^{-2}, 4.54 x 10^{-3}, 4.54 x 10^{-4} or 4.54 x 10^{-5} ng (Appendix A.4.31). The mean initial *R. solani* AG3-PT DNA amounts (T0) were determined as 0.7 pg/g dried soil for the untreated control (nil inoculated treatment) and 615,376 pg/g dried soil for the *R. solani* AG3-PT inoculated treatment.

Soil microbial activity

Soil microbial activity as measured by soil dehydrogenase activity was determined using the method described in Section 3.2.4.7 (Chapter 3).

4.2.4.2 Potato plant assessments

At 35 days after potato planting

Four replicates (four potato plants) in each treatment were destructively harvested. The plants (Figure 4.1C) were harvested by carefully removing them from the bags, and the roots, stolons and stems were carefully washed, blotted dry on tissue paper, and laid on a table surface for disease evaluations.

Severity of stem canker (Figure 4.2A) was evaluated using a 0-6 scale (Gibson and Falloon, 2016); Appendix A.3.2, Chapter 3): where 0 = no symptoms; 1 = 1-10% of stem surface area affected; 2 = 10-30% surface area affected; 3 = 30-50% surface area affected; 4 = 50-80% of surface area affected; 5 = 80-100% of stem surface area affected; or 6 = stem dead.

Severity of stolon disease (Figure 4.2B) was assessed using the scale of Atkinson *et al.* (2010): where 0 = no symptoms; 1 = one or two stolons with lesions; 2 = one or two stolons girdled; 3 = three stolons girdled; or 4 = four or more stolons girdled.

Severity of root disease (Figure 4.2C) was rated using a 0-5 scale (Larkin and Griffin, 2007): where 0 = no symptoms; 1 = brown discoloration of roots; 2 = distinct canker lesions, covering <50% root circumference; 3 = severe cankers, covering >50% of root circumference; 4 = canker completely girdling root and covering >50% of total root surface; or 5 = roots and stems completely nipped off, 100% root surface covered with cankers, and/or death of plant.

At harvest (134 days after potato planting)

The remaining four replicates of each treatment were harvested as described for the 35 days assessment. Severity of stem canker (Figure 4.3A) was assessed using scale described above.

Potato tubers from each treatment replicate were visually assessed for severity of black scurf (Figure 4.3B) using the following scale (adapted from a scale for Ascochyta blight on pea leaves; S. L. H Viljanen, Plant and Food Research, personal information; Appendix A.4.32): where 0 = no symptoms; 1 = 1% of tuber surface affected; 2 = 5% of surface affected; 3 = 10% of surface affected; 4 = 15% of surface affected; 5 = 20% of surface affected; 6 = 30% of surface affected, 7 = 45% of surface affected; or 8 = 60% of tuber surface affected.

Symptoms of powdery scab on the potato tubers (Figure 4.4A) and root galls (Figures 4.4B, C) caused by *Sp. subterranea*, and stem lesions caused by *Colletotrichum coccodes* (Figure 4.5), were also observed at harvest. Powdery scab severity the tubers was assessed using the scale of Falloon *et al.* (1995); Appendix A.4.33): where 0 = no symptoms; 1 =1-5% of scab surface affected; 2 = 5-10% of surface affected; scab covered on tuber; 3 = 10-15% of surface affected; 4 = 15-20% of surface affected; 5 = 20-33% of surface affected; 6 = 33-46% of surface affected; 7 = 46-60% of surface

affected; 8 = 60-73% of surface affected; 9 = 73-86% of surface affected; or 10 = 86-100% of tuber surface affected.

Severity of *Spongospora* root galls was assessed using the 0-4 scale of Sinton *et al.* (2016): where 0 = no galls; 1 = <5 galls/plant; 2 = 5-20 galls/plant; 3 = 20-50 galls/plant; or 4 = >50 galls/plant.

The incidence of dead stems caused by *C. coccodes* was counted. To confirm infection by *C. coccodes* the dead stems were observed under a stereo microscope for the presence of black microsclerotia (Figure 4.5A), on the external stem tissues and internally after dissection of the stems. In addition, the samples were observed under a compound microscope for the presence of acervuli with setae (Figure 4.5B) and typical *C. coccodes* conidia (Figure 4.5C).

The disease severities (DS) for stems, stolons, and tubers were calculated using the formula described by Atkinson *et al.* (2010) (Section 3.2.4.5, Chapter 3).

Other parameters were also assessed, including the number of days taken to plant emergence, numbers of stems and stolons on plants, plant height, root dry biomass, shoot dry biomass, total dry biomass (root + shoot), numbers of tuber initials (tubers with diameter ≤10 mm), numbers of tubers (tubers with diameter >10 mm), total fresh tuber weight/plant and mean tuber weight/plant. The harvested potato roots and shoots were oven-dried at 60°C for 3 days before weighing to determine dry biomass.

4.2.5 Data analyses

The raw data were firstly examined for assumptions of normal distribution using GenStat software (Version 18.1.0.17005; VSN International Ltd, Hemel Hempstead, United Kingdom). If the data did not satisfy assumptions of normality, they were appropriately transformed prior to statistical analyses.

The means of amounts of *R. solani* DNA, DHA, time to plant emergence, plant height, numbers of stems or stolons, root and shoot dry biomass, total tuber weight, weight/tuber, and stem/tuber disease incidence and severity between treatments, were used to compare differences as well as interactions between treatments and sampling times (if present), using Tukey's Honestly Significant Difference (HSD) test at P=0.05.

Possible correlations between potato growth parameters, soil microbial activity and amounts of *R. solani* DNA were determined using GenStat software.

4.3 Results

4.3.1 Amounts of *Rhizoctonia solani* AG3-PT DNA

The statistical analyses are shown in Appendix A.4.2, and the results are summarised in Table 4.3. Initially, the mean amount of *R. solani* AG3-PT DNA in soil from the untreated control was 0.7 pg/g dried soil, and from the inoculated treatments was 615,376 pg/g dried soil. There was a significant interaction (P<0.001) between treatments and sampling times for the amounts of *R. solani* DNA in soil. The amounts in the positive control treatment at the three sampling times (26,900-175,522 pg/g dried soil) were significantly than from all the other treatments. Before potato planting (T1), there was no significant difference in the amounts of DNA between the biofumigant, allyl ITC or and fungicide treatments compared with the untreated control (0.2-34 pg/g dried soil). At 35 days after planting (T2), DNA amount in the allyl ITC-treated soil (75 pg/ g dried soil) increased and was significantly greater than from the untreated (0.2 pg/g dried soil) or 'Caliente' mustard treatment (6 pg/g dried soil). At harvest (T3) *R. solani* DNA amount (337 pg/g dried soil) was significantly greater from the fungicide treatment compared to the other treatments (0.2-75 pg/g dried soil), except for the positive control. At T3, the *R. solani* DNA amount from the allyl ITC treatment (74 pg/g dried soil) was also greater than in the untreated control (0.7 pg/g dried soil).

Across the treatments, there was a significant effect (P<0.001) of sampling time on the amount of *R. solani* AG3-PT DNA. Before potato planting, the mean amount of DNA (632 pg/g dried soil) was less than that at 35 days after planting (1,082 pg/g dried soil), or at harvest (1,226 pg/g dried soil). Across the sampling times, there was a significant effect (P<0.001) of treatment on amount of *R. solani* DNA. The positive control treatment gave the greatest mean amount of *R. solani* DNA (89,033 pg/g dried soil), followed by the fungicide treatment (119 pg/g dried soil). The amount of pathogen DNA from the allyl ITC treatment (54 pg/g dried soil) was greater than from the untreated control or the 'Caliente' mustard treatment.

Table 4.3 Mean amounts of *Rhizoctonia solani* AG3-PT DNA (pg/g dried soil) in soil after different biofumigant and fungicide treatments, assessed before potato planting (T1), at 35 days after planting (T2) or at harvest (134 days after planting).

Treatment	Sampling time			Mean of treatment
	T1	T2	T3	
Untreated control	0.2 g	0.2 g	0.7 g	0.5 D
Positive control	26,900 c	147,252 b	175,522 a	89,033 A
'Caliente' mustard	8 g	6 g	20 fg	11 D
Brown mustard	21 fg	31 fg	28 fg	27 CD
'Nemat' arugula	25 fg	14 fg	19 fg	19 CD
Allyl isothiocyanate	14 fg	75 ef	74 ef	54 C
Fungicides	34 fg	13 fg	337 d	119 B
Mean of sampling time	632 Z	1,082 Y	1,226 X	
P values: <0.001 (Treatment); <0.001 (Sampling time); <0.001 (Treatment x Sampling time)				

Means within each column or row followed by the same letter are not significantly different (*P*=0.05), according to Tukey's HSD test. Data were log(X+1) transformed prior to statistical analyses, and are presented as back-transformed means.

4.3.2 Soil microbial activity (dehydrogenase activity)

The statistical analyses are shown in Appendix A.4.3, and the results are summarised in Table 4.4. There was a significant interaction (*P*<0.001) between treatment and sampling time for soil DHA. At all the sampling times, the soil DHA from the untreated control (no *R. solani* inoculum) was significantly less (mean = 1.14-1.82 µg/g dried soil.hr^{-1}) compared with the DHA from all the other treatments. Before biofumigant planting (T0), there were variations between treatments, apart from the untreated control. This could be due to sampling errors or natural variations between samples. Before potato planting (T1), except for the fungicide treatment (2.92 µg/g dried soil.hr^{-1}), soil microbial activities from the other treatments were less and decreased compared with those at T0. The DHA at 35 days after potato planting (T2) and at harvest (T3), from all treatments apart from the untreated control, were significantly less than at the T1 and T0 assessments, and there was no significant difference in the DHAs at T2 and T3 assessments between any of these treatments (1.36-1.54 µg/g dried soil.hr^{-1}).

There was a significant effect (*P*<0.001) of sampling time on soil microbial activity. Mean DHA at T0 (3.40 µg/g dried soil.hr^{-1}) was greater than at all other assessment times, followed by that at T1 (2.11 µg/g dried soil.hr^{-1}) which was greater than at T2 or T3 (1.40 µg/g dried soil.hr^{-1} for both). There was a significant effect (*P*<0.001) of treatment on soil microbial activity. Apart from the untreated control

treatment, 'Caliente' mustard and 'Nemat' arugula treatments resulted in the lowest DHAs (2.03-2.06 µg/g dried soil.hr^{-1}), which were significantly different those for all the other treatments apart from brown mustard (2.11 µg/g dried soil.hr^{-1}).

Table 4.4 Mean soil dehydrogenase activity (µg/g dried soil.hr^{-1}) after different biofumigant or fungicide treatments, assessed before biofumigant planting (T0), before potato planting (T1), at 35 days after potato planting (T2) or at harvest (134 days after potato planting, T3).

Treatment	Sampling time				Mean of treatment
	T0	T1	T2	T3	
Untreated control	1.82 f	1.29 hi	1.14 i	1.16 i	1.34 D
Positive control	3.88 ab	2.33 e	1.39 gh	1.52 g	2.18 AB
'Caliente' mustard	3.77 bc	1.95 f	1.41 gh	1.37 gh	2.03 C
Brown mustard	3.52 c	2.30 e	1.48 g	1.44 gh	2.11 BC
'Nemat' arugula	3.98 ab	1.79 f	1.39 gh	1.50 g	2.06 C
Allyl isothiocyanate	4.13 a	2.43 e	1.47 gh	1.36 gh	2.23 AB
Fungicides	3.06 d	2.92 d	1.54 g	1.50 g	2.19 AB
Mean of sampling time	3.40 X	2.11 Y	1.40 Z	1.40 Z	
P values: <0.001 (Treatment); <0.001 (Sampling time); <0.001 (Treatment x Sampling time)					

Means within each column or row followed by the same letter are not significantly different (*P*=0.05), according to Tukey's HSD test. Data were sqrt(X+1) transformed prior to statistical analyses, and are presented as back-transformed means.

4.3.3 Disease assessments on potato plants

4.3.3.1 Stem canker, tuber black scurf, and stolon and root disease

There were significant effects of the treatment on severity of stem canker severity (Figure 4.2A) (*P*<0.001), stolon disease (Figure 4.2B) (*P*<0.001) and root disease (Figure 4.2C) (*P*=0.01) at 35 days after planting (Appendices A4.4-A4.6). The untreated control gave less severe stem canker severity (mean = 3.9% stem surface area affected) compared with all the other treatments. 'Caliente' mustard, 'Nemat' arugula and the fungicide treatments reduced stem canker severity (50-58% reduction) relative to the positive control, followed by brown mustard (34% reduction) (Table 4.5). All the treatments apart from allyl ITC reduced severity of stolon canker compared with the positive control. The fungicide and 'Caliente' mustard treatments reduced stolon disease by the greatest amounts, decreasing stolon severity compared with the other biofumigant treatments. Further, the fungicide treatments significantly reduced stolon canker severity compared with the 'Caliente'

mustard treatment, and were not significantly different from the untreated control treatment. There were no significant differences in the severities root disease between treatments (mean score = 0.7-1.11), apart from the root severity score in the untreated control, which was less compared with the positive control or brown mustard treatments.

Figure 4.2 Stem canker, stolon and root lesions on potato plants 35 days after planting: stem canker (A); stolon nipping off (B); brown root discolorations or cankers (C), indicated by blue arrows.

At harvest, there were significant effects (P<0.001; Table 4.5; Appendices A4.7-A4.8) of the treatments on severity of stem canker (Figure 4.3A) and black scurf (Figure 4.3B). All treatments reduced stem canker severity compared with the positive control and the untreated control treatments, with the mean stem canker severity from the positive control (56.1% stem surface affected) being greater than from untreated control treatment (36.8%). Of the remaining treatments, the fungicide treatment (21.1%) reduced stem canker severity compared with all the other treatments apart from the brown mustard treatment (24.9%). The positive control gave the greatest stem canker severity (56.1%), followed by the untreated control (36.8%).

There were no symptoms of black scurf on tubers from the fungicide treatments (Table 4.5). The untreated and positive control treatments gave the greatest black scurf severity (mean = 6.6-8.4% of tuber surface affected). All the other treatments significantly reduced black scurf severity compared with the untreated control, and all treatments apart from 'Nemat' arugula reduced black scurf severity compared with the positive control treatment.

A B

Figure 4.3 Symptoms of stem canker and black scurf on potato plants at harvest: Stem canker indicated by blue arrow (A); black scurf on tubers (B).

Table 4.5 Mean severities of stem canker, stolon or root diseases on potato plants grown in *R. solani* (AG3-PT) inoculated soil, after treatments with different biofumigant plants ('Caliente' mustard, brown mustard or 'Nemat' arugula), allyl isothiocyanate, fungicides or control treatments, at 35 days after potato planting or at harvest (134 days after planting), under shadehouse conditions. Each datum is a mean of four replicate plants.

Treatment	35 days after planting			At harvest	
	Stem canker severity (%)	Stolon disease severity (%)	Root disease score	Stem canker severity (%)	Black scurf severity (%)
Untreated control	3.9 d[(1)]	7.8 d[(1)]	0.7 b[(2)]	36.8 b[(3)]	8.4 a[(4)]
Positive control	18.3 a	34.9 a	1.2 a	56.1 a	6.6 ab
'Caliente' mustard	7.6 c	15.4 c	1.0 ab	26.6 cd	2.5 c
Brown mustard	12.1 b	24.6 b	1.2 a	24.9 de	1.7 cd
'Nemat' arugula	7.6 c	25.4 b	1.0 ab	30.7 c	4.7 b
Allyl isothiocyanate	17.1 a	33.4 a	1.1 ab	29.3 cd	1.0 d
Fungicides	9.2 bc	8.1 d	1.0 ab	21.1 e	0 e
P	<0.001	<0.001	<0.001	<0.001	<0.001

Means within each column followed by the same letter are not significantly different (*P*=0.05), according to Tukey's HSD test. Data were transformed prior to statistical analyses, and are presented as back-transformed means.

[(1)] Data were sqrt(X/100) transformed

[(2)] Data were sqrt(X) transformed

[(3)] Data were arcsine(sqrt(X/100)) transformed

[(4)] Data were sqrt((X+1)/100) transformed

4.3.3.2 Diseases caused by other pathogens

In addition to symptoms on the potato plants likely to be caused by *R. solani*, symptoms caused by other pathogens, including powdery scab on tubers and root galls caused by *Sp. subterranea* (Figure 4.4A) and black dot caused by *Colletotrichum coccodes* (Figure 4.5A), were also observed on potato stems, roots and/or tubers at harvest.

All the biofumigant plant, allyl ITC and fungicide treatments significantly reduced powdery scab severity on tubers (mean severity = 1.3-5.0% tuber surface affected) compared with both the positive and untreated control treatments (9.4-12.3%) (Table 4.6; Appendix A.4.9). 'Caliente' mustard, brown mustard, 'Nemat' arugula or allyl ITC treatments reduced powdery scab severity compared with the fungicide treatments, but not the brown mustard treatment. However, there was no significant effect (P=0.069; Appendix A.4.10) of treatment on the mean root gall score.

There was a significant effect (P<0.001) of treatments on the incidence of dead stems caused by *C. coccodes* (Appendix A.4.11). The fungicides, allyl ITC, 'Caliente' mustard, brown mustard and 'Nemat' arugula treatments (24.7-34.4%) reduced the incidence of dead stems caused by *C. coccodes* compared with the positive control treatment (47.4%) (Table 4.6). All treatments apart from 'Nemat' arugula also reduced the incidence of dead stems (24.7-31.1%) compared with the untreated control (34.4-42.3%).

Table 4.6 Mean disease symptoms caused by *Spongospora subterranea* or *Colletotrichum coccodes on* potato plants grown in *Rhizoctonia solani* (AG3-PT) inoculated soil after treatment with different biofumigant plants ('Caliente' mustard, brown mustard or 'Nemat' arugula), allyl isothiocyanate, fungicides and control treatments, at potato tuber harvest for plants grown under shadehouse conditions.

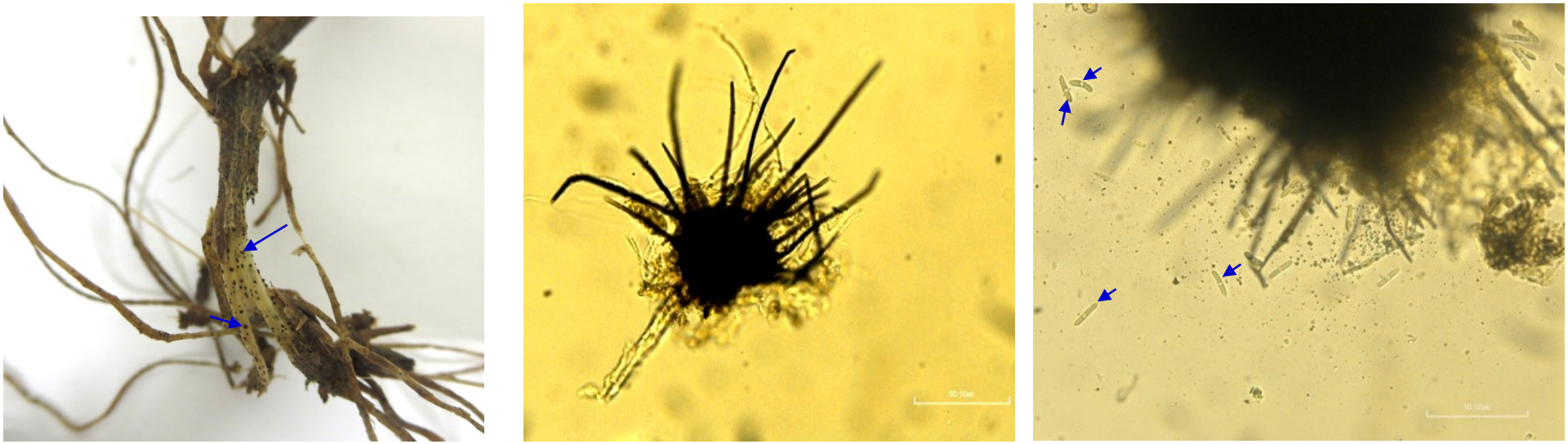
Treatment	Disease caused by *Spongospora subterranea*		Incidence of dead stems caused by *Colletotrichum coccodes* (%)
	Powdery scab severity (%)	Root gall score	
Untreated control	12.3 a[(1)]	1.7 a[(2)]	42.3 ab[(1)]
Positive control	9.4 a	2.0 a	47.4 a
'Caliente' mustard	2.0 c	1.0 a	31.1 cd
Brown mustard	3.0 bc	1.2 a	30.4 cd
'Nemat' arugula	1.6 c	1.2 a	34.4 bc
Allyl isothiocyanate	1.3 c	1.2 a	27.6 cd
Fungicides	5.0 b	1.2 a	24.7 d
P	<0.001	0.069	<0.001

Means within each column followed by the same letter are not significantly different (P=0.05) according to Tukey's HSD test. Data were transformed prior to statistical analyses, and are presented as back-transformed means.

[(1)] Data were sqrt(X/100) transformed; [(2)] Data were sqrt(X+1) transformed

A B C

Figure 4.4 Symptoms caused by *Spongospora subterranea*: powdery scab on tubers (A), root galls (B), and a root gall observed under a stereo microscope (C). Scale bar = 50 μm.



A B C

Figure 4.5 Disease symptoms caused by *Colletotrichum coccodes*: potato stem rot with black microsclerotia indicated by blue arrows (A), acervulus with setae (B), Typical *C. coccodes* conidia (blue arrows) observed under a compound microscope (C). Scale bars = 50 μm.

4.3.4 Effects of biofumigant plants on potato plant growth

4.3.4.1 Potato plant characteristics

There were no significant differences between treatments for the means of numbers of days to plant emergence (10-13 days) (Table 4.7; Appendix A.4.12), numbers of stems/plant (8.5-10.7 stems/plant at 35 days after planting (DAP)), 8.4-12.3 stems/plant at harvest) (Table 4.7; Appendices A4.15-A4.16), number of stolons (4.7-5.0 stolons per stem at 35 DAP) (Appendix A.4.17) or root : shoot ratio (0.105-0.130) at 35 DAP (Table 4.7; Appendix A.4.21).

At 35 DAP, all treatments apart from the untreated control significantly increased plant height compared with the positive (inoculated) control (mean height = 80.5 cm). The 'Caliente' and brown mustard treatments, allyl ITC and fungicide treatments (178.0-206.8 cm) significantly increased plant height compared with the untreated control (83.2 cm) (Table 4.7; Appendix A.4.13). At harvest, only the allyl ITC and fungicide treatments (respectively, 206.8 cm and 192.1 cm) increased plant heights compared with the positive (inoculated) and untreated control treatments (169.5-171.3 cm) (Table 4.7; Appendix A.4.14).

At 35 DAP, the fungicides was the only treatment to significantly increased root dry weight (mean = 2.7 g) compared with the positive control treatment (1.9 g) (Table 4.7; Appendix A.4.18). All the other treatments increased shoot dry weights and total plant dry biomass compared with the positive control (14.6 g for shoot dry weight and 16.5 g for total biomass). In addition, the shoot dry weight of plants from the allyl ITC and fungicide treatments (25.4-27.0 g) were greater than from all the other treatments, apart from the untreated control treatment (23.3 g) (Table 4.7; Appendix A.4.19). Similarly, total dry biomasses from the allyl ITC and fungicide treatments were 28.1-29.5 g, which were greater than from other treatments, except for the untreated control (25.9 g) (Table 4.7; Appendix A.4.20).

At harvest, only the fungicide treatment significantly increased mean root dry weight (2.3 g) compared with the untreated control treatment, or the 'Caliente' mustard or brown mustard (1.6-1.7 g) treatments (Table 4.7; Appendix A.4.22). 'Caliente' mustard, allyl ITC, and the fungicide treatments increased shoot dry weights (69.8-83.4 g), and total dry biomasses (71.6-85.6 g) compared with the positive and untreated control treatments (respectively, 52.8 and 55.4 g for shoots and 54.7 and 57.2 g for total biomass) (Table 4.7; Appendices A4.23-A424). The root : shoot ratios were increased in the positive control treatment (0.036) compared with all the other treatments apart from the untreated control (0.031) and 'Nemat' arugula (0.030) treatments (Table 4.7; Appendix A.4.25).

4.3.4.2 Tuber yields

There were significant effects of the treatments on the mean numbers of tuber initials (P<0.001; Table 4.8; Appendix A.4.26), numbers of tubers (P<0.001; Table 4.8; Appendix A.4.27), total tuber weights (P<0.001; Table 4.8; Appendix A.4.28) and average weights/tuber (P<0.001; Table 4.8; Appendix A.4.29). The fungicide treatment (10.2) increased the numbers of initials compared with all the other treatments apart from the positive control (7.2). Allyl-ITC (1.7) and 'Caliente' mustard (1.7) treatments reduced the numbers of tuber initials compared with the untreated control treatment (5.7). The untreated control, positive control, 'Nemat' arugula and fungicide treatments gave greater numbers of tubers (19.7-20.7) compared with the other treatments (14.2-15.2). The fungicide treatment increased the total tuber weight (634 g) compared with the untreated and positive controls, and all the other treatments. 'Caliente' mustard, brown mustard and allyl ITC also increased total tuber weights (495.1-538.7 g) compared with the untreated and positive controls. 'Caliente' mustard, brown mustard, allyl ITC and fungicide treatments significantly increased the mean weights/tuber (32.2-36.6 g) compared with all the other treatments (22.7-23.2 g).

Table 4.7 Mean time (days) to potato plant emergence, plant heights (cm), numbers of stems and stolons, root, shoot or total plant dry biomasses (g), and root : shoot ratios, from different biofumigant plant, fungicide or control treatments at 35 days after potato planting (DAP) and harvest (134 DAP). Each datum is a mean for four replicate plants.

Treatment	Emergence (day)	Plant height (cm)		Number of stems		Number of stolons (35 DAP)	35 DAP				At harvest			
		35 DAP	Harvest	35 DAP	Harvest		Root dry weight (g)	Shoot dry weight (g)	Total biomass (g)	Root:shoot ratio	Root dry weight (g)	Shoot dry weight (g)	Total biomass (g)	Root:shoot ratio
Untreated control	11 a[(1)]	83.2 bc[(1)]	171.3 c[(1)]	10.7 a[(1)]	11.2 a[(1)]	4.7 a[(1)]	2.6 ab[(1)]	23.3 ab[(1)]	25.9 ab[(1)]	0.112 a[(2)]	1.7 b[(1)]	55.4 de[(1)]	57.2 d[(1)]	0.031 ab[(2)]
Positive control	11 a	80.5 c	169.5 c	10.2 a	9.9 a	4.8 a	1.9 b	14.6 d	16.5 d	0.130 a	1.9 ab	52.8 e	54.7 d	0.036 a
'Caliente' mustard	11 a	94.3 a	182.3 bc	10.5 a	11.2 a	4.8 a	2.6 ab	20.7 bc	23.4 bc	0.128 a	1.6 b	74.5 ab	76.0 ab	0.021 c
Brown mustard	10 a	101.5 a	178.0 bc	9.7 a	9.0 a	5.0 a	2.5 ab	21.2 bc	23.7 bc	0.119 a	1.7 b	61.7 cd	63.4 cd	0.027 b
'Nemat' arugula	11 a	91.8 ab	172.9 c	10.2 a	12.3 a	4.9 a	2.1 ab	18.6 c	20.7 c	0.115 a	1.8 ab	60.8 de	62.6 cd	0.030 ab
Allyl isothiocyanate	11 a	98.5 a	206.8 a	10.5 a	8.4 a	4.9 a	2.5 ab	27.0 a	29.5 a	0.095 a	1.8 ab	69.8 bc	71.6 bc	0.027 b
Fungicides	13 a	94.7 a	192.1 ab	8.5 a	10.1 a	4.9 a	2.7 a	25.4 a	28.1 a	0.105 a	2.3 a	83.4 a	85.6 a	0.027 b
P	0.156	<0.001	<0.001	0.283	0.372	0.897	0.028	<0.001	<0.001	0.122	0.008	<0.001	<0.001	<0.001

Means within each column followed by the same letter are not significantly different (*P*=0.05), according to Tukey's HSD test. Data were transformed prior to statistical analyses, and are presented as back-transformed means.

[(1)] Data were sqrt(X) transformed

[(2)] No data transformation

Table 4.8 Mean numbers of tuber initials, tubers, and total or mean tuber weight/plant (g), for potato plants grown in *Rhizoctonia solani* AG3-PT-inoculated soil after treatment with different biofumigant plants ('Caliente' mustard, brown mustard or 'Nemat'arugula), allyl isothiocyanate or fungicides.

Treatment	Number of initials	Number of tubers	Total tuber weight (g)	Weight of a tuber (g)
Untreated control	5.7 bc	20.0 a	446.9 d	22.4 b
Positive control	7.2 ab	19.7 a	446.5 d	22.7 b
'Caliente' mustard	1.7 d	14.7 b	538.7 b	36.6 a
Brown mustard	3.7 cd	14.2 b	495.1 bc	34.9 a
'Nemat' arugula	3.9 cd	20.7 a	475.7 cd	23.2 b
Allyl isothiocyanate	1.7 d	15.2 b	506.7 bc	33.4 a
Fungicides	10.2 a	19.7 a	634.0 a	32.2 a
P	<0.001	<0.001	<0.001	<0.001

Means within column followed by the same letter are not significantly different (*P*=0.05), according to Tukey's HSD test. All data were sqrt(X) transformed prior to statistical analyses, and are presented as back-transformed means.

4.3.5 Correlations between potato plant growth parameters, disease severity scores, amounts of soil *Rhizoctonia solani* AG3-PT DNA and soil microbial activity

At 35 DAP (Table 4.9), amounts of *R. solani* AG3-PT DNA in soil were positively correlated with stem canker and stolon disease severity, and were negatively correlated with plant height, root dry weight, shoot dry weight and total plant dry biomass. There was no correlation between soil microbial activity and amounts of *R. solani* AG3-PT DNA. Soil microbial activity (DHA) was positively correlated with stem canker severity, root disease score and plant height, and negatively correlated with number of stems.

At harvest (Table 4.10), amounts of *R. solani* AG3-PT DNA were positively correlated with stem canker severity, black scurf severity, powdery scab severity, root gall score, *C. coccodes* incidence and DHA level, and negatively correlated with shoot dry weight, total plant dry biomass and weight/tuber. Soil microbial activity was negatively correlated with black scurf severity.

Table 4.9 Correlations between different potato plant parameters, diseases, soil microbial activity and amounts of *Rhizoctonia solani* DNA, at 35 days after potato planting. Statistically significant correlation coefficients (R) are indicated in bold.

Variable	Correlation coefficient (R)										
	1	2	3	4	5	6	7	8	9	10	11
1. Stem canker severity (%)	-										
2. Stolon severity (%)	**0.77*** **	-									
3. Root disease score	**0.60*** **	**0.43***	-								
4. Plant height (cm)	0.07	0.07	0.18	-							
5. Number of stem	-0.14	0.14	0.06	-0.17	-						
6. Root dry weight (g)	-0.24	**-0.44***	-0.12	0.27	0.00	-					
7. Shoot dry weight (g)	-0.19	**-0.37***	-0.20	**0.45*** **	-0.14	**0.57*** **	-				
8. Total dry biomass (g)	-0.07	**-0.40***	-0.21	**0.45****	-0.13	**0.64*** **	**1.00*** **	-			
9. Dehydrogenase activity (DHA)	**0.52****	0.25	**0.45****	**0.53****	**-0.36***	0.03	0.12	0.12	-		
10. DNA amount (pg/g dried soil)	**0.54****	**0.46****	0.24	**-0.56*** **	0.07	**-0.51****	**-0.70*** **	**-0.71*** **	-0.02	-	
11. Number of stolon	0.18	0.15	0.14	0.12	**-0.37***	0.13	0.11	0.13	0.29	0.00	-

*: different at $P \leq 0.05$; **: different at $P \leq 0.01$; ***: different at $P \leq 0.001$

Table 4.10 Correlations between different potato plant parameters, diseases, soil microbial activity and amounts of *Rhizoctonia solani* DNA at harvest (134 days after planting). Statistically significant correlation coefficients (R) are indicated in bold.

Variable	Correlation coefficient (R)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Stem canker severity	-														
2. Black scurf severity	**0.72*** **	-													
3. Powdery scab severity	**0.52** **	**0.54** **	-												
4. Root gall score	**0.53** **	**0.49** **	**0.45** **	-											
5. *Colletotrichum* incidence	**0.82** **	**0.84*** **	**0.62*** **	**0.46** **	-										
6. Number of stem	0.01	0.23	0.07	0.14	0.17	-									
7. Root dry weight (g)	-0.08	-0.26	0.10	0.14	-0.16	0.17	-								
8. Shoot dry weight (g)	**-0.72*** **	**-0.81*** **	**-0.42***	**-0.46** **	**-0.74*** **	0.02	**0.36***	-							
9. Total dry biomass (g)	**-0.71*** **	**-0.81*** **	**-0.41***	**-0.45** **	**-0.73*** **	0.02	**0.39***	**1.00*** **	-						
10. Number of initial	0.16	0.05	**0.58*** **	**0.44***	0.19	0.10	**0.43***	-0.18	-0.17	-					
11. Number of tuber	0.31	**0.41***	**0.43***	0.29	**0.39***	0.27	0.21	-0.14	-0.12	**0.58*** **	-				
12. Total tuber weight (g)	**-0.69*** **	**-0.81*** **	-0.27	**-37***	**-0.72*** **	-0.04	0.27	**0.86*** **	**0.86*** **	0.07	-0.12	-			
13. Weight of a tuber (g)	**-0.64*** **	**-0.77*** **	**-0.52** **	**-46** **	**-0.71*** **	-0.22	-0.07	**0.62*** **	**0.61*** **	**-0.42***	**-0.85*** **	**0.59*** **	-		
14. DHA	0.01	**-0.35***	-0.29	-0.12	-0.08	-0.06	0.23	0.18	0.18	0.03	0.06	0.25	0.12	-	
15. DNA level (pg/g dried soil)	**0.86*** **	**0.34***	**0.43***	0.48	**0.60*** **	-0.05	0.13	**-0.43***	**-0.42***	0.31	0.22	-0.31	**-0.38***	**0.35***	-

*: different at $P \leq 0.05$; **: different at $P \leq 0.01$; ***: different at $P \leq 0.001$

4.4 Discussion

The biofumigant plant types ('Caliente' and brown mustards), allyl ITC and fungicide treatments all reduced amounts of soil *R. solani* AG3-PT inoculum, and gave corresponding reductions in severity of stem canker, stolon disease, and black scurf on tubers. The treatments also increased shoot dry weights, total tuber weights and mean tuber weights. The increases to plant mass resulting from the 'Caliente' mustard and brown mustard treatments recorded in this study are similar to those found by Snapp *et al.* (2007). They reported that incorporation of mustard shoots increased potato tuber yields and reduced tuber disease scores compared with untreated experimental controls. The increases in plant yield parameters were probably due to decreased amounts of disease, and not to improved crop nutrition due to the additions of organic matter and increased microbial activity, through amendment of soil with biofumigant plant tissues. The reductions in disease in the potato plants were similar or greater than those measured from the fungicide and allyl ITC control treatments.

Although the aim of this research was to evaluate the effects of biofumigant treatments on *R. solani* AG3-PT, there were also reductions of powdery scab (caused by *Sp. subterranea*) severity on tubers and incidence of dead stems caused by *C. coccodes*, from the treatments with 'Caliente' and brown mustards. A study conducted by Sinton *et al.* (2016) showed that there were several soilborne pathogens, including *R. solani* (AG2-1 and AG3), *Sp. subterranea, St. scabies*, *C. coccodes*, *Verticillium dahliae*, root lesion nematodes (*Pratylenchus neglectus*, *P. crenatus*) and root knot nematode (*Meloidogyne fallax*), detected in soil samples collected from 18 New Zealand fields prior to potato plantings. These identifications were obtained using the commercial qPCR 'PreDicta Pt' soil testing service (Primary Industries and Regions South Australia, 2015). Thus, an ideal effective biofumigant should have activity against this range of soilborne pathogens. This also implied that *in vitro* or greenhouse experiments focussing on single plant diseases may not adequately reflect biofumigant efficacy for potato field crops as they are usually affected by multiple pathogens.

Inoculation of soil with *R. solan*i with or without amendment with biofumigant tissues increased soil microbial activity (DHA), compared with the untreated treatment. This result is similar to those from experiments described in Chapter 3. In general, across all of the biofumigant and fungicide treatments, the DHAs did not differ compared with the positive (inoculated) control. This indicated that DHA activity *per se* was not associated with the *R. solani* inoculum. However, DHAs were less from the untreated control treatment compared with all other treatments, indicating that soil organic matter incorporation, probably the barley grains with the *R. solani* inoculum but also the biofumigant plant tissue amendments resulted in increased DHA measures. As discussed in Chapter 3, soil DHA is associated with soil organic matter (SOM) (Wolińska and Stępniewska, 2012). The decline of DHA from the initial levels at T0 and T1 to those at T2 and T3 was probably a result of in

the utilisation of the soil organic matter, and accompanying reduction in overall activity. In addition, the increase in *R. solani* DNA amounts in the treatment with fungicides, and to lesser extent with the ITC, compared with the biofumigant treatments at harvest, indicates that the biofumigant treatments maintained suppression of *R. solani*. This was probably due to modification of soil microbial communities rather than short-term effects of toxic volatiles. Further study, possibly using techniques such as PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) (Omirou *et al.*, 2011; Wang *et al.*, 2014), may identify the actual changes in the community structure of the soil microorganisms under biofumigation effects.

Although amounts of *R. solani* DNA were greater from the inoculated positive control compared with any of the biofumigant or fungicide treatments, the amounts after the initial incubation period prior to potato planting (26,900 pg/g dried soil) were considerably less than that at the start of the experiment, immediately after inoculum incorporation (615,376 pg/g dried soil). Similarly, Larkin and Griffin (2007) reported that when bare soil was inoculated with cracked wheat colonised with *R. solani,* there was a sharp reduction in *R. solani* over time. Decreases in *R. solani* inoculum were also detected in the experiments described in Chapter 3, where rapid decline in DNA amounts occurred between initial inoculation and after 28 days incubation in the positive pathogen control treatments. These results indicate that there is a maximum 'carrying capacity' ("the ecological space available for maintenance and persistence" (van Veen *et al.*, 1997)) for pathogen inoculum in soils. After introduction of fungus propagules into soil, and in the absence of suitable hosts, other studies have shown that there was initial decline in pathogen populations, towards the 'carrying capacity' of soils (van Veen *et al.*, 1997; Kirkegaard *et al.*, 2000; Gupta *et al.*, 2010; Strunnikova *et al.*, 2015). This 'carrying capacity' is dependent on suitable nutrient sources and competition by resident microbial populations (van Veen *et al.*, 1997). Larkin and Griffin (2007) hypothesized that *R. solani* inoculum levels naturally decline due to the absence of a suitable potato host, or because of physical, chemical or biological characteristics of the soil. However, after the initial sharp decline in *R. solani* AG3-PT DNA amounts, they increased in subsequent assessments probably because a susceptible host, (potato) was present, and the pathogen inoculum increased.

In the present study, the fungicide and biofumigant treatments reduced the amounts of *R. solani* DNA, both compared with the initial inoculum levels and also compared with the positive controls at the three subsequent sampling times. Therefore, in addition to the natural inoculum decline in the positive control, the treatments themselves also effectively reduced *R. solani* inoculum. The fungicides azoxystrobin and pencycuron have been reported as being effective at reducing and/or killing *R. solani* inoculum, and this reduces infection on potato stems or tubers (Bains *et al.*, 2002; Djébali and Belhassen, 2010; Wharton and Wood, 2013; Malik *et al.*, 2014; Özer and Bayraktar, 2015; Potatoes New Zealand, 2017). Larkin and Griffin (2007) reported that incorporation of *Brassica* crops,

such as Indian mustard, rapeseed, canola, yellow mustard, radish, or turnip, reduced inoculum of *R. solani* (AG3) in soil compared with untreated soil. Similarly, the results from experiments in Chapter 3 of this thesis illustrated that the biofumigant plants 'Caliente' mustard, brown mustard and 'Nemat' arugula reduced *R. solani* DNA amounts when incorporated into soil.

Although fungicides were effective at reducing *R. solani* DNA levels and subsequent disease, continual uses raises the risk of developing fungicide-resistant pathogen strains, which has been reported overseas (Djébali *et al.*, 2014). In the present study, increased *R solani* populations were measured from the fungicide treatments towards the end of the experiment (134 days of planting). This indicated that the fungicide treatment was losing activity against *R. solani*. The fungicides azoxystrobin and pencycuron are normally recommended to be applied once to potato crops, prior potato planting (New Zealand Novachem Agrichemical Manual, 2015). This single application may not be sufficient to control the Rhizoctonia diseases for complete growing seasons. Therefore, further research could assess the efficacy of fungicides in combination with non-pesticide strategies, to provide long term control and to minimise risks of pesticide resistance.

In the present research, the *R. solani* AG3-PT DNA amounts were low (0.2-337pg/ g dried soil) after all treatments apart from the positive inoculated control. However, disease symptoms were observed in the potato plants at 35 DAP and at harvest. This indicated that although these treatments reduced *R. solani* inoculum to low levels, these were still sufficient to infect the potato plants and cause visible symptoms. Some studies have shown that although no *R. solani* was detected by qPCR in soil samples from field sites, disease symptoms of stem canker and tuber black scurf still occurred (Brierley *et al.*, 2016; Sinton *et al.*, 2016). This could be because *R. solani* inoculum is unevenly distributed in soil (Tsror, 2010; Brierley *et al.*, 2016). Moreover, potato diseases usually occur in patches, rather than being evenly distributed across fields (Tsror, 2010; Anees *et al.*, 2010; Crop Protection Compendium, 2015; Brierley *et al.*, 2016). Sampling methods may account for issues with accurate estimation of *R. solani* in soil at field scales. However, in the present study, the *R. solani* AG3-PT colonised barley grains were thoroughly mixed into the soil before setting up the experiments, and the soil was sampled at five positions from each replicate pot. Therefore, the inoculation and intensive sampling methods used would have reduced possible variations and increased the chances to detection of *R. solani*.

Rhizoctonia infection of potato is caused by several *R. solani* AGs, such as AG2-1, AG4, AG5, AG8 and AG9, resulting in disease symptoms on potato plants and tubers (Tsror, 2010; Anees *et al.*, 2010; Das, 2013; Das *et al.*, 2014; Crop Protection Compendium, 2015; Brierley *et al.*, 2013, 2016; Sinton *et al.*, 2016). *Rhizoctonia solani* AG3-PT, AG2-1, and AG5 are reported from potato tubers or potato soils in New Zealand (Das *et al.*, 2014; Sinton *et al.*, 2016). Whether the soil used in the present study was naturally infested with *R. solani* AG2-1 or AG5 is not known, but results presented in Chapter 2 of this

thesis have shown that a *R. solani* AG2-1 isolate was less sensitive to biofumigation than 8 out of 9 *R. solani* AG3-PT isolates tested. However, the fungicides used in this study, azoxystrobin and pencycuron, were likely to be equally effective at controlling all *R. solani* AG inocula, and this may account for the more effective control of *R. solani* infection of potato by the fungicide treatments compared with the biofumigant treatments.

Rhizoctonia solani DNA amounts were positively correlated with severity of stem canker and stolon disease at 35 DAP, and with severity of stem canker and black scurf at harvest. Thus, the high inoculum levels of *R. solani* AG3-PT, as indicated by amounts of soil DNA, resulted in severe disease on stems and tubers. These results concur with the findings of Ritchie *et al.* (2013) and Brierley *et al.* (2016). They reported that increasing soil inoculum density of *R. solani* AG3-PT resulted in increased diseases on stems, stolons and tubers. However, Atkinson *et al.* (2010) did not find the relationship between soil *R. solani* AG3-PT inoculum and diseases on potato stolons or stems. Thus, the relationship between soil inoculum of *R. solani* and diseases on potato plants may not only depend on *R. solani* AG3-PT inoculum, but also be influenced by soil conditions.

In the present research, the biofumigants ('Caliente' and brown mustards and 'Nemat' arugula) and the allyl ITC treatment reduced powdery scab severity on potato tubers compared with the untreated and positive control treatments. These results are similar to those of Larkin and Griffin (2007), whereby Indian mustard (*B. juncea*) was the most effective compared with other biofumigant crops, rapeseed and canola (*B. napus* species), for reducing powdery scab on potato tubers in field trials. However, the mechanism by which biofumigant crops reduce powdery scab is unclear. It has been suggested that *Sp. subterranea* propagules (resting spores, zoospores) were weakened by biofumigation, and because they are intolerant to environmental factors such as chemicals or microbial activity (Larkin and Griffin, 2007). In the present study, the fungicides (especially azoxystrobin) had activity against powdery scab, and all of the treatments apart from brown mustard reduced *Spongospora* diseases. This result concurs with those of Bittara *et al.* (2009), who showed that azoxystrobin applied at 4 and 8 week after potato planting reduced powdery scab severity on potato tubers compared with the untreated controls, in a field trial.

Infections by *C. coccodes* (causing black dot on potato) were observed on the potato plants in the present experiment. 'Caliente' mustard, brown mustard, allyl ITC and fungicides reduced the incidence of dead stems compared with the positive (inoculated) and untreated controls. Azoxystrobin reduces *C. coccodes* mycelium growth *in vitro*, and infections on potato stems or tubers in greenhouse and field trials (Harding *et al.*, 2005). Azoxystrobin soil applications before potato planting are likely to not only reduce diseases caused by *R. solani*, but also decrease the incidence of dead stems caused by *C. coccodes*. An *in vitro* study showed that some ITCs inhibited the mycelium growth of *C. coccodes*, with 2-phenylethyl ITC being the most effective (Taylor *et al.*, 2014). The

mycelium growth of *C. coccodes* was also shown to be effectively suppressed by toxic volatiles released from leaves, roots and seed meals of *B. juncea*, *B. napus*, and *Raphanus sativus*. Of these biofumigants, seed meal of *B. juncea* showed the greatest efficacy (de Boer *et al.*, 2003; Harding *et al.*, 2005). Therefore, based on the results of the present and previous studies, further research should be conducted to evaluate the potential of biofumigation to control this *C. coccodes.*

In summary, the selected biofumigant plants, particularly 'Caliente' mustard and brown mustard, suppressed infection by *R. solani* AG3-PT, and potentially other AGs present in the soil, of potato stems, stolons and tubers, to levels comparable to those achieved from the fungicide treatment. In addition, the biofumigant treatments reduced and maintained the *R. solani* AG3-PT inoculum at low levels during the experiment. The biofumigant treatments also reduced the severity of powdery scab caused by *Sp. subterranea*, and incidence of dead stems caused by *C. coccodes*. Consequently, there were increases of plant yield parameters from in the biofumigant treatments. Repeated study is required to confirm those outcomes. However, there was no differential effect of biofumigant treatments on microbial activity (as assessed by DHAs). This indicated DHA did not identify potential differences in microbial communities between treatments, or the potential non-target impacts of the treatments on beneficial microbial communities, which could boost or reduce potential biofumigation impacts. Thus, further research, using molecular tools such as PCR-DGGE, is required to determine the diversity of soil microbial communities which have been reported to contribute roles during and after biofumigation processes (Cohen *et al.*, 2005; Cohen and Mazzola, 2006; Omirou *et al.*, 2011; Wang *et al.*, 2014). Moreover, the results reported here were based on an experiment using artificial inoculation under shadehouse conditions. Study of the effects of biofumigation on natural pathogen inoculum in field experiments is required to confirm the practical potential for biofumigation for potato disease management.

Chapter 5

Effects of cover crops in potato rotation systems on soil microbial communities

5.1 Introduction

Besides brassicaceous species (e.g. mustards, radish, turnip, canola) (Kirkegaard *et al.*, 2000; Larkin and Griffin, 2007; Snapp *et al.*, 2007; Mattner *et al.*, 2008; Montfort *et al.*, 2010; Reddy, 2012; Ngala *et al.*, 2014; Handiseni *et al.*, 2016, 2017; Ríos *et al.*, 2016), other non-*Brassica* plants such as oat, ryegrass, wheat, barley and Sudan grass (planting and incorporation), can also reduce potato disease caused by *Rhizoctonia solani* (Larkin and Griffin, 2007) or soilborne nematodes (Reddy, 2012). However, most biofumigation research has focused on pathogen suppression efficiency, and has usually overlooked effects on non-target soil microbial communities. Some studies have shown that biofumigation stimulated populations of beneficial soil microbes which in turn could increase the control efficiency of soilborne pathogens (Cohen *et al.*, 2005; Cohen and Mazzola, 2006; Ascencion *et al.*, 2015). Thus, biofumigation efficiency should include consideration of effects on non-target soil microbes, as well as the target pathogens.

Soil microbial communities have been studied by culturing on a wide range of media (Hill *et al.*, 2000; Cohen *et al.*, 2005). However, this approach only estimates very small proportions (less than 0.1%) of total soil microbial communities. Determination of sequences of 16S ribosomal RNA (rRNA) genes in prokaryotes and 5S or 18S rRNA genes in eukaryotes can improve estimation of total soil microbial communities by more than 90% (Hill *et al.*, 2000). PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), based on sequences of the rRNA genes, has been developed and applied to study the diversity of soil microbial communities, mainly for bacteria and fungi (Muyzer *et al.*, 1993; Nübel *et al.*, 1996; Hill *et al.*, 2000; Vainio and Hantula, 2000; Gomes *et al.*, 2001; Vandenkoornhuyse *et al.*, 2002; Rumberger and Marschner, 2003, 2004; Postma *et al.*, 2008; Muhling *et al.*, 2008; Karpouzas *et al.*, 2010; Omirou *et al.*, 2011; Wang *et al.*, 2014; Hanschen *et al.*, 2015; Mocali *et al.*, 2015).

Results from experiments described in previous Chapters of this thesis have shown that addition of biofumigant crops increases soil microbial activity (dehydrogenase activity; DHA), but the results indicated that this was mainly due to carbon inputs rather than an effect of biofumigation *per se*. However, whether the biofumigant plant incorporations altered the microbial community and

functional diversity compared with carbon inputs from other crop incorporations is unknown. The aim of the experiments described here was to evaluate the effects of cover crops in rotation systems with potatoes on soil microbial communities, by assessing total microbial activity (DHA), the functional diversity with respect to carbon utilisation (using the MicroResp^{TM} system) and microbial community diversity (using PCR-DGGE) of the representative groups of measurable soil microbes, including total fungi, arbuscular mycorrhizal fungi and Proteobacteria.

5.2 Materials and methods

5.2.1 Sampling of field soils

5.2.1.1 Field trials in 2015/16

Soil sampling was carried out from two potato field trials in the Timaru and Ashburton districts (Canterbury, New Zealand). These trials were set up in association with the Sustainable Farming Fund (SFF) project "The impact of different potato crop rotations on soil-borne diseases and soil quality", conducted by the New Zealand institute for Plant & Food Research Ltd, Lincoln, New Zealand. These trials were carried out during the 2015/16 growing season.

The trial at Timaru (Appendix A.5.1) had two separate 1 ha blocks, one that was unplanted (no cover crop) and the other was sown with 'Caliente' mustard. These were considered as "treatments" in this trial. The trial at Ashburton (Appendix A.5.1) consisted of three separate 1 ha blocks, one that was unplanted (no cover crop), one with oat, and the third was sown with 'Caliente' mustard. These were considered as "treatments" in this trial.

The cover crops (oat or 'Caliente' mustard) were sown during the winter in 2015, and the resulting green manure crops were mulched and incorporated into the soils in September 2015. One month later (October 2015), soil samples (1 kg in total) were collected from ten positions along a W-shaped transect (marked with flags and recorded with GPS coordinates in each 1 ha block), before planting with potatoes. Similar samples were taken again 3 months later (January 2016). The soil samples were obtained using a 15 cm depth standard soil sampler. The individual soil samples were collected at fixed GPS positions. The unplanted treatments were sprayed twice with herbicides, and then left until incorporation of cover crops. Weeds grew in the unplanted blocks but they were not removed. And the unplanted blocks were mulched in the same way with the cover crop blocks. The treatments in each trial all received the same crop management regimes (fertiliser and agrichemical applications) during potato crop planting and growth.

5.2.1.2 Field trial in 2016/17

During the 2016/17 growing season, one field trial was set up in the Timaru district (also part of the SFF project) by the Foundation for Arable Research and Plant & Food Research, Lincoln. The trial

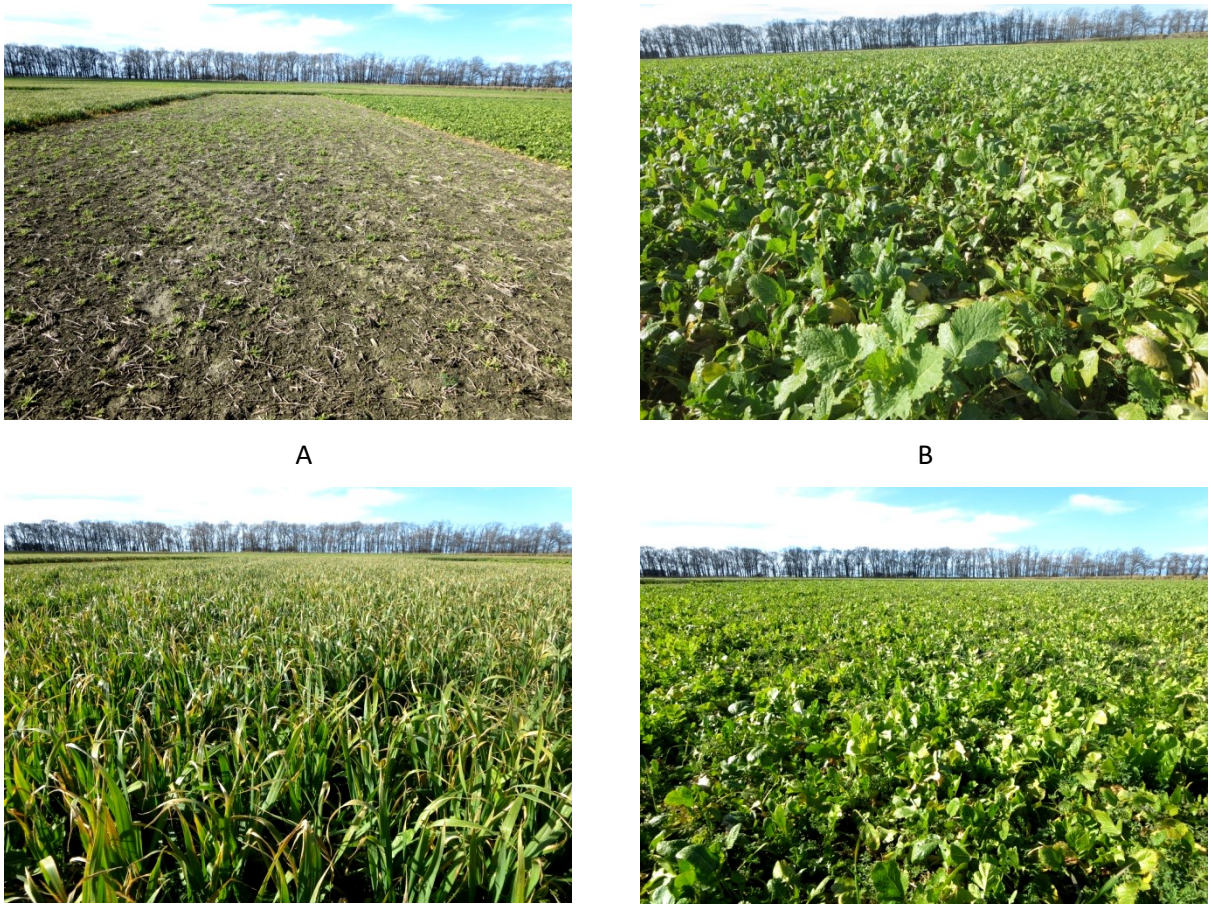
consisted of four treatments, including fallow (unplanted control) (Figure 5.2A), 'Caliente' mustard (Figure 5.2B), oat (Figure 5.2C), or 'Graza' radish (Figure 5.2D), with eight replicates (blocks) giving a total of 32 plots (each plot was 12 m wide x 40 m long) (Figure 5.1; Appendix A.5.3). The cover crops ('Caliente' mustard, oat or radish) were sown in autumn (March 2016), and in early spring (September 2016) they were mulched and incorporated into the soil. Although the "fallow" treatment was sprayed twice with glyphosate herbicide, weeds and volunteer radish plants grew in these plots. These plants were also incorporated into the soil in unplanted plots in the same way as those in the cover crop treatment plots. All treatments received the same crop management regime during potato crop planting and growth. Before being incorporated into the soils, the cover crops were harvested to estimate the potential biomass which was conducted by Plant & Food Research staff (Sinton *et al.*, 2017). A cover crop sample in each plot was randomly taken from a 4 m x 0.5 m quadrat. For radish plants both roots and shoots were harvested, while only shoots (aboveground parts) of 'Caliente' mustard and oat were taken. This work determined means of 0.7 tonne (of dry matter)/ha of volunteer radish in the unplanted treatment, 3.1 t/hectare of 'Caliente' mustard (shoots), 3.8 t/ha of oat (shoots), and 4.7 t/ha of 'Graza' radish (roots plus shoots).

Soil samples were collected before the cover crops were sown (T0), at 138 days after cover crop sowing (T1), 8 days after the cover crops incorporated into soil (T2), 42 days after cover crop incorporation (before potato planting) (T3), 89 days after potato planting (T4), and 167 day after potato planting (at potato harvest) (T5) (Table 5.1).

At T0 (before cover crop sowing), soil samples were taken in a W-shaped transect from each block, using a 10-cm hand trowel. Thirty two soil samples were taken from positions along the transect, and these samples were bulked, resulting in a total of eight samples (corresponding to the eight trial blocks) for this sampling time. The soil samples from each block were mixed thoroughly, and a subsample of 500 g was taken for analyses. Soil samples at this sampling time were used as references and not included in the statistical analyses.

For the remaining sampling times (T1-T5), 15 soil samples were taken from 15 positions in each plot (32 plots in total) across a W-shaped transect, also using a 10-cm hand trowel (approx. 1 kg across the 15 positions). This gave 32 soil samples for each sampling time. At T1 (138 days after cover crop sowing), T4 (89 days after potato planting) and T5 (at potato harvest), soil samples were collected in root rhizosphere of cover/potato crops. The samples from each plot were thoroughly mixed and a subsample of 500 g was taken for analyses.

Figure 5.1 Overall view of the cover crop treatments in the Timaru trial (2016/17), including fallow (unplanted control), 'Caliente' mustard, oat or 'Graza' radish. This photo was captured by a drone (image provided by Steven Dellow and Sarah Sinton, Plant & Food Reasearch, Lincoln).



A B C D

Figure 5.2 Plots of unplanted control (A), 'Caliente' mustard (B), oat (C) and 'Graza' radish (D) at 138 days after cover crop planting (T1).

Table 5.1 Soil sampling times and the crop planting conducted at the Timaru field trial (2016/17).

Sampling time/planting	Date	Season
T0	7th March 2016	Autumn
Cover crop planting	30th March 2016	Autumn
T1	15th August 2016	Winter
Cover crop incorporation	7th September 2016	Spring
T2	15th September 2016	Spring
T3 (Before potato planting)	19th October 2016	Spring
Potato planting	19th October 2016	Spring
T4	16th January 2017	Summer
T5 (Potato harvest)	4th April 2017	Autumn

5.2.2 Soil processing and assessments

5.2.2.1 Soil processing

The collected soil samples were sieved through a 2 mm mesh (Figure 5.3) to remove plant debris and stones, and then thoroughly homogenised by hand. The mixed samples were each divided into subsamples for storing at -80°C (50 g for each sample for subsequent microbial diversity analyses), and at 4°C (100 g for each samples and stored within 1 week) for determination of soil community level physiological profiles and measurement of soil microbial activity.

Figure 5.3 Collected field soil samples were sieved through a 2 mm mesh.

5.2.2.2 Soil microbial assessments

Dehydrogenase activity (DHA) measurement

For soil samples from the Timaru and Ashburton field trials (2015/16), five replicates per treatment for each sampling time were selected for DHA analyses. For samples from the Timaru field trial (2016/17), all eight replicates (blocks) per treatment at each sampling time were used. The DHA analytical method used was as described in Appendix A.3.8 (Chapter 3). For each soil sample two replicates were set up.

Identification of carbon source utilisation profiles

For soil samples from the Timaru and Ashburton field trials (2015/16), five replicates per treatment at each sampling time were selected for analyses of soil community level physiological profiles. All soil samples from each sampling time in the Timaru field trial (2016/17) were used.

Soil community level physiological profiles were assessed using the MicroResp™ system (The James Hutton Institute, Aberdeen, Scotland). The protocol was conducted based on the descriptions of Campbell *et al.* (2003), the manufacturer, and Wakelin *et al.* (n.d.). The system is principally based on colour change of the pH indicator dye cresol, which is red at alkali pH, and becomes yellow at acidic pH (Rowell, 1995; Campbell *et al.*, 2003). The MicroResp™ system consists of two plates placed face to face during an incubation period, including a deep well plate (1.2 mL per well) containing the soil samples and carbon sources, and a detection plate (microplate) with each well containing 150 μL mixture of pH indicator dye (cresol red) (12.5 μg/mL), potassium chloride (150 mM), sodium bicarbonate (2.5 mM) and 1% Noble agar (Campbell *et al.*, 2003).

For each field site, the optimum moisture content (range 20-40%) was initially determined for the soil used in the experiments, following the methods outlined by Wakelin *et al.* (n.d.).

Soil samples were loaded into deep well plates using the loading device. Before adding the carbon sources, the soil filled deep-well plates were incubated at 20°C for 7 days to ensure complete utilisation of available carbon sources in the soil. Twenty two carbon sources (L-arabinose, D-fructose, D-galactose, D-glucose, D-xylose, maltose, sucrose, raffinose, citric acid, glycolic acid, tartaric acid, glycerol 50%, D-(+) glucosamine hydrochloride, urea, triton X100, L-proline, glycine, L-alanine, L-serine, arginine, cysteine and tyrosine) (Sigma-Aldrich, New Zealand) were used, with their selection based on common soil carbon sources often present in crop tissues and/or root exudates. These compounds were diluted with Millipore filtered water, and added to each plate well at 30 mg/mL soil water (volume added based on optimum moisture of each soil sample). Millipore filtered water was used as a control (Lob, 2014). Before sealing to the deep well plates, the detection plates containing the pH indicator medium were first read using a spectrophotometer microplate reader at a wavelength of 590 nm. The two plates were then sealed using a silicone rubber seal with air holes

allowing CO_2 to escape from the deep well plate to be absorbed by the detection plate. Carbon dioxide released from metabolism of the carbon sources in the soil in the deep wells reduced the pH and resulted in colour changes of the cresol from red to yellow in the gel detection plates. The two plates were held together using a metal clamp, and incubated at 25°C for 3 hours. The absorbance of the detection plates was again read at 590 nm using a spectrophotometer microplate reader (Campbell *et al.*, 2003). The change in absorbance was calculated for each soil treatment/carbon source. For each soil sample two replicates were set up.

Soil microbial community analysis using PCR-DGGE

The effects of cover crop on the diversity of non-target soil microbial communities, including α-proteobacteria; β-proteobacteria, γ-proteobacteria, total fungi, and arbuscular mycorrhizal fungi (AMF), were assessed using the PCR-DGGE technique.

For soil samples from the Timaru and Ashburton field trials (2015/16), three replicates per treatment at each sampling time were selected for analysis. For soil samples from the Timaru field trial (2016/17), four replicates (blocks) per treatment at each sampling time were used.

Soil DNA extraction

Soil samples were prepared as pellets as described by Section 3.2.4.1 (Chapter 3). The pellets were used for DNA extraction using the PowerSoil® DNA isolation kit (MoBio Laboratories, Carlsbad, California, USA), following the manufacturer's instructions.

PCR amplification

All the PCR were carried out using the FastStart™ Taq DNA Polymerase kit (Roche, Germany). The 25 μL reaction included sterile Millipore filtered water, 2.5 μL of 10× PCR buffer (1× of concentration) with 20 mM $MgCl_2$ (2 mM), 0.5 μL of 20 mM dNTPs (200 μM), 1 μL of 10 μM each forward/reverse primer (400 nM), 0.25 μL of FastStart™ Taq DNA Polymerase 5U/μL (1.25U) and 1 μL soil DNA template. For the nested PCRs, the primary PCR was conducted as described, and then 1 μL of the primary PCR product was used as DNA template for the secondary PCR. The primers and thermal cycles used for the proteobacteria, total fungi and AMF are shown in Tables 5.2 and 5.3.

The PCR products were separated in 1.5% agarose gels (in 1× TAE, Tris-Acetate-EDTA) at 100 V for 45 minutes (Cripps-Guazzone, 2014; Thanh and Khoo, 2014). Each gel was stained in ethidium bromide solution (0.5 μg/mL, Biorad, USA) for 20 minutes, and washed by soaking in tap water for 10 minutes to remove unbound ethidium bromide. The gels were visualised in a UV transilluminator (UNITEC Cambridge, Total Lab Systems Ltd, New Zealand) (Cripps-Guazzone, 2014).

Denaturing gradient gel electrophoresis (DGGE)

Denaturing gradient gel electrophoresis was carried out using a Cipher DTSX system (C.B.S Scientific, USA). The DGGE protocols for α-proteobacteria, β-proteobacteria, γ-proteobacteria, total fungi, and AMF were developed and used in the Plant Pathology group, Department of Pest-management and Conservation (Faculty of Agriculture and Life Sciences, Lincoln University). Briefly, 8% polyacrylamide gels (in 1× TAE, Appendix A.5.4) were used for α-proteobacteria, β-proteobacteria, total fungi and AMF, and 7% polyacrylamide gels (in 1× TAE, Appendix A.5.5) were used for γ-proteobacteria, with different denaturing gradients (Table 5.4). The PCR products (5 μL PCR plus 3 μL loading dye (Appendix A.5.8) were loaded into polyacrylamide gels, and run at different parameters of temperatures, hours, and voltages depending on the soil microbial groups (Table 5.4). After completion of electrophoresis, both sides of the gels were washed with reverse osmosis (RO) water, then fixed by shaking in 250 mL 1× fixative solution (Appendix A.5.10) for 10 minutes. Each gel was stained for 10 minutes in silver solution containing 250 mL of Cairn's solution and 0.5 g silver nitrate (Appendix A.5.11). Each gel was then washed twice with RO water, and shaken for 2 minutes with 200 mL RO water, and then developed for 40 minutes (or until bands were clearly visible) in developer solution (Appendix A.5.12). The gel was shaken again for 5 minutes in 250 mL 1× fixative solution (Appendix A.5.10). The gels were then photographed immediately (Appendices A.5.14-A.5.18) and the photographs used for the analyses. Each gel was then shaken for 2 minutes in 200 mL RO water, and preserved in Cairn's solution (A.5.13) for 7 minutes, and the gel was covered with a piece of cellophane, and dried for 6 hours at 60°C.

Table 5.2 Group-specific 16S ribosomal RNA gene primers and thermal cycles used in the nested PCRs for different target groups of soil proteobacteria.

Target group	Primer	Sequence (5'-3')	Reference
α-proteobacteria	F203α	CCGCATACGCCCTACGGGGGAAAGATTTAT	Nübel *et al.* (1996);
	L1401	GCGTGTGTACAAGACCC	Gomes *et al.* (2001);
		Primary PCR (F203α & L1401): 94°C for 5 minutes; 30 cycles: 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minute; 72°C for 10 minutes	da Silva *et al.* (2013)
	341f-GC	**40 bp GC clamp**[(1)]-CCTACGGGAGGCAGCAG	Muyzer *et al.* (1993);
	518r	ATTACCGCGGCTGCTGG	Muhling *et al.* (2008)
		Secondary PCR (341f-GC & 518r): 96°C for 4 minutes; 30 cycles: 96°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute; 74°C for 10 minutes	
β-proteobacteria	Beta359f	GGGGAATTTTGGACAATGGG	Ashelford *et al.* (2002);
	Beta682r	ACGCATTTCACTGCTACACG	Muhling *et al.* (2008)
		Primary PCR (Beta359f & Beta682r): 96°C for 4 minutes, 30 cycles: 96°C for 1 minute, 63°C: 1 minute, 74°C for 1 minute; 74°C: 10 minutes	
	518f-GC	**40 bp GC clamp**[(1)]- CCAGCAGCCGCGGTAAT	Ashelford *et al.* (2002);
	Beta682r	ACGCATTTCACTGCTACACG	Muyzer *et al.* (1993);
		Secondary PCR (518f-GC & Beta682r): 96°C for 4 minutes, 30 cycles: 96°C for 1 minute, 60°C for 1 minute, 74°C for 1 minute; 74°C for 10 minutes	Muhling *et al.* (2008)
γ-proteobacteria	Gamma395f	C**M**ATGCCGCGTGTGTGAA (where **M**: A or C)	Muhling *et al.* (2008)
	Gamma871r	ACTCCCCAGGCGGTC**D**ACTTA (where **D**: A or G or T)	
		Primary PCR (Gamma395f & Gamma871r): 96°C for 4 minutes, 30 cycles: 96°C for 1 minute, 54°C for 1 minute, 74°C for 1 minute; 74°C for 10 minutes	
	518f-GC	**40 bp GC Clamp**[(1)]-CCAGCAGCCGCGGTAAT	Muyzer *et al.* (1993);
	Gamma785r	CTACCAGGGTATCTAATCC	Lee *et al.* (1993);
		Secondary PCR (518f-GC & Gamma785r): 96°C for 4 minutes, 30 cycles: 96°C for 1 minute, 56°C for 1 minute, 74°C for 1 minute 74°C for 10 minutes	Muhling *et al.* (2008)

40 bp GC-clamp[(1)]**:** 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3' (Muyzer *et al.*, 1993)

Table 5.3 Group-specific 18S ribosomal RNA gene primers and thermal cycles used in the nested PCRs for total soil fungi and arbuscular mycorrhizal fungi (AMF) groups.

Target group	Primer	Sequences (5'-3')	References
Total fungi	AU2	TTTCGATGGTAGGATAG**D**GG (where **D**: A or G or T)	Vandenkoornhuyse *et al.* (2002)
	AU4	**R**TCTCACTAAGCCATTC (where **R**: A or G)	
		Primary PCR (for AU2 & AU4): 95°C for 3 minutes; 35 cycles: 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute; 72°C for 7 minutes	
	FR1-GC	**40 bp GC clamp**[(1)]-A**I**CCATTCA ATCGGTA**I**T (where **I**: inosine)	Vainio and Hantula (2000)
	FF390	CGATAACGAACGAGACCT	
		Secondary PCR (FF390 & FR1-GC): 95°C: 2 minutes; 8 cycles: 95°C for 30 seconds, 55°C: 30 seconds (touchdown 1°C per cycle), 72°C for 1 min; 27 cycles: 95°C for 30 seconds, 47°C for 30 seconds, 72°C for 1 minute; 72°C for 7.5 minutes	
AMF	AML1	ATCAACTTTCGATGGTAGGATAGA	Lee *et al.* (2008)
	AML2	GAACCCAAACACTTTGGTTTCC	
		Primary PCR (AML1 & AML2): 95°C for 3 minute; 35 cycles: 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute; 72°C for 7 minute	
	NS31-GC	**39 bp GC clamp**[(2)]-TTGGAGGGCAAGTCTGGTGCC	Simon *et al.* (1992);
	Glo1	GCCTGCTTTAAACACTCTA	Kowalchuk *et al.* (2002)
		Secondary PCR (NS31-GC & Glo1): 95°C: 3 min; 35 cycles: 94°C for 45 seconds, 52°C for 45 seconds, 72°C: 1 minute; 72°C: 7 minute	Cornejo *et al.* (2004)

40 bp GC-clamp [(1)]**:** 5'-CCCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGCCG-3' (Vainio and Hantula, 2000)

39 bp GC clamp[(2)]**:** 5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGG-3' (Kowalchuk *et al.*, 2002)

Table 5.4 Gel gradient (%), running temperature, voltage and time parameters for denaturing gel gradient electrophoresis (DGGE) for different target groups of proteobacteria and fungi.

Target group	Gel gradient (%)	Gels run at		
		Temperature (°C)	Voltage (V)	Hours
α-proteobacteria	40-60	58	60	18
β-proteobacteria	40-55	58	60	18
γ-proteobacteria	40-60	58	60	18
Total fungi	25-55	58	90	16
AMF	30-45	58	90	16

5.2.3 Data analyses

5.2.3.1 DHA data

The DHA data were examined for normal distributions using GenStat software (Version 18.1.0.17005; VSN International Ltd, Hemel Hempstead, United Kingdom). If the data satisfied normal distributions, they were directly used for statistical analyses, otherwise they were sqrt(X) transformed prior to analysis. The means of treatments, sampling times and interaction between treatments x sampling times were separated using Tukey's Honestly Significant Difference (HSD) at P=0.05.

5.2.3.2 MicroResp™ data

These data were analysed using Primer software version 6.1.18 (Primer-E Ltd, Plymouth, United Kingdom). The data were firstly normalised, then a Euclidean distance resemblance was carried out. The resemblance data were used to conduct permutational multivariate analysis of variation (PERMANOVA+ version 1.0.8) to identify differences between treatments and sampling times (Lob, 2014). The non-metric multidimensional scaling (MDS) graphs were generated from the resemblance data to show similarities of carbon utilization profiles among treatments and sampling times. Pearson correlation tests were used to determine the contribution of individual C-substrates towards distances between samples for each of the carbon sources (Lob, 2014).

5.2.3.3 DGGE gels

The DGGE gels showing the soil microbial communities (α-proteobacteria, β-proteobacteria, γ-proteobacteria, total fungi, and AMF) were analysed using the Phoretix 1D Pro Gel (Totallab, United Kingdom) package, following the provider's instructions, to generate the binary data matrices (1 or 0 values) representing the presence/absence of bands on the DGGE gels. The binary data matrices were used to calculate resemblance using Jaccard distance, and the Jaccard resemblance data were

used to generate the similarities among treatments and sampling times. The Jaccard resemblance data were also used to conduct PERMANOVA (Section 5.2.3.2) to test for differences among treatments and sampling times.

For the data from the Timaru and Ashburton field trials (2015/16), multidimensional scaling (MDS) graphs were generated to visualise similarity of the soil microbial communities between treatments or sampling times.

For the data from the Timaru trial (2016/17), MDS graphs were generated to visualise similarity of each soil microbial community between sampling times. Because the similarity of each soil microbial community between treatments did not cluster together using MDS graphs, the unweighted pair group method with arithmetic mean (UPGMA) graphs using the Phoretix 1D Pro Gel software were generated.

The means of species richness (total bands for each treatments in DGGE gels) of each soil microbial community from the different treatments or sampling times were separated using Tukey's HSD at *P*=0.05.

The peak heights which reflected band intensities in densitometric curves shown in the Phoretix 1D Pro Gel software were used to calculate the structural diversity (Shannon-Weaver diversity index, or H) of the soil microbial communities, based on the formula described by Boon *et al.* (2002):

$$H = -\sum_{i}^{n} \frac{p_i}{N} \log \frac{p_i}{N}$$

Where:

- p_i is the peak height at i^{th} band

- N: the sum of all peak heights

The means of Shannon-Weaver diversity indices of each soil microbial community from the different treatments or sampling times were separated using Tukey's HSD at *P*=0.05.

5.3 Results

5.3.1 Effects of cover crops on soil microbial activity

5.3.1.1 Timaru field trial (2015/16)

Statistical analyses for data from this experiment are shown in Appendix A.5.19, and the results are summarised in Table 5.5. There was a significant effect (*P*=0.002) of treatment on soil DHA, with DHA in the soil from the 'Caliente' mustard treatment (mean = 1.37 μg/g dried soil) being greater than that from the unplanted treatment (0.90 μg/g dried soil). There was no significant effect (*P*=0.915) of sampling time on soil DHA (overall mean = 1.13 μg/g dried soil). There was no significant interaction (*P*=0.543) between treatment and sampling time on soil DHA (mean ranged from 0.87 to 1.40 μg/g dried soil).

Table 5.5 Mean dehydrogenase activity (μg/g dried soil.hr^{-1}) of the microbial communities in field soils that were either unplanted or after incorporation of 'Caliente' mustard, and assessed before potato planting (T0) or 90 days after potato planting (T1), in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	0.93[(1)]	0.87	0.90 b[(2)]
'Caliente' mustard	1.40	1.31	1.37 a
Mean of sampling time	1.13	1.13	
P values: 0.002 (Treatment), 0.915 (Sampling time), 0.543 (Treatment x Sampling time)			

[(1)] All the means without letters are not significantly different

[(2)]Means within the column followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

5.3.1.2 Ashburton field trial (2015/16)

Statistical analyses for data from this experiment are shown in Appendix A.5.20, and the results are summarised in Table 5.6. There was a significant effect (*P*=0.09) of treatment on soil DHA. DHA in the 'Caliente' mustard treatment (0.47 μg/g dried soil) was greater than that in the unplanted treatment, but did not differ significantly from that for the oat treatment (0.41 μg/g dried soil). There was no significant effect (*P*=0.069) of sampling time on soil DHA (0.35-0.44 μg/g dried soil). There was no significant interaction effect (*P*=0.054) between treatment and sampling time on soil DHA (range = 0.28-0.52 μg/g dried soil).

Table 5.6 Mean dehydrogenase activity (μg/g dried soil.hr^{-1}) of the microbial communities in field soils that were either unplanted or after incorporation of 'Caliente' mustard or oat, and assessed before potato planting (T0) or 90 days after potato planting (T1) in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	0.28[(1)]	0.33	0.30 b[(2)]
'Caliente' mustard	0.52	0.43	0.47 a
Oats	0.51	0.31	0.41 ab
Mean of sampling time	0.35	0.44	
P values: 0.009 (Treatment), 0.069 (Sampling time), 0.054 (Treatment x Sampling time)			

[(1)] All the means without letters are not significantly different

[(2)]Means within the column followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

5.3.1.3 Timaru trial (2016/17)

Statistical analyses for data from this experiment are shown in Appendix A.5.21, and the results are summarised in Table 5.7. There was a significant interaction (*P*<0.001) between treatment and assessment time on the soil microbial activity (DHA), mostly associated with a significant increase in microbial activity from the cover crop treatments ('Caliente' mustard, oat or 'Graza' radish) compared with the unplanted treatment at 8 days (T1) after cover crop incorporation, and 89 days (T4) after potato planting. However, there was no significant difference in cover crop treatments at T1 and T3. At harvest (T5), DHA levels in soils from the oat and 'Graza' radish treatments were significantly higher compared with that from the unplanted treatment.

Across sampling time, DHA in soils from the cover crop treatments (mean = 1.59-1.64 μg/g dried soil.hr^{-1}) were significantly (*P*<0.001) greater than that from the unplanted treatment. Across the treatments, there was a significant effect (*P*<0.001) of sample time on soil DHA level, which was greatest at T2 (8 days after cover crop incorporation) (1.90 μg/g dried soil.hr^{-1}) and T3 (42 days after cover crop incorporation) (1.73 μg/g dried soil.hr^{-1}) compared with all other sampling times.

Table 5.7 Mean dehydrogenase activity (μg/g dried soil.hr^{-1}) of the microbial communities in soils that were either unplanted or associated with incorporation of 'Caliente' mustard, oat or 'Graza' radish, and assessed at six sampling times (T0: Before cover crop planting; T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/Before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) in the Timaru field trial (2016/17).

Treatment	Sampling time						Mean of treatment
	T0	T1	T2	T3	T4	T5	
Unplanted	2.16[(1)]	1.41 cd[(2)]	1.44 cd	1.53 bcd	0.67 f	0.92 ef	1.18 B
'Caliente' mustard	2.16	1.65 abc	2.05 ab	1.74 abc	1.43 cd	1.11 de	1.59 A
Oat	2.16	1.36 cde	2.08 a	1.81 abc	1.55 bcd	1.44 cd	1.64 A
'Graza' radish	2.16	1.38 cde	2.05 ab	1.84 abc	1.40 cd	1.49 cd	1.62 A
Mean of sampling time	2.16	1.45 Y	1.90 X	1.73 X	1.25 Z	1.23 Z	
P values: <0.001 (Treatment), <0.001 (Sampling time), <0.001 (Treatment x Sampling time)							

[(1)] Data not included in the analysis

[(2)] Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

5.3. 2 Effects of cover crops on soil microbial functionality

5.3.2.1 Ashburton trial (2015/16)

There was a significant effect (*P*=0.001) of sampling time on the carbon utilisation profiles (CUP) of soil microbial communities (Table 5.8, Figure 5.4). There was no effect of treatment (*P*=0.400) or interaction between treatment and sampling time (*P*=0.276) on CUP. Pearson correlation tests (Appendix A.5.22) showed that some substrates, including glucose, urea, fructose and glycerol 50%, greatly contributed the differences in the CUP between the two sampling times (Figure 5.4).

Table 5.8 Permutational MANOVA analysis for the carbon utilisation profiles of the soil microbial communities after incorporation of different cover crop (either unplanted or 'Caliente' mustard or oat) assessed before and 90 days after potato plantings, using the MicroResp™ system in the Ashburton field trial (2015/16).

Source	P(perm)
Main test	
Treatment	0.400
Sampling time	0.001
Treatment x Sampling time	0.276

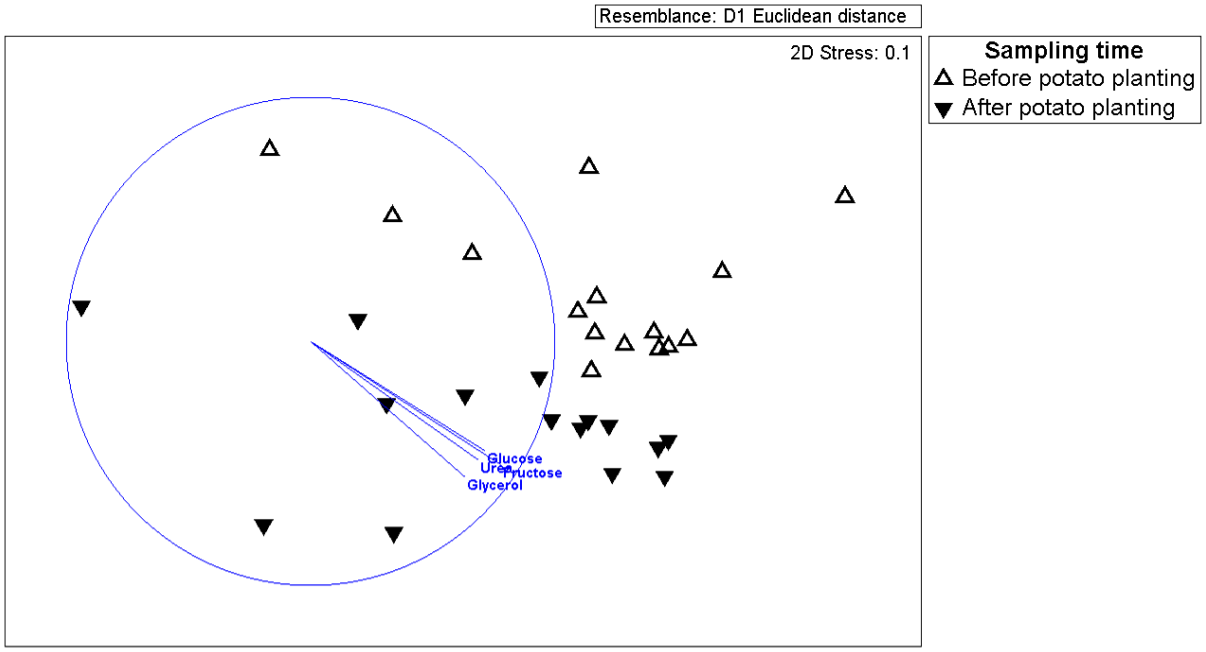


Figure 5.4 Non-metric multi-dimensional scaling (MDS) plot showing the pattern of utilisation of 22 carbon sources by soil microbial communities after incorporation of different cover crops (either unplanted or 'Caliente' mustard or oat) assessed before and 90 days after potato planting, as determined using the MicroResp™ system in the Ashburton field trial (2015/16). Blue vectors show the indicated carbon sources.

5.3.2.2 Timaru field trial (2016/17)

There was a significant interaction (*P*=0.002) between treatment and sampling time on the CUP of the soil microbial communities (Table 5.9, Figure 5.5). Cover crop treatments (*P*=0.013) and sampling times (*P*=0.001) significantly affected CUP of the soil microbial communities.

The pair-wise tests showed that there were significant differences in CUP of the microbial communities in soils from the oat and 'Graza' radish (*P*=0.04) and oat and unplanted (*P*=0.003) treatments. There were no significant differences in CUP between any of the other treatments, although there was an indication (*P*=0.109) that the CUP of microbial communities differed between the oat and 'Caliente' mustard treatments. The pair-wise tests indicated that there was a significant difference in CUP between all assessment times (Table 5.9).

The Pearson correlation test showed that all 22 carbon sources strongly contributed to the differences in the CUP between treatments and sampling times (Appendix A.5.23). The differences in the CUP between sampling times were strongly affected by CUP at T4 (89 days after potato planting) (Figure 5.5).

Table 5.9 Permutational MANOVA analysis for the carbon utilisation profiles of microbial communities in soils associated with incorporation of different cover crop treatments (either unplanted or 'Caliente' mustard, oat or 'Graza' radish), assessed at 5 sampling times (T1: 138 days after cover crop plantings; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting), using the MicroResp™ system in the Timaru field trial (2016/17).

Source of variation	*P* (perm)
Main test	
Treatment	0.013
Sampling time	0.001
Treatment x Sampling time	0.002
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' mustard	0.350
Unplanted treat x Oat	0.003
Unplanted treat x 'Graza' radish	0.213
'Caliente' mustard x Oat	0.109
'Caliente' mustard x 'Graza' radish	0.244
Oat x 'Graza' radish	0.004
Pair-wise test for sampling time	
T1 x T2	0.001
T1 x T3	0.001
T1 x T4	0.001
T1 x T5	0.001
T2 x T3	0.001
T2 x T4	0.001
T2 x T5	0.001
T3 x T4	0.001
T3 x T5	0.001
T4 x T5	0.001

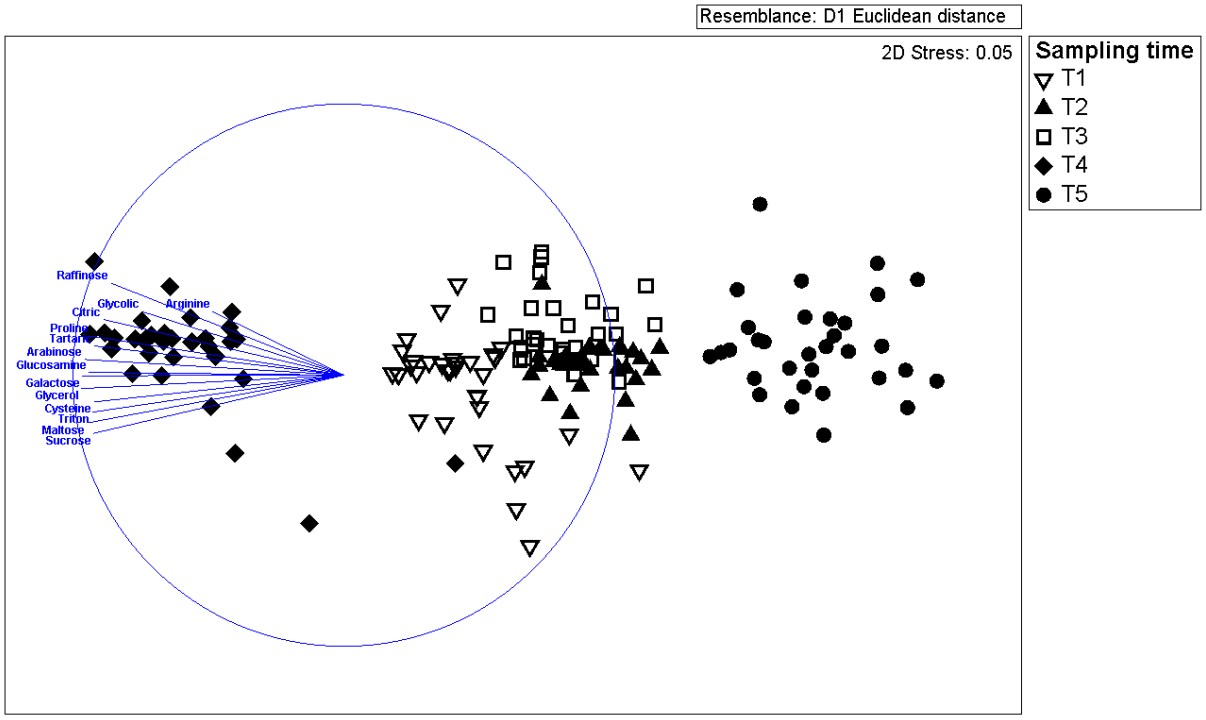


Figure 5.5 Non-metric multi-dimensional scaling (MDS) plot showing the pattern of utilisation of 22 carbon sources by the soil microbial communities associated with incorporation of different cover crop treatments (either unplanted or 'Caliente' mustard, oat or 'Graza' radish), at five sampling times (T1: 138 days after cover crop plantings; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting), using the MicroResp™ system in the Timaru field trial (2016/17). Blue vectors show indicated carbon sources.

5.3.3 Effects of cover crops on soil microbial communities

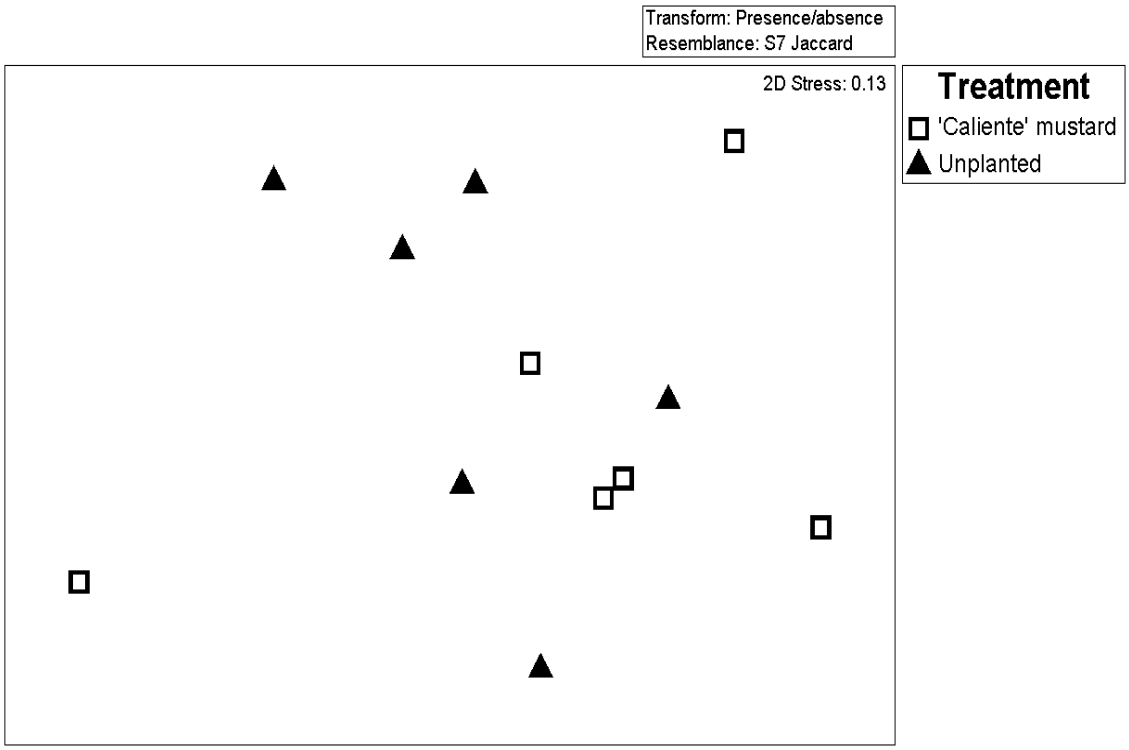
5.3.3.1 Timaru field trial (2015/16)

Similarities

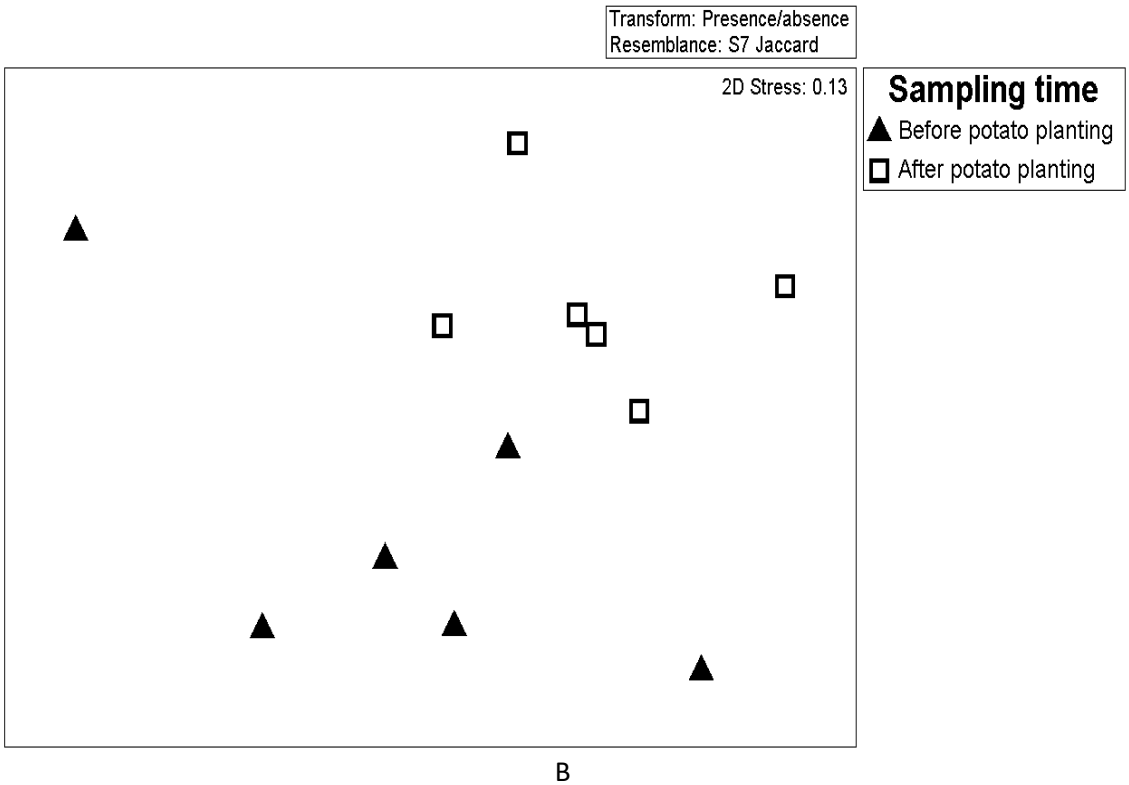
Cover crop treatments significantly affected total the soil community structures of fungi (*P*=0.021, Figure 5.6A), Betaproteobacteria (*P*=0.018, Figure 5.7A) and Gammaproteobacteria (*P*=0.008, Figure 5.8A) (Table 5.10). Sampling time influenced the community structures for fungi (*P*=0.002, Figure 5.6B), Betaproteobacteria (*P*=0.004, Figure 5.7B) and Gammaproteobacteria (*P*=0.002, Figure 5.8B). There were significant interactions between treatments and sampling times for the community structures of AMF (*P*=0.031), Betaproteobacteria (*P*=0.027) and Gammproteobacteria (*P*=0.015).

Table 5.10 Permutational MANOVA analysis for similarities in the community structures of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil after incorporation of 'Caliente' mustard or wheat, assessed before and at 90 days after potato plantings using PCR-DGGE in the Timaru field trial (2015/16).

Community	*P* values (PERMANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.021	0.002	0.639
AMF	0.394	0.368	0.031
Alphaproteobacteria	0.173	0.232	0.434
Betaproteobacteria	0.018	0.004	0.027
Gammaproteobacteria	0.008	0.002	0.015

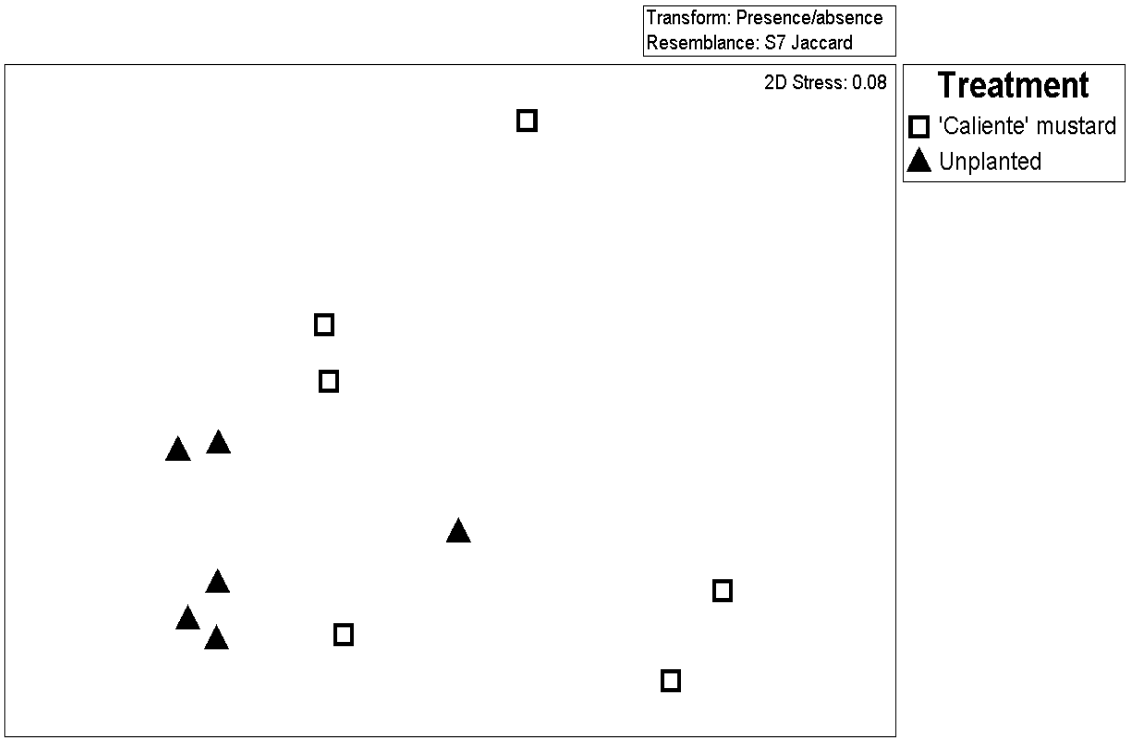


A



B

Figure 5.6 Non-metric multi-dimensional scaling (MDS) plots for total fungi community structure in soil that was either unplanted or after incorporation of 'Caliente' mustard (A), and assessed before (T0) and 90 days after potato planting (T1) (B) using PCR-DGGE in the Timaru field trial (2015/16).



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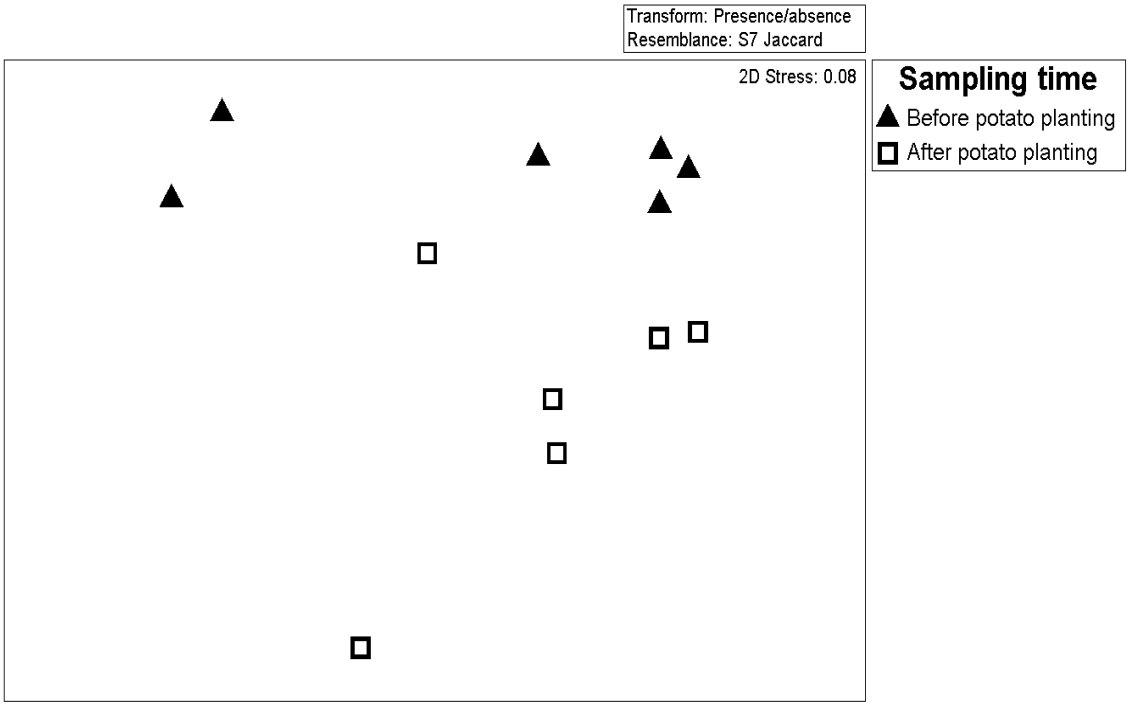
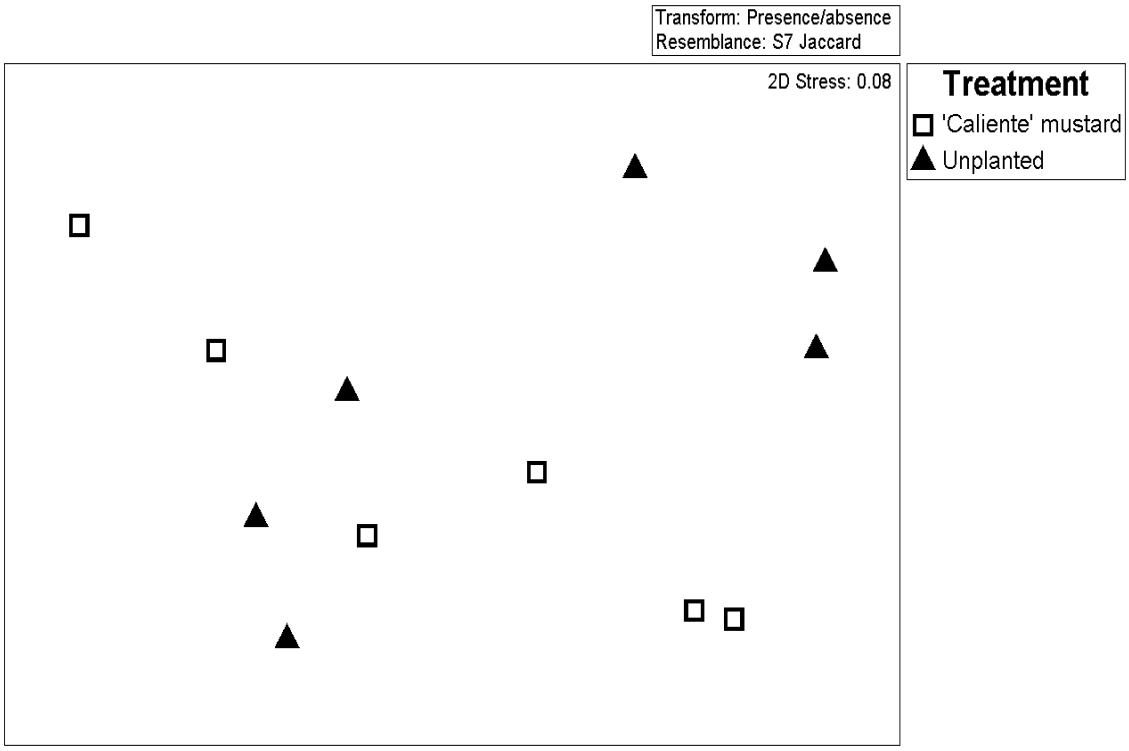


B

Figure 5.7 Non-metric multi-dimensional scaling (MDS) plots showing Betaproteobacteria community structure in soil that was either unplanted or after incorporation of with 'Caliente' mustard or unplanted (A), and assessed before (T0) and 90 days after potato plantings (T1) (B) using PCR-DGGE in the Timaru field trial (2015/16).



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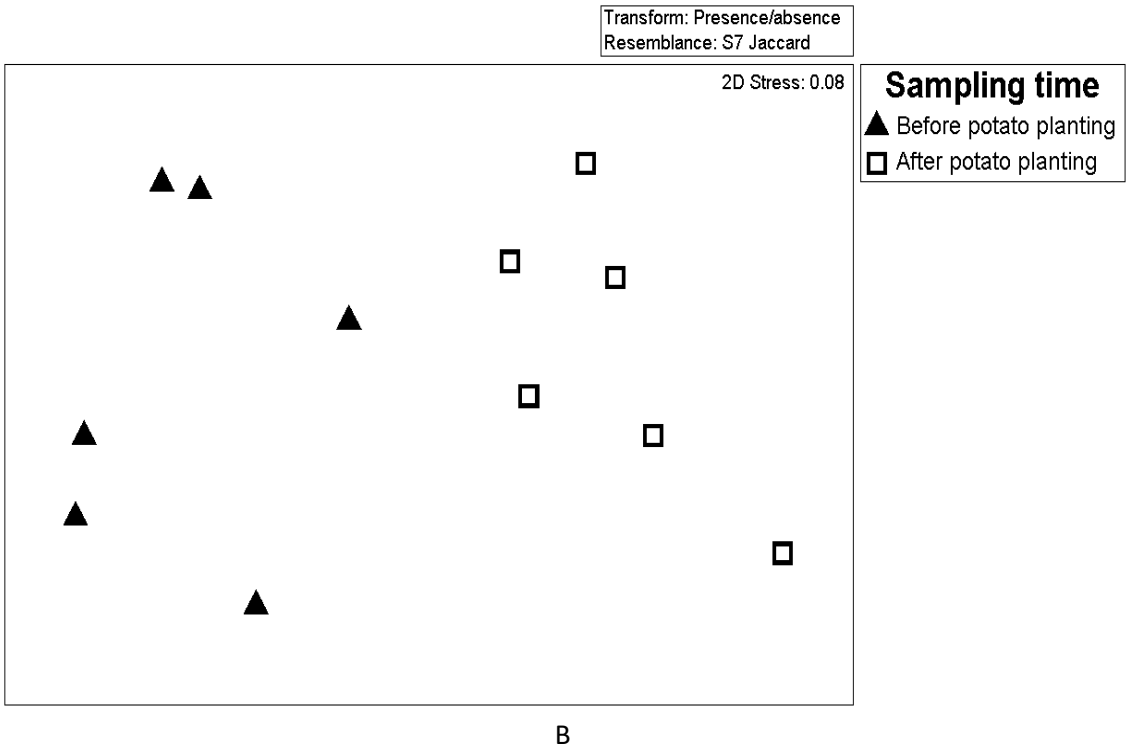


B

Figure 5.8 Non-metric multi-dimensional scaling (MDS) plots showing Gammaproteobacteria community structure in soil that was either unplanted or after incorporation with 'Caliente' mustard (A), assessed before and 90 days after potato planting (B) using PCR-DGGE in the Timaru field trial (2015/16).

Richness

There were significant effects of treatment (*P*=0.001) and sampling time (*P*<0.001) on the richness of the total fungi community (Table 5.11). The 'Caliente' treatment reduced the richness of fungi (mean of richness = 38.6) compared with the unplanted treatment (46.1). The fungus community at T0 (before potato planting) was richer (47.6) than at T1 (90 day after potato planting) (37.4) (Appendix A.5.24).

Richness of the Betaproteobacteria community was significantly affected by the cover crop treatment (*P*=0.007) (Table 5.11). The 'Caliente' treatment increased richness of this group (38.0) compared with the unplanted treatment (31.6) (Appendix A.5.25).

The interaction between treatment and sampling time significantly affected the Gammaproteobacteria (*P*=0.002) community richness (Table 5.11). At T0, the 'Caliente' mustard treatment (23.4) increased the richness of these bacteria in comparison with the unplanted treatment (16.6), whereas the 'Caliente' mustard treatment (18.6) reduced the richness of these bacteria compared with the unplanted treatment (22.9) (Appendix A.5.26).

Table 5.11 Analysis of variance table for the richness of the communities of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil that was either unplanted or after incorporation of wheat or 'Caliente' mustard, and assessed before (T0) and 90 days after potato plantings (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Community	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.001	<0.001	0.907
AMF	0.214	0.297	0.177
Alphaproteobacteria	0.716	0.249	0.383
Betaproteobacteria	0.007	0.350	0.061
Gammaproteobacteria	0.265	0.412	0.002

Shannon-Weaver diversity index

Treatment (*P*=0.016) and sampling time (*P*=0.002) significantly affected the diversity indices of the total fungi communities (Table 5.12). The 'Caliente' mustard treatment (mean diversity index = 1.45) reduced the diversity of total fungi compared with the unplanted treatment (1.51). At T0, the total fungus diversity index was greater (1.51) than that at T1 (1.41) (Appendix A.5.27**)**.

The cover crop treatment significantly influenced the Betaproteobacteria community diversity index (*P*=0.003) (Table 5.12). The 'Caliente' mustard treatment reduced the diversity of bacteria (1.32) compared with the unplanted treatment (1.41) (Appendix A.5.28).

The diversity index of the Gammaproteobacteria community was significantly affected (*P*=0.030) by the interaction between treatment and sampling time (Table 5.12). The diversity index of these bacteria was least after the 'Caliente' mustard treatment (1.07) before potato planting (T0) compared with the unplanted treatment (1.20) (Appendix A.5.29).

Table 5.12 Analysis of variance table for the Shannon-Weaver diversity indices of the communities of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil that was either unplanted or after incorporation of 'Caliente' mustard, and assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Community	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.016	0.002	0.208
AMF	0.111	0.582	0.145
Alphaproteobacteria	0.997	0.468	0.419
Betaproteobacteria	0.003	0.869	0.063
Gammaproteobacteria	0.302	0.711	0.030

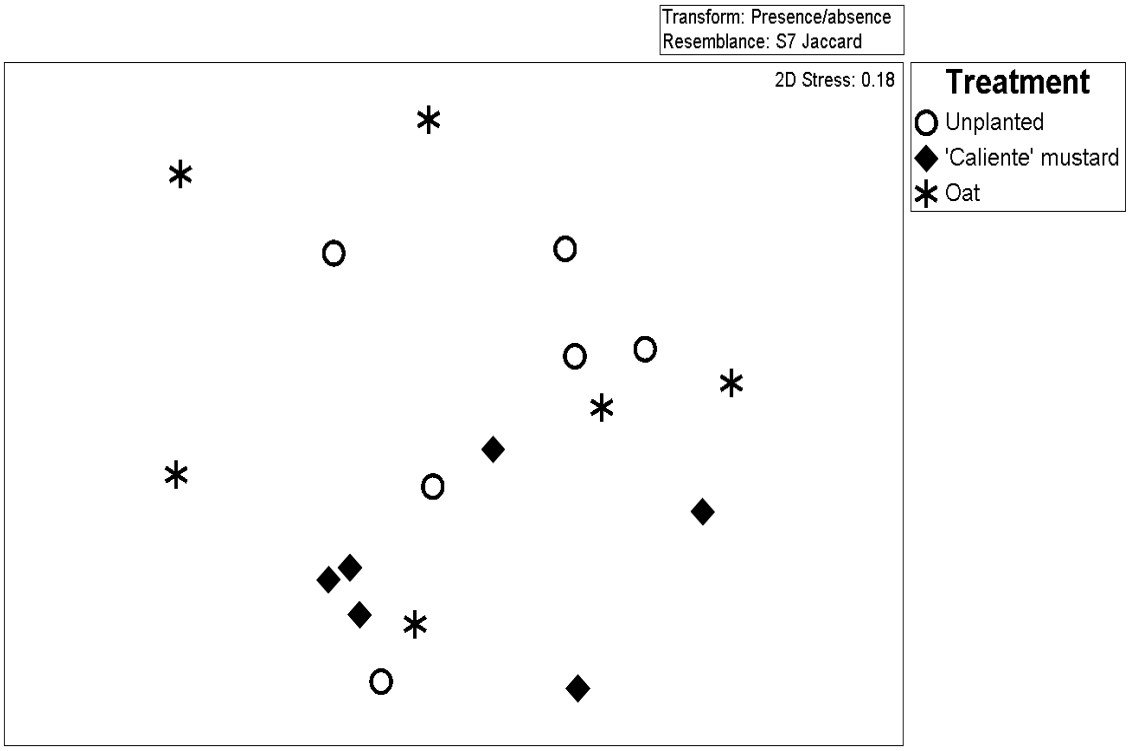
5.3.3.2. Ashburton field trial (2015/16)

Similarities

Cover crop treatment significantly affected the fungi (*P*=0.038, Figure 5.9A) and Betaproteobacteria community structures (*P*=0.030, Figure 5.12A) (Table 5.13). Sampling time influenced all five soil microbial community structures (*P*=0.001-0.005) (Figures 5.9B, 5.10, 5.11, 5.12B, 5.13). The interactions between treatment and sampling time had significant effects on all Proteobacteria community structures (*P*=0.001-0.036). For the pair-wise tests, the 'Caliente' mustard treatment affected the fungi community structure compared to the unplanted (*P*=0.02) and oat (0.044) treatments. The 'Caliente' mustard (*P*=0.048) and oat (*P*=0.02) treatments also affected the Betaproteobacteria community structures compared with the unplanted treatment.

Table 5.13 Permutational MANOVA analysis for similarities in community structures of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil that was either unplanted or after incorporation of 'Caliente' mustard or oat, and assessed before (T0) and 90 days (T1) after potato planting using PCR-DGGE in the Ashburton field trial (2015/16).

Source of variation	*P* values (PERMANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Main test			
Fungi	0.038	0.001	0.203
AMF	0.191	0.003	0.540
Alphaproteobacteria	0.224	0.001	0.014
Betaproteobacteria	0.030	0.001	0.001
Gammaproteobacteria	0.523	0.005	0.036
Pair-wise test for treatment	Fungi	Betaproteobacteria	
Unplanted x 'Caliente' mustard	0.020	0.048	
Unplanted x Oat	0.252	0.020	
'Caliente' mustard x Oat	0.044	0.217	



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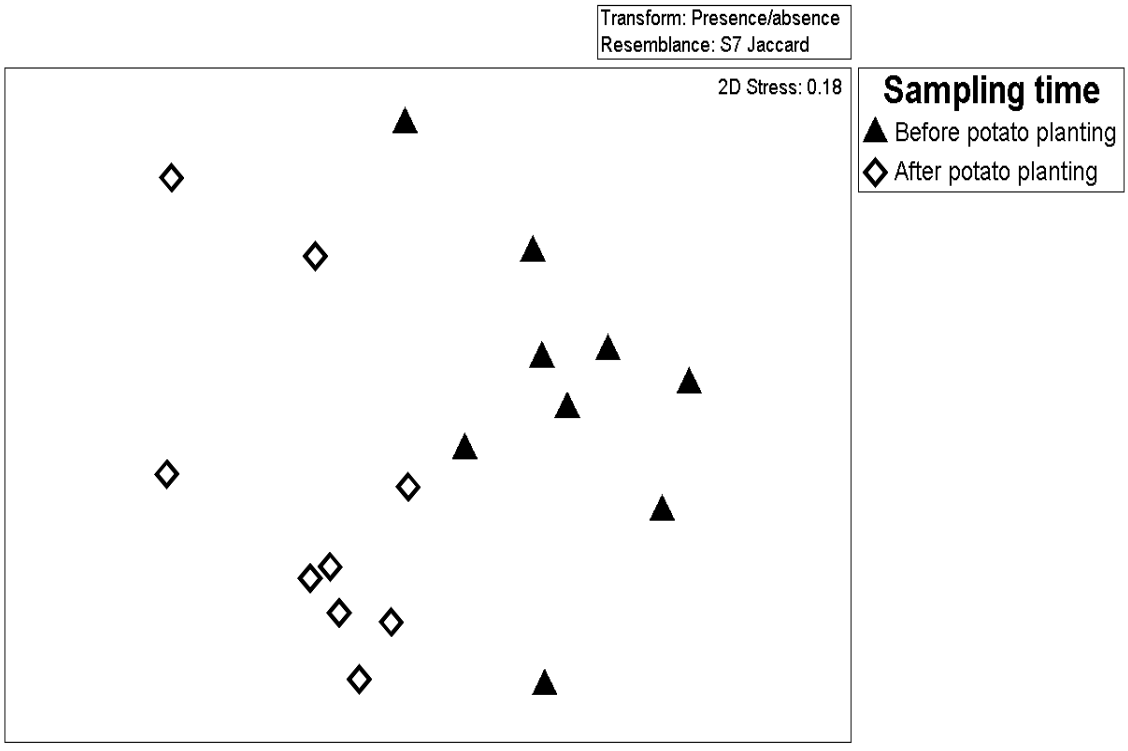


B

Figure 5.9 Non-metric multi-dimensional scaling (MDS) plots for the total fungi community structure in soil that was either unplanted or after incorporation of 'Caliente' mustard or oat (A), and assessed before (T0) and 90 days after potato planting (T1) (B) using PCR-DGGE in the Ashburton field trial (2015/16).

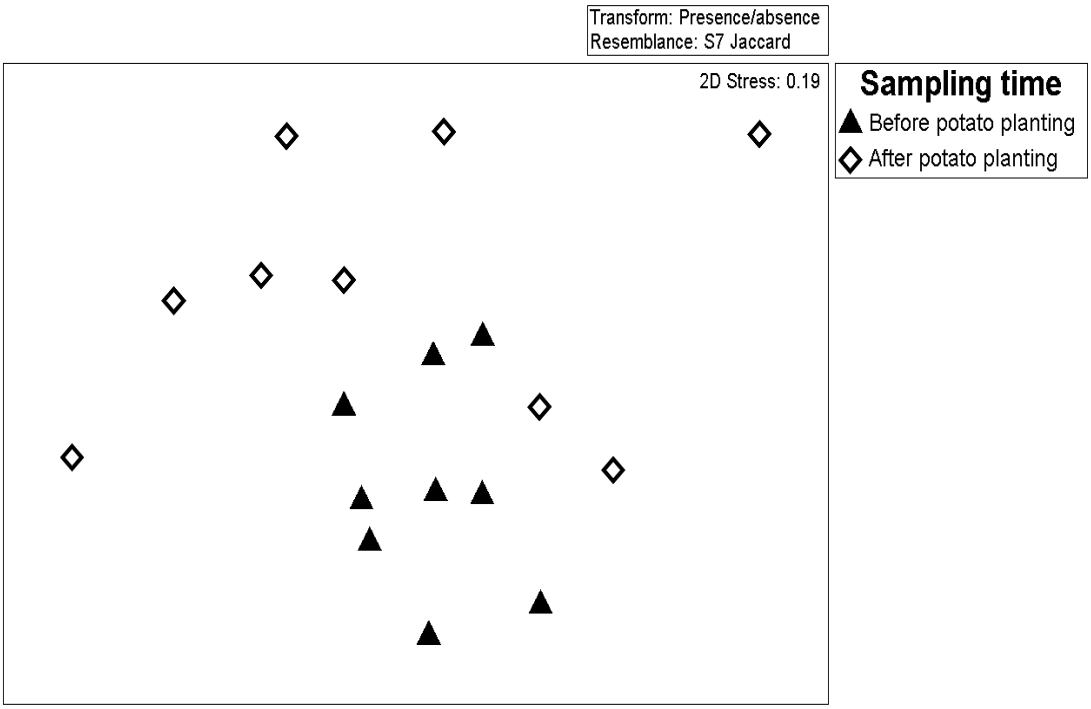


Figure 5.10 Non-metric multi-dimensional scaling (MDS) plots showing arbuscular mycorrhizal fungi community structure in soil after incorporation of cover crops, and assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

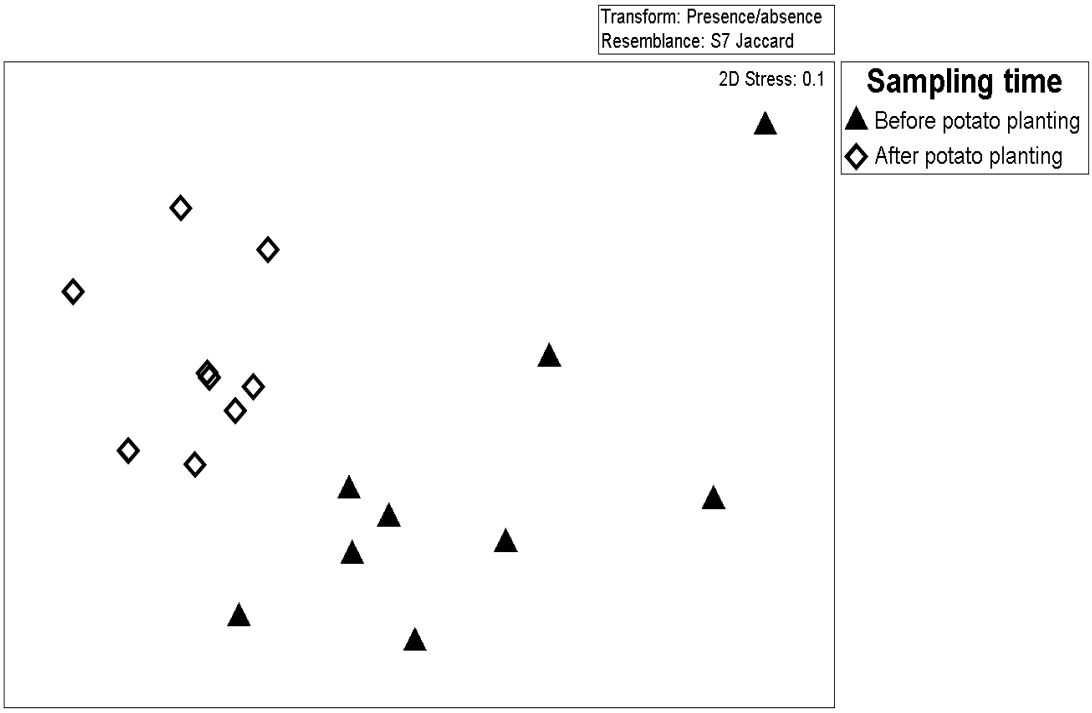
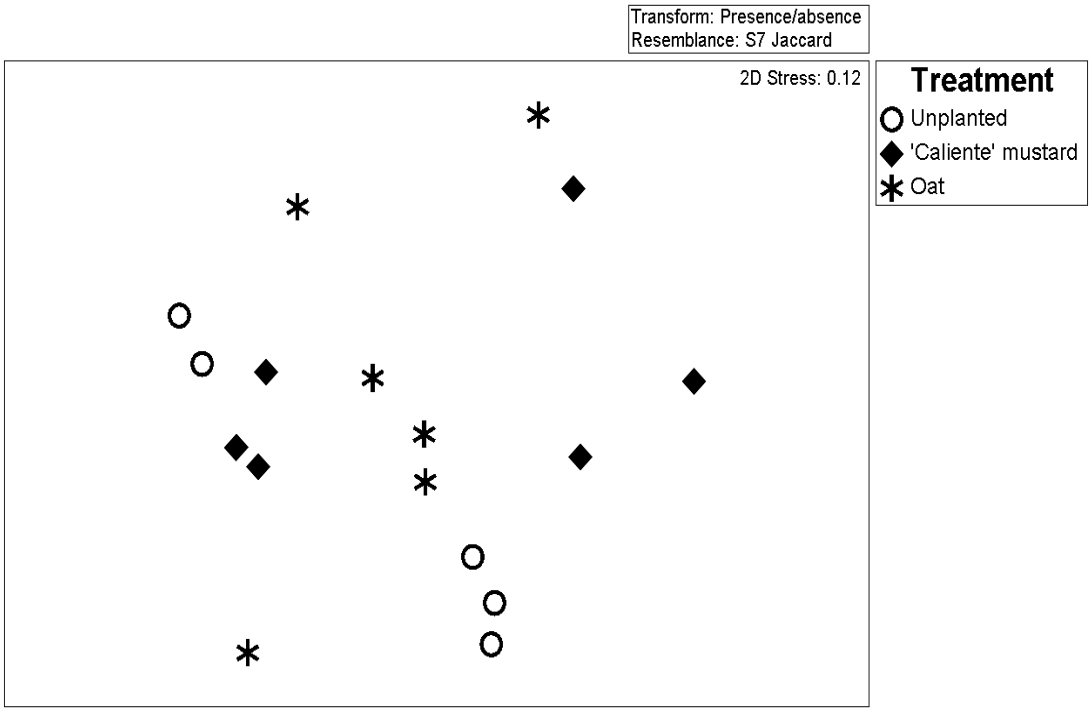


Figure 5.11 Non-metric multi-dimensional scaling (MDS) plots showing Alphaproteobacteria community structure in soil after incorporation of cover crops, and assessed before (T0) and 90 days after potato plantings (T1) using PCR-DGGE in the Ashburton field trial (2015/16).



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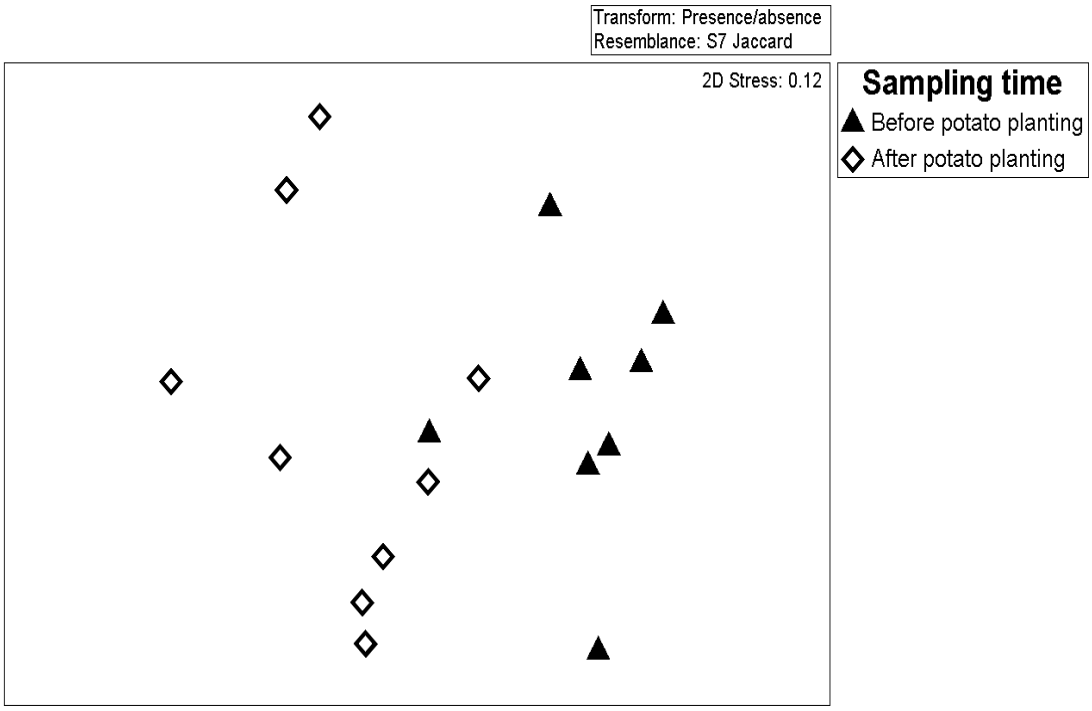

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Figure 5.12 Non-metric multi-dimensional scaling (MDS) plots showing Betaproteobacteria community structure in soil that was either unplanted or after incorporation of 'Caliente' mustard or oat (A), and assessed before (T0) and 90 days after potato planting (T1) (B) using PCR-DGGE in the Ashburton field trial (2015-2016).

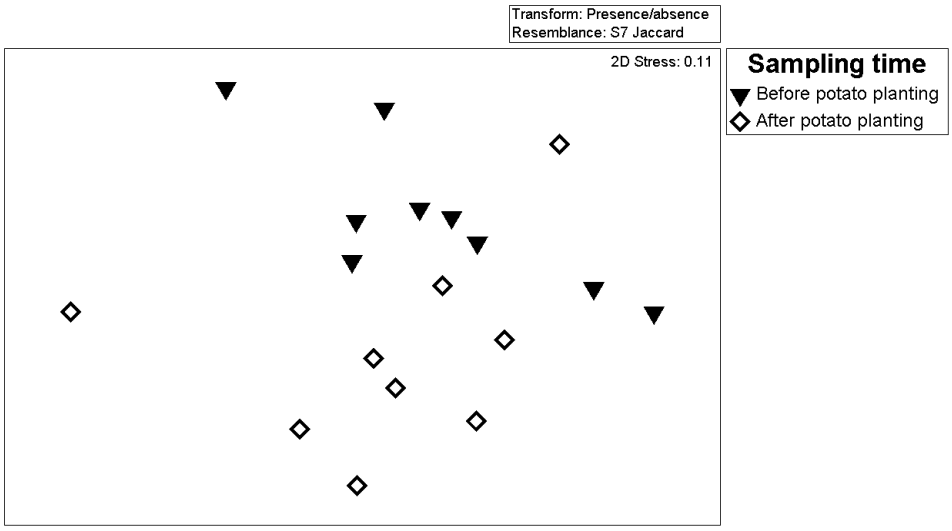

Figure 5.13 Non-metric multi-dimensional scaling (MDS) plots showing Gammaproteobacteria community structure in soil that was either unplanted or after incorporation of cover crops, and assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015-2016).

Richness

The cover crop treatments did not affect the community richness of any of the five soil microbial groups (Table 5.14). However, sampling time significantly affected the richness of the communities of total fungi (*P*=0.003) and arbuscular mycorrhizal fungi (*P*=0.001). Sampling at T0 (before potato planting) increased the richness of the total fungi (mean = 47.9) and AMF (35.5) communities compared with that at T1 (43.7 for the total fungi and 23.4 for the AMF communities) (Appendices A.5.30 and A.5.31).

The community richness indices for Betaproteobacteria (*P*=0.003) and Gammaproteobacteria (*P*=0.040) were influenced by the interaction between treatment and sampling time (Table 5.14). The unplanted treatment at T2 gave the least richness of bacteria (22.4) (Appendix A.5.32). The richness of Gammaproteobacteria indices after the 'Caliente' mustard treatment (T0), and the unplanted treatment (T1) (mean for both = 9.3) were significantly less than that after the Caliente treatment (T1) (14.8) (Appendix A.5.33)

Table 5.14 Analysis of variance for the community richness of total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil that was either unplanted or after incorporation of 'Caliente' mustard or oat, assessed before (T0) and 90 days after potato plantings (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Group	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.431	0.003	0.326
AMF	0.141	0.001	0.854
Alphaproteobacteria	0.117	0.124	0.648
Betaproteobacteria	0.149	0.236	0.003
Gammaproteobacteria	0.196	0.224	0.040

Shannon-Weaver diversity index

Cover crop treatments significantly affected the community diversity indices of Alphaproteobacteria (*P*=0.043) (Table 5.15). The 'Caliente' mustard treatment reduced the diversity index of these bacteria (mean index = 1.61) compared with the unplanted treatment (1.65) (Appendix A.5.36).

Sampling time affected the community diversity indices of total fungi (*P*=0.001), AMF (*P*=0.004) and Betaproteobacteria (*P*=0.028) (Table 5.15). Sampling time at T0 (before potato planting) increased the community diversity indices of these three microbial groups, compared with that at T1 (90 days after potato planting) (Appendices A.5.34, A.5.35, and A.5.37).

There was a significant interaction between treatment and sampling time on the community diversity indices of total fungi (*P*=0.007), Alphaproteobacteria (*P*=0.047) and Betaproteobacteria (*P*=0.022) (Table 5.15). The indices for total fungi at T0 (mean = 1.55) were greater than that at T1 (Appendix A.5.34). The diversity index of Alphaproteobacteria after the 'Caliente' mustard treatment (T1) (1.57) was less than those for the unplanted treatment (at T0 and T1) and 'Caliente' mustard (T0) treatment (1.64-1.65) (Appendix A.5.36). The diversity index of Betaproteobacteria for the unplanted treatment at T0 (1.38) was greater than that at T1 (1.23) (Appendix A.5.37).

Table 5.15 Analysis of variance table for the Shannon-Weaver community diversity indices for total fungi, arbuscular mycorrhizal fungi (AMF), Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, in soil that was either unplanted or after incorporation of 'Caliente' mustard or oat, and assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Group	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.790	0.001	0.007
Arbuscular mycorrhizal fungi	0.192	0.004	0.472
Alphaproteobacteria	0.043	0.055	0.047
Betaproteobacteria	0.374	0.028	0.022
Gammaproteobacteria	0.649	0.842	0.316

5.3.3.3 Timaru field trial (2016/17)

Similarities

Total fungi communities

There were significant effects of treatment, sampling time (*P*=0.001) and their interaction (*P*=0.001) on the total fungi community structures (Table 5.16). Both cover crop treatments affected the fungi community structures compared with the unplanted treatment (Table 5.16 and Appendix A.5.38). The fungi community structures from the 'Caliente' mustard treatment also differed from those from the 'Graza' radish treatment. Pair-wise tests showed that the fungi community structures differed at all the assessment times, with the replicates for each of the five sampling times grouping together (Figure 5.14).

Table 5.16 Permutational MANOVA analysis for the similarities in the total fungi community structures associated with incorporation of different cover crop treatments (either unplanted , 'Caliente' mustard, oat or 'Graza' radish) at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Source of variation	*P* values(PERMANOVA)
Main test	
Treatment	0.001
Sampling time	0.001
Treatment x Sampling time	0.001
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' mustard	0.027
Unplanted treat x Oat	0.055
Unplanted treat x 'Graza' radish	0.025
'Caliente' mustard x Oat	0.318
'Caliente' mustard x 'Graza' radish	0.030
Oat x 'Graza' radish	0.147
Pair-wise test for sampling time	
T1 x T2	0.041
T1 x T3	0.028
T1 x T4	0.032
T1 x T5	0.021
T2 x T3	0.027
T2 x T4	0.025
T2 x T5	0.035
T3 x T4	0.034
T3 x T5	0.030
T4 x T5	0.028

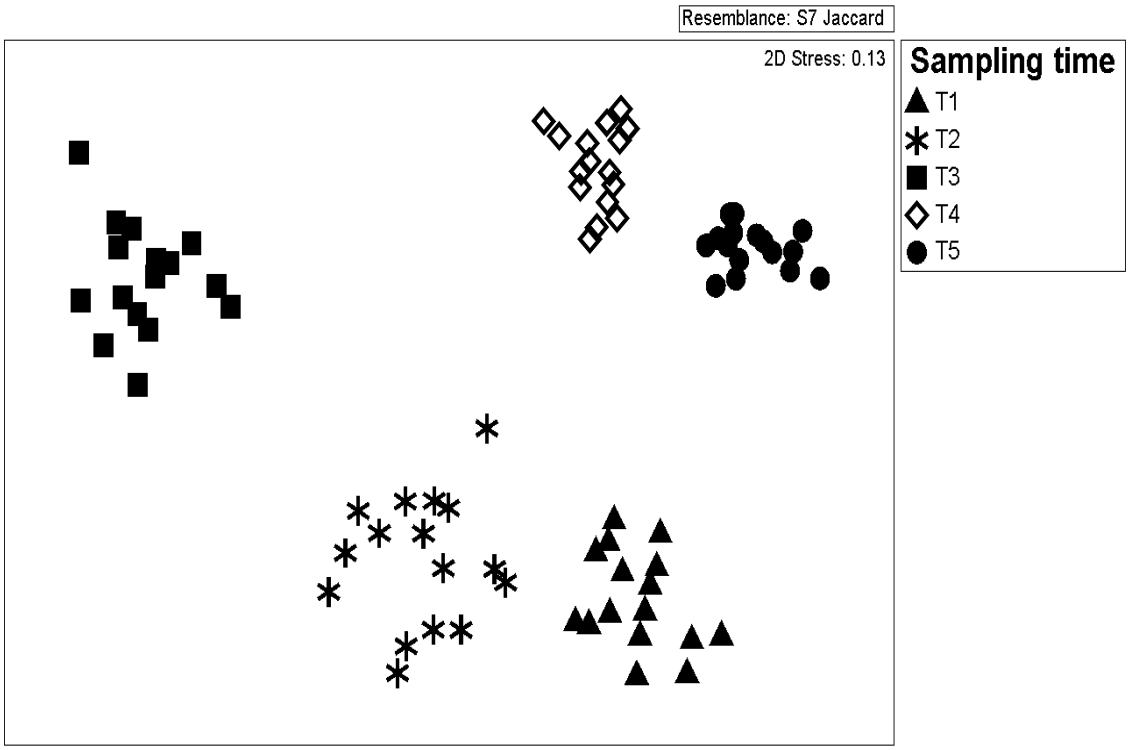

Figure 5.14 Non-metric multi-dimensional scaling (MDS) plots showing total fungi community structures assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting), using PCR-DGGE in the Timaru field trial (2016/17). Data across treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish).

Arbuscular mycorrhizal fungi communities

Arbuscular mycorrhizal fungi (AMF) communities were significantly affected by the cover crop treatments (*P*=0.011) (Appendix A.5.39), sampling time (*P*=0.001) (Figure 5.15), and the cover crop x sampling time interaction (*P*=0.001) (Table 5.17). The 'Graza' radish treatment affected the AMF community structures compared with the unplanted (*P*=0.006) and mustard (*P*=0.005) treatments. Pair-wise tests showed that the AMF community structures differed (*P*=0.001) between all assessment times, with the separate replicates for each of the five sampling times grouping together (Figure 5.15).

Table 5.17 Permutational MANOVA analysis table for the similarities of the arbuscular mycorrhizal fungi (AMF) community structures after different cover crops treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish), assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Source of variation	*P* values(PERMANOVA)
Main test	
Treatment	0.011
Sampling time	0.001
Treatment x Sampling time	0.001
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' mustard	0.393
Unplanted treat x Oat	0.118
Unplanted treat x 'Graza' radish	0.006
'Caliente' mustard x Oat	0.582
'Caliente' mustard x 'Graza' radish	0.005
Oat x 'Graza' radish	0.158
Pair-wise test for sampling time	
T1 x T2	0.001
T1 x T3	0.001
T1 x T4	0.001
T1 x T5	0.001
T2 x T3	0.001
T2 x T4	0.001
T2 x T5	0.001
T3 x T4	0.001
T3 x T5	0.001
T4 x T5	0.001

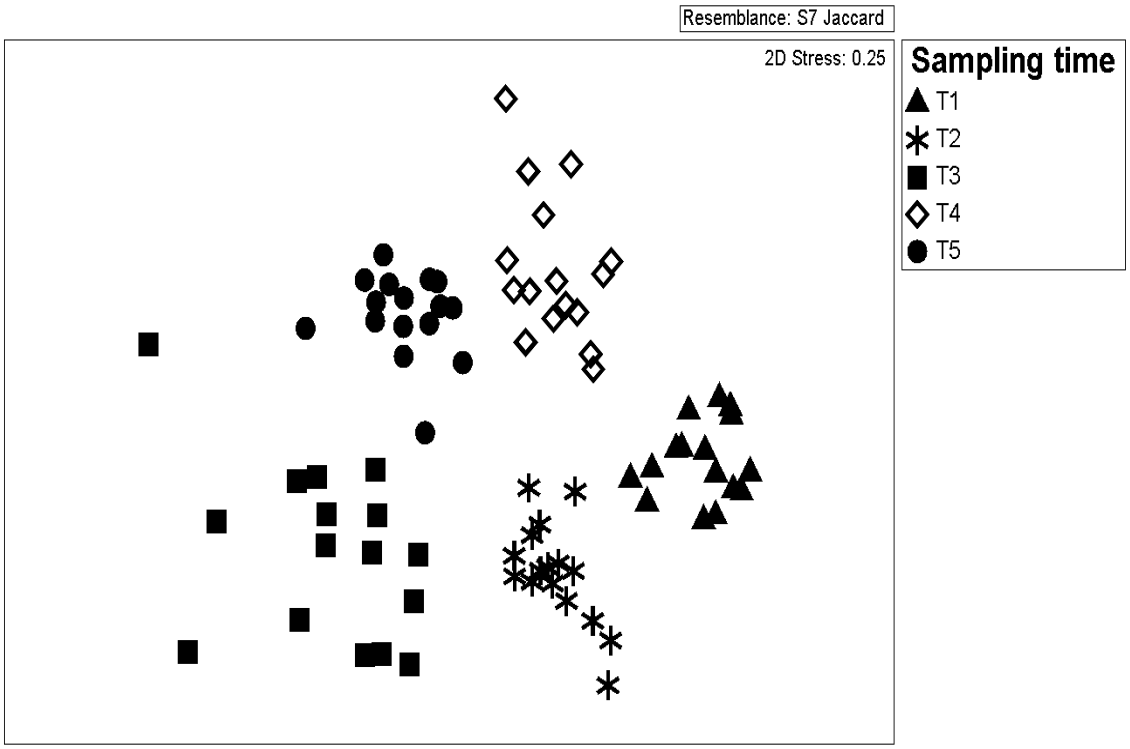

Figure 5.15 Non-metric multi-dimensional scaling (MDS) plots showing the arbuscular mycorrhizal fungi (AMF) community structures assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016-2017). Data across treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish).

Alphaproteobacteria communities

There were significant effects of the treatments (Appendix A.5.40), sampling times (*P*=0.001) (Figure 5.16) and their interaction (*P*=0.001) on Alphaproteobacteria communities. Pair-wised comparisons showed that the community structures differed between all of the treatments and between all of the assessment times (Table 5.18).

Table 5.18 Permutational MANOVA analysis table for the similarities in the Alphaproteobacteria community structures after different cover crop treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish), assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Source of variation	*P* values(PERMANOVA)
Main test	
Treatment	0.001
Sampling time	0.001
Treatment x Sampling time	0.001
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' mustard	0.001
Unplanted treat x Oat	0.001
Unplanted treat x 'Graza' radish	0.001
'Caliente' mustard x Oat	0.008
'Caliente' mustard x 'Graza' radish	0.001
Oat x 'Graza' radish	0.011
Pair-wise test for sampling time	
T1 x T2	0.001
T1 x T3	0.001
T1 x T4	0.001
T1 x T5	0.001
T2 x T3	0.001
T2 x T4	0.001
T2 x T5	0.001
T3 x T4	0.001
T3 x T5	0.001
T4 x T5	0.001

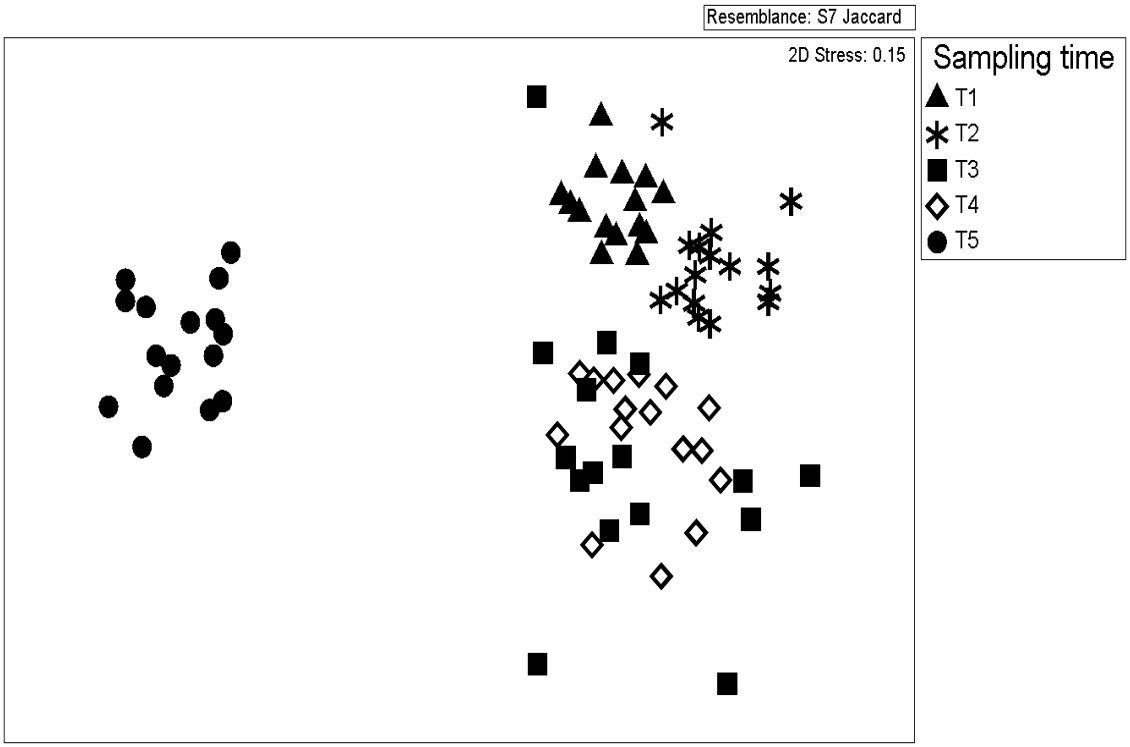

Figure 5.16 Non-metric multi-dimensional scaling (MDS) plots showing the Alphaproteobacteria community structures assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016-2017). Data across treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish).

Betaproteobacteria communities

There were significant effects of the treatments (*P*=0.001) (Appendix A.5.41), sampling times (*P*=0.001) (Figure 5.17) and their interactions (*P*=0.001) on the Betaproteobacteria communities. These indices produced from the cover crop treatments differed from the unplanted treatment, but did not differ from each other. The Betaproteobacteria community structures differed for all assessment times (Table 5.19).

Table 5.19 Permutational MANOVA analysis table for the similarities in the Betaproteobacteria community structures after the different cover crops treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish), assessed at five sampling times (T1: 138 days after cover crop plantings; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Source of variation	*P* values(PERMANOVA)
Main test	
Treatment	0.001
Sampling time	0.001
Treatment x Sampling time	0.001
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' mustard	0.001
Unplanted treat x Oat	0.005
Unplanted treat x 'Graza' radish	0.001
'Caliente' mustard x Oat	0.257
'Caliente' mustard x 'Graza' radish	0.200
Oat x 'Graza' radish	0.312
Pair-wise test for sampling time	
T1 x T2	0.004
T1 x T3	0.001
T1 x T4	0.001
T1 x T5	0.001
T2 x T3	0.001
T2 x T4	0.001
T2 x T5	0.001
T3 x T4	0.001
T3 x T5	0.001
T4 x T5	0.001

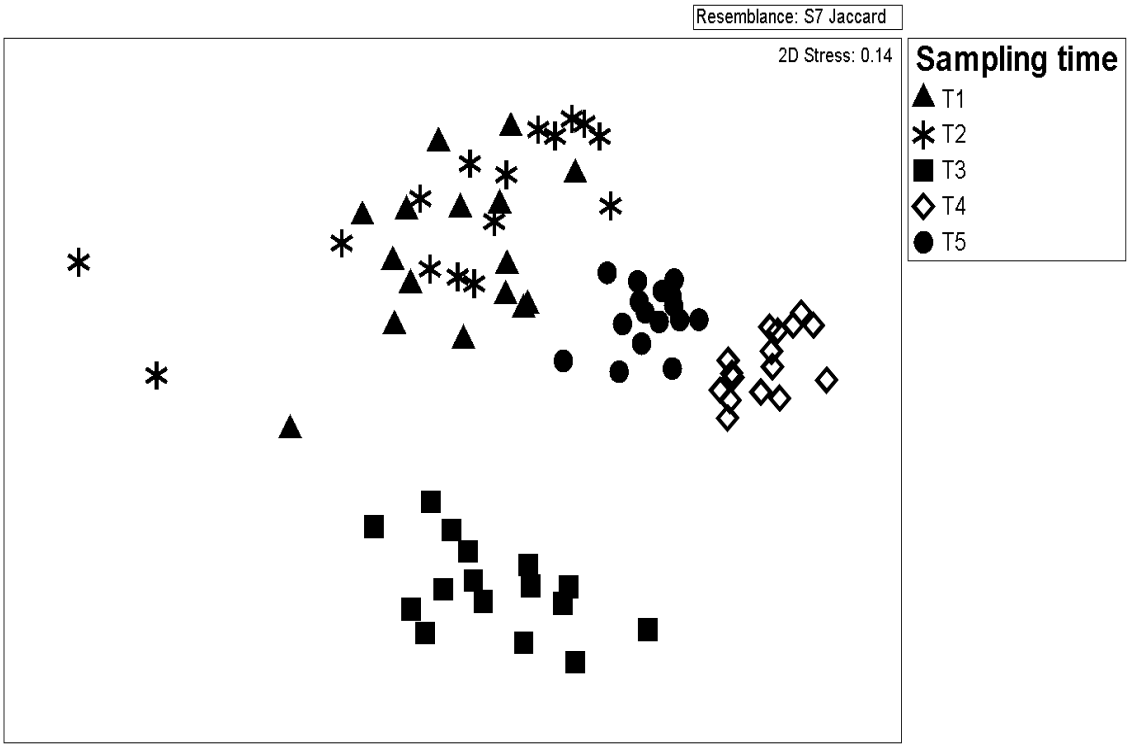

Figure 5.17 Non-metric multi-dimensional scaling (MDS) plots for the Betaproteobacteria community structures assessed at by five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting), using PCR-DGGE in the Timaru field trial (2016-2017). Data across treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish).

Gammaproteobacteria communities

There were significant effects of the treatments (*P*=0.001) (Appendix A.5.42), sampling times (*P*=0.001) (Figure 5.18) and their interactions (*P*=0.001) on the Gammaproteobacteria communities. The community structures of these bacteria from the cover crop treatments differed from the unplanted treatment, but did not differ from each other (Table 5.20). Pair-wise tests showed that the community structures differed (*P*=0.001) between all the assessment times.

Table 5.20 Permutational MANOVA analysis table for the similarities of the Gammaproteobacteria community structures after different cover crop treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish) assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Source of variation	*P* values(PERMANOVA)
Main test	
Treatment	0.001
Sampling time	0.001
Treatment x Sampling time	0.001
Pair-wise test for treatment	
Unplanted treatment x 'Caliente' Mustard	0.001
Unplanted treat x Oat	0.001
Unplanted treat x 'Graza' radish	0.001
'Caliente' mustard x Oat	0.209
'Caliente' mustard x 'Graza' radish	0.098
Oat x 'Graza' radish	0.005
Pair-wise test for sampling time	
T1 x T2	0.001
T1 x T3	0.001
T1 x T4	0.001
T1 x T5	0.001
T2 x T3	0.001
T2 x T4	0.001
T2 x T5	0.001
T3 x T4	0.001
T3 x T5	0.001
T4 x T5	0.001

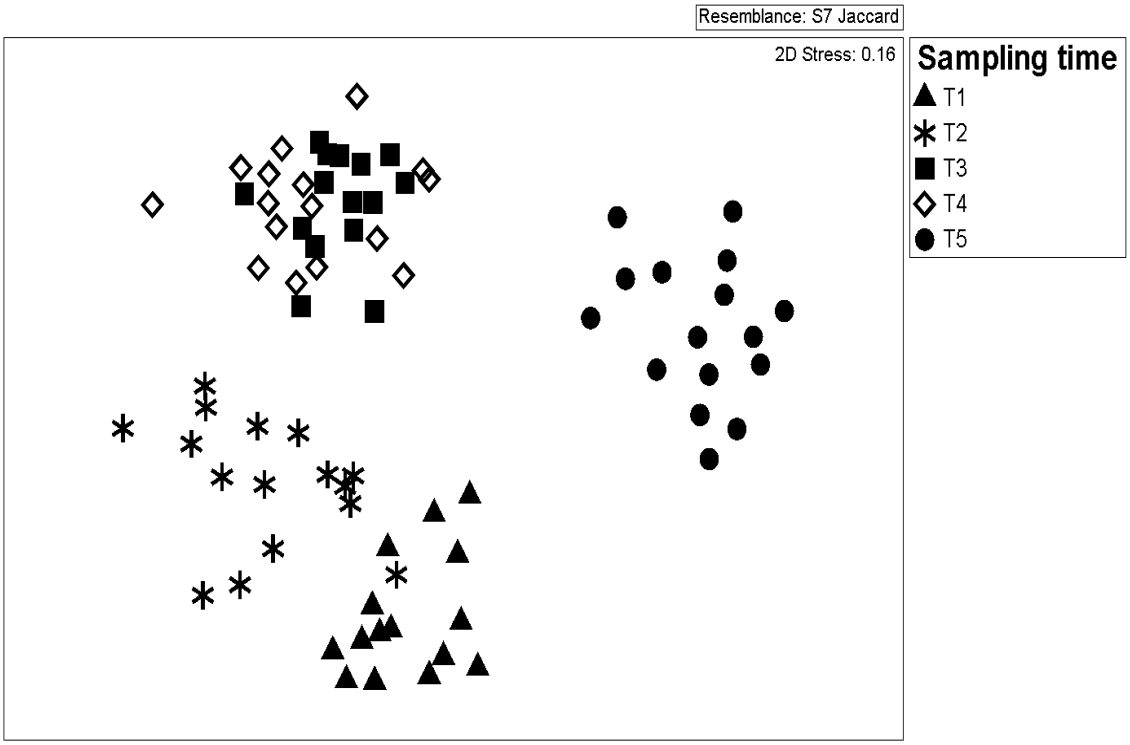

Figure 5.18 Non-metric multi-dimensional scaling (MDS) plots showing the Gammaproteobacteria community structures assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting), using PCR-DGGE in the Timaru field trial (2016/17). Data across treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish).

Richness

There were no significant effects of treatment on the community richness of AMF (*P*=0.816), Alphaproteobacteria (*P*=0.205) or Betaproteobacteria (*P*=0.913) (Table 5.21). However, there were significant effects of treatment on richness of total fungi (*P*=0.007) and Gammaproteobacteria (*P*=0.004). The 'Caliente' mustard and oat treatments (mean richness = 36.6) were less rich in their total fungi community than in the unplanted treatment (38.8) (Appendix A.5.43). The oat treatment also reduced the richness of Gammaproteobacteria (14.8) compared with the unplanted or 'Graza' radish treatments (16.2) (Appendix A.5.47).

Sampling time affected the community richness of all measured soil microbial groups (*P*=0.003 to *P*<0.001) (Table 5.21). Soil samples at T3 were less rich in total fungi (27.3), compared with other sampling times (Appendix A.5.43). Soil samples at T1-T3 had reduced AMF richness (23.1-24.0) compared with that at T5 (28.2) (Appendix A.5.44). Samples at T2 were less rich in Alphaproteobacteria (37.2) than at T1 (40.6) or T5 (40.9) (Appendix A.5.45). Samples at T1 to T3 were less rich in Betaproteobacteria (20.0-21.6) than at T4 (31.0) (Appendix A.5.46). Samples at T1 had the

least richness (12.2) of Gammaproteobacteria compared with the other sampling times (Appendix A.5 .47).

There was significant interaction between treatment and sampling time on the richness of the total fungi (*P*=0.020) and Gammaproteobacteria (*P*=0.002) (Table 5.21). All treatments at T3 had the least richness in total fungi (26.5-30.1) compared with other sampling times (Appendix A.5.43). All treatments at T1 ha the least richness of the Gammaproteobacteria (11.7-12.2) compared with other treatments at other sampling times, apart from the unplanted (T2), oat (T2), 'Caliente' mustard (T4) and oat (T4) (12.4-14.7) (Appendix A.5.47).

Table 5.21 Analysis of variance table for the richness of total fungi, Arbuscular mycorrhizal fungi, Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria communities after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Group	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	0.007	<0.001	0.020
Arbuscular mycorrhizal fungi	0.816	0.003	0.162
Alphaproteobacteria	0.205	0.001	0.820
Betaproteobacteria	0.913	<0.001	0.422
Gammaproteobacteria	0.004	<0.001	0.002

Shannon-Weaver diversity indices

The treatments only influenced the community diversity indices for total fungi (*P*<0.001) (Table 5.22). The 'Caliente' mustard (mean index = 1.43) and oat (1.42) treatments reduced the indices for total fungi compared with the unplanted treatment (1.47) (Appendix A.5.48).

Sampling time affected the diversity indices for all five microbial communities (*P*<0.001) (Table 5.22). Soil samples at T3 had the lowest diversity index for total fungi (1.30) compared with other soil samples at other sampling times (Appendix A.5.48). The diversity indices for AMF at T1 to T3 (0.94 to 0.96) were lower than those at T5 (1.13) (Appendix A.5.49). The index for Alphaproteobacteria at T3 (1.43) was the least compared with those at T1, T4 or T5 (1.50 to 1.54) (Appendix A.5.49). The indices for Betaproteobacteria at T1 to T3 (1.20 to 1.23) were less than those at T4 and T5 (1.30 and 1.36)

(Appendix A.5.51). The indices for Gammaproteobacteria at T1 (0.95) was the least compared with other sampling times (Appendix A.5.52).

The treatment and sampling time interaction affected the communities of total fungi (*P*=0.045) and Gammaproteobacteria (*P*<0.001) (Table 5.22). The indices for total fungi from all treatments at T3 (1.26 to 1.34) were least compared with all treatments at the other sampling times (Appendix A.5.48). The indices for Gammaproteobacteria from all treatments at T1 (0.92 to 0.96) were the least compared with other treatments at other sampling times, apart from the unplanted treatment at T2, the 'Caliente' mustard treatment at T4, oat at T2 to T4, or 'Graza' radish at T2 and T3 (mean indices = 0.99-1.06) (Appendix A.5.52).

Table 5.22 Analysis of variance table for the community diversity indices for total fungi, Arbuscular mycorrhizal fungi, Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria after the different cover crop treatments (unplanted, 'Caliente' mustard, oat or 'Graza' radish), assessed at five sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Group	*P* values (ANOVA)		
	Treatment	Sampling time	Treatment x Sampling time
Total fungi	<0.001	<0.001	0.045
Arbuscular mycorrhizal fungi	0.156	<0.001	0.089
Alphaproteobacteria	0.496	<0.001	0.608
Betaproteobacteria	0.913	<0.001	0.636
Gammaproteobacteria	0.110	<0.001	<0.001

5.4 Discussion

This research evaluated the effects of cover crop treatments on three aspects of non-target soil microbial communities, including general microbial activity (DHA), functional diversity (carbon utilisation; MicroResp^{TM}) and community diversity (using PCR-DGGE).

The three field trials were carried out in locations with different cropping histories, and previous research has shown that these were likely to have produced differences in the starting composition of soil microbial communities at each site (Dunfield and Germida, 2001; Berg and Smalla, 2009; Ashworth *et al.*, 2017; Finney *et al.*, 2017; Martínez-García *et al.*, 2018). For example, a study using 16S rRNA microarray (PhyloChip) technique, DeAngelis *et al.* (2008) reported that Actinomycetes and Alphaproteobacteria taxa are rhizosphere competent for wild oat (*Avena fatua*). A more recent study

using next generation sequencing (NGS) technology showed that the greatest taxa diversities in roots were fungi for wheat (*Triticum aestivium*), arbuscular mycorrhizal fungi (AMF) for hairy vetch (*Vicia villosa*) and clover (*Trifolium incarnatum*), and undefined saprotrophs for oat (*A. sativa*) (Benitez *et al.*, 2016).

DHA levels across the three field trials were greatest in the assessments after cover crop incorporation into the soils, and this was not affected by the respective cover crop treatments. These results are supported by the similar increases in DHA levels associated with carbon soil inputs from either *R. solani* colonised barley grain fragments or biofumigant tissue, as measured in experiments described Chapters 2 and 3 of this study. As discussed previously, microbial activity as measured by DHA is related to soil organic carbon levels (Wolińska and Stępniewska, 2012), and is associated with activity of total microbial communities (Wolińska and Stępniewska, 2012; Paudel *et al.*, 2016). DHA therefore provides limited information with regards to the functional diversity of soil microbial communities such as the ability to utilise different carbon sources (Campbell *et al.*, 2003).

The microbial carbon utilisation profile (CUP) of soil reflects the ability to consume a carbon source by soil microorganisms (Campbell *et al.*, 2003). In the present study, the CUP was strongly influenced by sampling time, corresponding to changes in carbon source inputs during the field trials at Ashburton (2015/16) and Timaru (2016/17). The results indicated that sampling time affected both the quantity of carbon sources (mean (tons dry matter/ha) = 0.7 (roots + shoots) for unplanted, 3.1 (shoots) for 'Caliente' mustard, 3.8 (shoots) for oat, and 4.7 (roots + shoots) for 'Graza' radish) (Sinton *et al.*, 2017) introduced into the soils in the cover crop amendment, compared with those present during crop growth, as well as differences in organic matter composition. After incorporation into soils, the raw materials of cover crops are normally degraded by soil microorganisms to form organic matter with high molecular weight compounds, such as lignins, humic acids and lipids (Rinnan and Bååth, 2009; Condron *et al.*, 2010; Schimel and Schaeffer, 2012). Increased soil organic matter has been reported to enhance catabolic activity of soil microbial communities (Nsabimana *et al.*, 2004; Jin *et al.*, 2010; Martínez-García *et al.*, 2018). Thus, different cover crop residues (at T2 and T3), including those from 'Caliente' mustard, oat or 'Graza' radish, provided different qualities and quantities of organic matter, after incorporation (Kuo *et al.*, 1997; Rodríguez-Vila *et al.*, 2016; Ghimire *et al.*, 2017; Hu *et al.*, 2018). Moreover, oat as a monocot has different carbon types, with C/N (shoots) = 16-20 compared with the *Brassica* plants, such as forage radish having C/N = 16 (shoots)-32 (roots) (Ketterings *et al.*, 2011). The decomposition rate of organic materials based on the ratio of C/N, showed that the greater C/N ratio the lower decomposition rate (Kumar and Goh, 2002; Talgre *et al.*, 2011). Thus, these could have changed the carbon substrate utilisation profiles of the microbial communities (Brant *et al.*, 2006; Carney *et al.*, 2007; Wu *et al.*, 2017).

Release of root exudates (low molecular weight organic compounds (Balendres *et al.*, 2016) during growth of cover or potato crops, is also likely to have affected the soil microbial community diversity and abundance (Shi *et al.*, 2011; Wang *et al.*, 2017), and thereby influenced utilisation of carbon substrates (Helal and Sauerbeck, 1986; Eisenhauer *et al.*, 2017). Root exudates differ between plant species (Rovira, 1956), varieties/cultivars (Balendres *et al.*, 2016), and growth stages (Rovira, 1956; Balendres *et al.*, 2016). For example, Rovira (1956) reported that oat root exudates contain 14 different amino acids, which is less than those (22 amino acids) from pea, and oat and pea roots only excreted glucose and fructose in the first 10 days of growth. Balendres *et al.* (2016) reported that some compounds, including asparagine, glutamic acid, glutamine, proline, serine, pinitol, choline, trehalose, and tyramine, were commonly found in the root zones of four potato cultivars from tissue culture, and raffinose was specific for 'Iwa', dehydroascorbic and quinic acids for 'Agria' , adenosine for 'Gladiator', and maleic acid for 'Russet Burbank'. However, whether this is consistent across different experiment systems has not been tested. These root exudates could affect specific soil microbial communities of each plant species or cultivar (Walker *et al.*, 2003; Badri and Vivanco, 2009; Dennis *et al.*, 2010; Hu *et al.*, 2015). Therefore, soil microbial community structure and catabolic utilisation in the present study would have been influenced by the rhizospheres of living cover/potato crops. Moreover, the CUP was strongly affected at 89-90 days after potato planting, when different sampling times were compared. Root exudates from growing potato plants (Balendres *et al.*, 2016) are likely to stimulate soil microbial communities (İnceoğlu *et al.*, 2011) to a greater extent than those from the cover crops, which could have released toxic compounds (such as isothiocyanates from *Brassica* crops and saponin avenacin from oat) causing negative effects on soil microbial communities (Angus *et al.*, 1994; Carter *et al.*, 1999; Fontenla *et al.*, 1999; Kirkegaard *et al.*, 2000; Rumberger and Marschner, 2003, 2004). Different organic matter from cover crop biomass and root exudates are all likely to cause the variations seen in the CUPs between sampling times.

Cover crop treatments in the second Timaru field experiment (2016/17) changed soil microbial functionality irrespective of sampling time. This was associated with the CUP of the microbial community in the oat treatment differing from that in the unplanted, Caliente' mustard and 'Graza' radish treatments. However, the factors that caused these changes in microbial functionality are unclear. This was probably due to the differentiated microbial communities between cover crops (Benitez *et al.*, 2016; Finney *et al.*, 2017; Martínez-García *et al.*, 2018) or different quantity and composition of cover crop residues as discussed earlier. Furthermore, there were volunteer radish plants (a *Brassica* sp.) in the unplanted plots (≅ 14.9% biomass compared with the radish plots) (Sinton *et al.*, 2017), which could explain the similarities of CUP between the unplanted and 'Graza' radish and 'Caliente' mustard treatments. Although the soil microbial CUPs were not different between the unplanted and 'Graza' radish/'Caliente' mustard treatments, this may not mean that the microbial community structure taxa are the same, as microorganisms can have the same

functions (utilise the same carbon substrates) or be functionally redundant. To address this, molecular methods are a useful option (Lee *et al.*, 1993; Muyzer *et al.*, 1993; Nübel *et al.*, 1996; Vainio and Hantula, 2000; Kowalchuk *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002; Lee *et al.*, 2008; Muhling *et al.*, 2008; da Silva *et al.*, 2013).

Crop phenology may have influenced soil microbial structure (species composition), richness and diversity indices. In the Timaru field trial (2016/17), all sampling times affected the soil microbial community structures, and sampling times at T1-T3 reduced the richness and diversity indices of the five microbial community groups. Sampling time at T1, when the cover crops were 138 days from planting gave the least richness and diversity indices for AMF, Betaproteobacteria and Gammaproteobacteria communities. This effect may have resulted from toxic exudates, such as isothiocyanates from the living roots of 'Caliente' mustard and 'Graza' radish (Angus *et al.*, 1994; Rumberger and Marschner, 2003; Njeru *et al.*, 2014), or saponin avenacin from active oat roots (Deacon and Mitchell, 1985; Carter *et al.*, 1999). Later samplings (T2 and T3), when the cover crops were incorporated into soils, gave reduced richness and diversity indices for the total fungi, AMF, Alphaproteobacteria and Betaproteobacteria communities. The effects at these sampling times on the soil microbial communities could have resulted from toxic compounds released from the mulching and incorporation of fresh cover crops into soil (Deacon and Mitchell, 1985; Kirkegaard *et al.*, 2000; Stapleton *et al.*, 2010; Njeru *et al.*, 2014; Wang *et al.*, 2014; Hu *et al.*, 2015; Li *et al.*, 2017; Mazzola *et al.*, 2017). For instance, Njeru *et al.* (2014) found that planting and incorporation of Indian mustard (*B. juncea*) reduced the subsequent root colonisation of maize and soil mycorrhization potential by AMF compared with that from hairy vetch (*V. villosa*) incorporation or control treatments. In addition, AMF are symbiotic, so are reliant on crop partners, such as oat or potato, for carbon sources (Brundrett *et al.*, 1996). Thus, the AMF communities are highly affected by crop presence (oat at T1 or potato at T4 to T5), and crop maturity stage or photosynthetic rate (Bhattarai and Mishra, 1984; Liu *et al.*, 2013; Njeru *et al.*, 2014). AMF colonisation rates are positively correlated with host growth stage for potato (Bhattarai and Mishra, 1984) or maize (Njeru *et al.*, 2014). These factors would have affected AMF communities during progressive sampling times. Furthermore, seasonal environmental factors, such as soil moisture (Bottner, 1985; Kieft *et al.*, 1987) or temperature (Tang *et al.*, 2018; Tomohiro *et al.*, 2018), may have had secondary influences on the soil microbial community structures and functions in the present study. However, since the crop-associated factors differed at each of the assessment times, it is not possible to draw inference or conclusion regarding these effects.

Of the cover crop treatments, 'Caliente' mustard had the greatest effects on the fungus communities, but only reduced the richness and the diversity indices for total fungi in the two Timaru field trials. These results concur with the previous study of Wang *et al.* (2014), who showed that

biofumigation with rapeseed meal (*B. napus* 'Dwarf Essex') decreased richness and diversity indices for total soil microbial communities. Siebers *et al.* (2018) also reported that application rapeseed-derived ITCs into soil reduced diversity of the total fungi. Omirou *et al.* (2011) reported that incorporation of *B. oleracea* var. *italica* 'Marathon' temporarily changed the community structure of the Ascomycetes fungi. Mocali *et al.* (2015) showed that the total fungi community structure was strongly changed by incorporation of *B. carinata*. The variation between field trials could be due to the different *Brassica* crops used, or to the experimental conditions that applied. However, this study did not identify the species within the fungal communities, thus whether the changes of fungal communities resulted in decrease or increase of harmful microorganisms (pathogens) or beneficial organisms was not determined. Some genera of potential soilborne pathogens, including *Fusarium*, *Nectria*, and *Cladosporium*, were not suppressed after incorporation of *B. oleracea* var. *italica* 'Marathon' at 15 g/kg dry soil (Omirou *et al.*, 2011). A recent study showed that antagonistic fungus *Trichoderma* spp. was recovered from the soil treated with rapeseed-derived ITCs (Siebers *et al.*, 2018). Thus, further studies are required to understand the changes in species or genera levels of the total fungi affected by cover crop treatments.

Although 'Caliente' treatment modified the Proteobacteria community structures, it effects on the richness and diversity indices for this group were variable. The 'Caliente' mustard treatment affected Proteobacteria community structures in the two Timaru field trials, but only increased the richness and reduced the diversity index of Betaproteobacteria community in the Timaru (2015/16) trial, and reduced the diversity index of Alphaproteobacteria community in the Ashburton field trial (2015/16). These changes of Proteobacteria communities were similar to those reported by Mocali *et al.* (2015), where that bacteria community structure was strongly altered after incorporation of *B. carinata*. However, Rokunuzzaman *et al.* (2016) reported that biofumigation with green mustard (*B. juncea*) did not change the bacteria community structure. The difference between studies may be due to the varied experimental conditions or different *Brassica* cultivars. The enrichment of Betaproteobacteria community by 'Caliente' mustard seen in the present research partially concurs with previous studies (Li *et al.*, 2017; Wang *et al.*, 2014). These have shown that incorporations of rapeseed (*B. napus* 'Dwarf Essex') (Wang *et al.*, 2014), mustard (*B. napiformis* 'ErDao') or rape (*B. campestris* 'WoGuan-2') (Li *et al.*, 2017) increased the richness and diversity indices of the total bacterial communities. The reduction of the diversity indices of Alphaproteobacteria and Betaproteobacteria communities after 'Caliente' mustard incorporation was similar to that reported by Siebers *et al.* (2018), where the diversity index for total bacteria was decreased by incorporation of rapeseed-derived ITCs. The Alphaproteobacteria contains several functional genera, including phototrophic group (*Rhodobacter*), plant symbiont groups (*Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Azorhizobium*) (van Rhijn and Vanderleyden, 1995; Pini *et al.*, 2011) and groups pathogenic towards animals and plants (*Rickettsia*, *Brucella* and *Agrobacterium*) (Pini *et al.*, 2011). Betaproteobacteria harbors some genera such as

ammonia-oxidising bacteria (*Nitrosomonas*), and symbiotic nitrogen fixation bacteria (*Burkholderia*) (Chen *et al.*, 2003; İnceoğlu *et al.*, 2010; Moulin *et al.*, 2015). Thus, the effects of 'Caliente' mustard on these two Proteobacteria groups are likely to affect soil nutrient cycles.

The oat treatment (non-brassicaceous crop) reduced the richness of the total fungi and Gammaproteobacteria communities in the Timaru field trial (2016/17). This was probably due to the release of toxic compounds from the living roots, or from oat shoots/leaves incorporated into the soils, or specific soil microbial communities for oat root rhizosphere. Intact oat roots release saponin avenacin (Deacon and Mitchell, 1985; Carter *et al.*, 1999). This compound is toxic to *Pythium* spp., reducing zoospore cyst wall formation and deactivating pre-encysted zoospores or active mycelium (Deacon and Mitchell, 1985). Stapleton *et al.* (2010) reported that incorporation of oat into soil suppressed the recovery of *Sclerotium rolfsii* and *P. ultimum* from soil and reduced tomato root galling (49-97%) caused by *Meloidogyne incognita*. However, the present results contrast with the study of Patkowska and Konopinski (2013), who reported that planting and incorporation of oat in to soil increased populations of antagonistic fungi and bacteria. The differences in these reported effects could be due to the different experimental methods and conditions. Patkowska and Konopinski (2013) only measured populations of cultivable fungi and bacteria on media, while the present study indentified both cultivable and non-cultivable total fungi and Gammaproteobacteria. DeAngelis *et al.* (2008) found that Gammaproteobacteria in wild oat roots was abundant compared with Alphaproteobacteria. Moreover, toxic exudates (e.g. saponin avenacin) (Deacon and Mitchell, 1985; Carter *et al.*, 1999) from root tips of wild oat could differently affect the richness of Proteobacteria communities (DeAngelis *et al.*, 2008), and presumably the Gammaproteobacteria community was more sensitive than other Proteobacteria groups. Further study is required to confirm this hypothesis. Wang *et al.* (2017) reported that plant species and plant genotypes/cultivars are main drivers for soil microbial communities in the rhizosphere. This current study used a domesticated oat species (*A. sativa*) which is different in species and genotypes with the wild oat (*A. fatua*). Thus, it is likely that there could different soil microbial communities in the rhizospheres between the domesticated and wild oats. In addition, avenacin released from oat (*A. sativa*) shaped the fungi community, which are resistant to avenacin, in the oat rhizosphere (Carter *et al.*, 1999). This could negatively affect to the total fungi community.

The 'Graza' radish treatment affected the AMF community structure, but not the AMF community richness or diversity indices in the Timaru field trial (2016/17). White and Weil (2010) reported that planting forage radish (*R. sativus* var. *longipinnatus*) as a cover crop did not reduce the colonisation of maize roots by AMF on in the subsequent season, compared with a non-cover crop treatment. Higo *et al.* (2015) also noted that the richness and diversity of AMF communities did not change depending on the rotation year of wheat (*Triticum aestivum*), rapeseed (*B. napus*), or fallow as

treatments, which are results similar to those in the present study. Since the present study did not look at the level of AMF colonisation or the abundance of mycorrhiza species, this may differ to the results of the DGGE analyses which indicate presence/absence. Furthermore, presence of AMF spores and mycelium (dormant and active propagules) in the soil may have affected these results. The PCR-DGGE technique does not differentiate between dormant (spores) and active propagules (mycelium) of AMF communities. Further experiments are required to confirm these results, and also to determine the effects of any change in the AMF community on the beneficial properties of these symbionts in relation to crop growth, and suppression of disease.

In the present study only presence/absence of microbial 'species'/taxa was determined using the PCR-DGGE method, and, as such no information regarding the effect of the treatments on the relative abundance of particular 'species'/taxa within the overall microbial communities was determined. Furthermore, care must be taken in interpreting the DGGE bands to represent single microbial species as one band can represent different bacteria or fungi species as a result of the same GC content (Muyzer *et al.*, 2004) resulting in the same band migration, or more than one band can be produced by a single species (Nakatsu *et al.*, 2000; Boon *et al.*, 2002). Moreover, low concentration of rDNA could result in a blurred or unclear band in the DGGE gel (Boon *et al.*, 2002). Thus, diversity indices calculated from DGGE gels should indicate relative indices, not absolute ones to show the level of diversity of the bacterial community (Eichner *et al.*, 1999; Boon *et al.*, 2002). However, DGGE-PCR has been reported to have some advantages such as low cost benefits (Christopoulos *et al.*, 2012) and highly sensitive to species with low numbers (Boon *et al.*, 2002). Recently, modern techniques, e.g. Illumina Miseq sequencing (Yim *et al.*, 2017; Siebers *et al.*, 2018), have provided the better solution for studying on soil microbial communities affected by biofumigation aiming to identify the species which are affected by the cover crops, and hence if there is an overall non-target impact.

In summary, each of the measures used in this study for soil microbial community analysis has limitations. However, using the data together provides a comprehensive assessment of the effects of the cover crop treatments on the soil microbial communities. The DHA was increased by the cover crop treatments. CUP of soil microbial communities was affected by sampling time and the oat treatment. The PCR-DGGE data showed that sampling times at T1 (138 days after cover crop planting) and T2-T3 (8-42 days after cover crop incorporation) strongly modified the structure, and decreased the richness and diversity indices of soil microbial communities. 'Caliente' mustard treatment altered the structures, reduced the richness and the diversity indices of the total fungi communities. In addition, 'Caliente' mustard affected Proteobacteria community structures, increased the richness of Betaproteobacteria community, and reduced the diversity indices of Alphaproteobacteria and Betaproteobacteria communities. 'Graza' radish treatment changed AMF

community structure. Oat treatment reduced the richness of the total fungi and Gammaproteobacteria communities. However, further research is needed to determine the changes in specific species or genera of fungi communities by sequencing the dominant bands from DGGE gels (Omirou *et al.*, 2011; Wang *et al.*, 2014) or using Illumina Miseq sequencing (Yim *et al.*, 2017; Siebers *et al.*, 2018). This research has demonstrated that biofumigant cover crops can cause considerable positive and negative effects on soil microbial populations. These effects on (non-pathogenic) soil microbes are likely to be important for the general organic processes in soils treated with these crops. Whether these (non-target) effects are important for productivity of potato crops has yet to be determined.

Chapter 6

Concluding discussion

The research outlined in this thesis embodies the first comprehensive study in New Zealand to assess the potential for biofumigation as a management strategy for control of diseases of potato caused by *Rhizoctonia solani*. The overall aims of this study were, firstly, to evaluate the potential for suppression of *R. solani* infection of potato using the selected biofumigant plants, and, secondly to determine whether the levels of pathogen suppression were sufficient to recommend widespread adoption of biofumigation for management of *R. solani* diseases in New Zealand potato cropping systems. *Rhizoctonia solani* AG3-PT is the predominant group causing black scurf on potato tubers in New Zealand (Das *et al.*, 2014), so this pathogenic *R. solani* group was the focus of the laboratory and shadehouse experiments described in Chapters 2, 3 and 4 of this thesis.

This work showed that the biofumigant plants, 'Caliente' mustard, brown mustard and 'Nemat' arugula, had the greatest potential for inhibiting the growth of *R. solani* AG3-PT and AG2-1 isolates (isolated from diseased potato plants in New Zealand) out of the ten species/cultivars tested (Chapter 2). The present findings partially agreed with international studies (Larkin and Griffin, 2007; Snapp *et al.*, 2007; Villalta *et al.*, 2016) in that 'Caliente' and brown mustard crops had the greatest ability to suppress mycelium growth of *R. solani* AG3-PT and AG2-1 isolates. In addition, 'Nemat' arugula, which has been reported to inhibit root-knot nematodes (*Meloidogyne* spp.) (Kokalis-Burelle *et al.*, 2013; Edwards and Ploeg, 2014), provided effective inhibition of the growth of *R. solani* isolates in the present study. This indicated that this cultivar should be explored as potentially having a broad control range of both soilborne pathogens and nematodes. In New Zealand, several previous studies showed that biofumigation with *Brassica* plants reduced clubroot (caused by *Plasmodiophora brassicae)* severity on cauliflower (Cheah *et al.*, 2006) or black foot (caused by *Cylindrocarpon* spp.) incidence on grapevine (Bleach, 2013). The authors only used two *Brassica* species in their studies, *B. juncea* and *B. napus* in Bleach (2013) or *B. rapa* and *B napus* in Cheah *et al.* (2006). This present work expanded that list to concurrently test ten plant species/cultivars, allowing clear identification of the most promising biofumigant plant genotypes for controlling *R. solani* on potato.

This work confimed some findings from oversea studies (Kirkegaard *et al.*, 1996; Yulianti *et al.*, 2006a; Snapp *et al.*, 2007; Mattner *et al.*, 2008) that shoots or shoots plus roots of the selected biofumigant plants gave greater inhibition of growth of *R. solani* isolates than roots alone (Chapter 2). The present study also supported the previous studies (Yulianti *et al.*, 2006a, 2006b) that the

inhibitory efficiency of the selected biofumigants on growth of the pathogen was positively correlated to the amounts of biofumigant tissues, with 5-10 g of macerated tissue per Petri plate (or 5-10% (w:w) in soil) giving the greatest inhibition of mycelium growth of *R. solani* isolates. However, the effective amounts seen in the present study were greater than that reported by Handiseni *et al.* (2017). This difference may have been due to the different *R. solani* isolates used, either because they originated from diseased potato plant tissues (this study) compared with from diseased rice tissues (Handiseni *et al.*, 2017) or due to the differences in the experimental conditions between the two studies. This also indicated that suppression of the pathogen by biofumigants might be harder to achieve in New Zealand soils, as they required greater amount of biofumigant materials. In addition, the present findings confirmed the previous knowledge (Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998; Mattner *et al.*, 2008) that biofumigant tissues harvested from plants at the mid flowering growth stage were more effective for reducing mycelium growth of *R. solani* isolates, than plants at other stages. This was likely due to greater amounts of total glucosinolates in mid-flowering tissues, and these compounds are known to be the precursors of toxic volatile isothiocyanates (Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998). Thus, these results indicated that biofumigant crops should be grown until midflowering before incorporation into soils to achieve maximum biofumigation potential for management of soilborne diseases.

This work showed for the first time that the *R. solani* AG2-1 isolated from potato was more tolerant to biofumigation compared with the eight (of nine) AG3-PT isolates in New Zealand (Chapter 2). This could be because *R. solani* AG2-1 has been reported to cause diseases on *Brassica* plants (Yulianti *et al.*, 2006a; Budge *et al.*, 2009b), so some biofumigant candidates could be hosts of this potato pathogen, and reduce their value for biofumigation in potato crop management. The present study also confirmed the overseas study that *R. solani* AG2-1 isolates were more tolerant to pure 2-phenylethyl isothiocyanate compared with *R. solani* AG3 isolates (Smith and Kirkegaard, 2002).

The present study is the first to show the conversion from mycelia to sclerotia of *R. solani* AG3-PT and AG2-1 isolates (from potato) under biofumigation treatments in New Zealand soils (Chapter 2). The conversion rate was variable depending on biofumigant amount and type. The results showed that low amount of the biofumigants, at 1% or 5% (w:w) loam soil incorporation of 'Caliente' mustard and 1% of brown mustard and 'Nemat' arugula, increased the conversion of *R. solani* mycelium into sclerotia. Although there were differences in experimental conditions, such biofumigant type, soil type or *R. solani* isolate, compared with the present study, Yulianti *et al.* (2006a) also reported that 1% (w:w) soil (Lacelin sand) incorporation of *B. nigra* and *Diplotaxis tenuifolia* (a brassicaceous weed) increased the sclerotia number of *R. solani* AG2-1 (isolated from diseased canola) compared with those at 10% (w:w) in Australia conditions. Sclerotia were found to be more tolerant with the biofumigation than mycelia (in Discussion Section of Chapter 2). Thus, these studies indicated that

incorporation of low and insufficient amounts of biofumigants, particularly in field application, may not suppress *R. solani* propagules, but increase risks of conversion of *R. solani* to resting bodies such as sclerotia, thus potentially reducing biofumigation efficiency.

This work was the first to apply the qPCR technique to monitor the changes of *R. solani* AG3-PT DNA under biofumigation treatments, 'Caliente' mustard, brown mustard and 'Nemat' arugula, in different soil edaphic factors (Chapter 3). The new findings showed that the most effective biofumigation treatments for reducing *R. solani* AG3-PT inoculum in a loam soil, was at pH 6.6, at 20°C and 40% soil WHC, and at a combination of 15°C and 40% WHC. These indicated that the biofumigation potential of these plants was complex and variable under different soil conditions. Thus, for the field application, such soil conditions together with other factors, e.g. biofumigant genotypes, maceration level, appropriate incorporation method or sufficient soil moisture (Kirkegaard, 2009), should be considered to obtain an optimum biofumigation efficacy. Larkin and Griffin (2007) also reported that the inoculum of *R. solani* AG3 (isolated from potato) in soil (field silk loam soil : sterile sand (3:1, v:v)) at biofumigation treatments (planting and incorporation) were reduced compared with the control. However, they used culture plating to calculate amounts of *R. solani* AG3 inoculum in soil, while the present study used qPCR to estimate amounts of *R. solani* AG3-PT DNA in soil which has been reported to be more accurate and sensitive (Lees *et al.*, 2002; Ophel-Keller *et al.*, 2008; Budge *et al.*, 2009a; Woodhall *et al.*, 2013). In addition, the present work confirmed the literature knowledge (Snapp *et al.*, 2007; Larkin and Griffin, 2007) that soils amended with macerated 'Caliente' mustard tissue (5%, w:w) had the least severe stem canker, and the greatest potato plant heights and dry biomass compared with those grown in soils from the *R. solani* inoculated control treatments. This indicated that under favourable soil conditions biofumigation treatments effectively suppressed *R. solani* AG3-PT inoculum, and thus, reduced the infection of the pathogen in subsequent potato planting.

The biofumigation effects of 'Caliente' mustard, brown mustard and 'Nemat' arugula, for suppression of *R. solani* infection of potato plants were demonstrated in a shadehouse experiment described in Chapter 4. The selected biofumigant plant tissue incorporations reduced and maintained *R. solani* AG3-PT inoculum at low levels during the experiment, suppressed infection by the pathogen, and potentially other AGs present in the soil. This disease suppression was on potato stems, stolons and tubers. These results are similar to those of Larkin and Griffin (2007) and Snapp *et al.* (2007) which showed that in US studies that biofumigation treatments reduced the incidence and severity of *R. solani* AG3 isolates on potato plants and increased tuber yields. However, the current study is the first use of qPCR to look at the dynamics of *R. solani* AG3-PT, and illustrated that, as expected, the *R. solani* DNA levels in soil was positively correlated with the severities of stem canker, stolon and tuber black scurf. The results also showed that the biofumigants had potential to control other soilborne

pathogens of potato. The severity of powdery scab (caused by *Sp. subterranea*) was reduced after the biofumigant treatments. Larkin and Griffin (2007) reported that *B. juncea* provided suppression of powdery scab on potato tubers in field trials. In the present study, biofumigant treatments also reduced the incidence of dead stems caused by *C. coccodes*. This was likely due to the reduction of *C. coccodes* by biofumigation treatment, therby decreasing the infection of the pathogen on potato plants. The potential for biofumigants to inhibit mycelium growth of *C. coccodes in vitro* was reported by de Boer *et al.* (2003) and Harding *et al.* (2005). Thus, these results indicated that the biofumigants tested here could have broad control ranges against soilborne pathogens on potato in New Zealand soils. Further studies should be carried out under field conditions to evaluate the practical biofumigation efficiency of the selected biofumigants for management of soilborne diseases in potato crops.

The effects of cover cops, including 'Caliente' mustard, oat or 'Graza' radish, in potato rotation systems, on soil microbial communities were assessed in the three field trials (Chapter 5). Measurements of soil microbial activity (DHA), soil microbial carbon utilisation profiles (MicroResp^{TM}), and microbial communities using PCR-DGGE were carried out. Incorporation of *B. juncea* green manures increased soil DHA levels (Paudel *et al.*, 2016), and DHA is generally reported to be related to the organic content in soil (Wolińska and Stępniewska, 2012). The DHA levels were enhanced in treatments incorporating 'Caliente' mustard, oat or 'Graza' radish (Chapter 5), which was consistent with the results from the *in vitro* and shadehouse experiments described in Chapters 3 and 4. DHA is a reliable indicator for soil microbial activity (Paudel *et al.*, 2016). However, DHA measurements only account for the total soil microbial activity (Wolińska and Stępniewska, 2012), and the method does not differentiate the composition changes of soil microbial populations occurring during biofumigation or biofumigant plant degradation. Thus, although this data concurred with previous reports it was clear that greater depth of analysis was required to understand the dynamics of soil microbial communities in response to biofumigation.

This work is the first to evaluate the soil carbon utilisation profile (CUP) of soil microbial communities under biofumigant cover crop treatments and subsequent soil sampling at different crop stages/crop types/crop residues in New Zealand field conditions (Chapter 5). The results showed that events at sampling times and the oat crop treatment affected the CUP of soil microbial communities. This could be due to the different biomass incorporation of the respective plant types (Ketterings *et al.*, 2011; Rodríguez-Vila *et al.*, 2016; Ghimire *et al.*, 2017; Hu *et al.*, 2018), or root exudates from living crops (Pérez and Ormeño-Nuñez, 1991; Mennan and Melakeberhan, 2006; Wichern *et al.*, 2007; Wadhwa and Narula, 2012; Balendres *et al.*, 2016).

This work is the first to use PCR-DGGE to look at the changes of soil microbial communities under different biofumigation cover crops in New Zealand field conditions (Chapter 5). The PCR-DGGE

results showed that the influence on soil microbial communities was complex and that specific microbial groups were differently affected by cover crops, trial sites, plant type and sampling time. For example, 'Caliente' mustard reduced the richness and diversity of total fungi community, reduced the diversity of Alphaproteobacteria and Betaproteobacteria communities, and increased the richness of Betaproteobacteria community; 'Graza' radish treatment changed the AMF community; oat treatment reduced the richness of the total fungi and Gammaproteobacteria communities. The five microbial communities selected for the present study provide the main roles in soil nutrient cycles or in plant pathogen antagonism (Brundrett *et al.*, 1996; Condron *et al.*, 2010; Omirou *et al.*, 2011; Wang *et al.*, 2014; Köberl *et al.*, 2017; Li *et al.*, 2017; Siebers *et al.*, 2018). For examples, the Alphaproteobacteria and Betaproteobacteria harbor most of nitrogen-fixation symbiont genera (Chen *et al.*, 2003; İnceoğlu *et al.*, 2010; Pini *et al.*, 2011; Moulin *et al.*, 2015); the total fungi contains both harmful (*Fusarium*, *Nectria* and *Cladosporium)* or beneficial (*Trichoderma*) groups (Omirou *et al.*, 2011); AMF is a beneficial group (Brundrett *et al.*, 1996); Gammaproteobacteria has the pathogen antagonistic groups (*Pseudomonas* and *Stenotrophomonas*) (Köberl *et al.*, 2017). Although planting and incorporation of cover crops in this study had some negative effects on the five microbial communities, the present study did not sequence the dominant PCR-DGGE bands to determine the particular changes in these community structures. It is therefore unknown if these changes are likely to affect more beneficial than harmful (pathogen) microbial communities. Thus, further research using sequencing of dominant DGGE bands (Omirou *et al.*, 2011; Wang *et al.*, 2014), or using Illumina Miseq sequencing (Yim *et al.*, 2017; Siebers *et al.*, 2018) is required to define changes in populations of particular microbial taxa resulting from field application of cover crop treatments.

In summary, this study has shown that the biofumigant plants, 'Caliente' mustard, brown mustard and 'Nemat', have considerable potential as biofumigants for reduction of *R. solani* inoculum in infested soils. Soil incorporation with these plants stimulated soil microbial activity, and subsequently decreased infection of potato plants by *R. solani*. In addition, selected biofumigants suppressed other soilborne potato pathogens, including *Sp. subterranea* and *C. coccodes*. These results give strong support for biofumigant plants and crops to be applied in field conditions as a management strategy to control economically important diseases of potato. Detailed field research, using appropriately controlled experimental approaches, and detailed assessment of diseases, are required to confirm if biofumigation adds value to potato crop management. These studies should also include assessments of the potential broad effects of biofumigation on "soil health", including the physical, chemical and biological components of soil environments that are important in productive field cropping agriculture.

Conference papers

Thanh, L. P., Falloon, R. E., Ridgway, H. J. & Jones, E. E. (2016). Biofumigation potential of *Brassica* crops for control of soilborne disease of potato caused by *Rhizoctonia solani.* Oral presentation at the 9th Australasian Soilborne Diseases Symposium, Lincoln University, New Zealand.

Thanh, L. P., Falloon, R. E., Ridgway, H. J. & Jones, E. E. (2017). Biofumigation potential of *Brassica* crops against *Rhizoctonia solani* disease of potato. Oral presentation at the Science Protecting Plant Health conference, Brisbane, Australia.

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Appendices

Appendices for Chapter 2

A.2.1 Nucleotide sequences amplified using primers ITS4 and ITS5 for ten selected *Rhizoctonia solani* isolates

LUPP2515

TGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGT
GAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAGTCATTGAACCCTTCTGTCTACTCAACTCATAT
AAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
GCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTT
TGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTC
CACTCAGCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAATAG
TCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA

LUPP2516

AGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGTGAGAC
AGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAGTCATTGAACCCTTCTGTCTACTCAACTCATATAAAAT
CAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCT
CCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTTTGATTT
TGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTCCACTCA
GCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAATAGTCCATT
GACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA

LUPP2517

AGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGTGAGAC
AGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAATCATTGAACCCTTCTGTCTACTCAACTCATATAAAAT
CAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCT
CCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTTTGATTT
TGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTCCACTCA
GCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAATAGTCCATT
GACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATA

LUPP2518

TGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGT
GAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAGTCATTGAACCCTTCTGTCTACTCAACTCATAT
AAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCA
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GCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTT
TGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTC
CACTCAGCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAATAG
TCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATAT

LUPP2519

TGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGT
GAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATAATAATAAATCATTGAACCCTTCTGTCTACTCAACTCATAT
AAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCA
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GCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTT
TGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTC
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TCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA

LUPP2520

TGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAACTTGT
GAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAGTCATTGAACCCTTCTGTCTACTCAACTCATAT
AAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTCGCA
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GCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTT
TGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGGTTC
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TCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA

LUPP2521

TTAATGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAAC
TTGTGAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATAATAATAAGTCATTGAACCCTTCTGTCTACTCAACTC
ATATAAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTC
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
CTTGCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTA
GTTTGATTTTGGTATTGGAGGTCTATTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGG
TTCCACTCAGCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAAGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAA
TAGTCCATTGACTTGGACACTATTATTATGATCTGATCTCAAATCAGGTAGGACTACCCGCTGAACTTAA

LUPP2522

GAGATCAGATCATAAAGGTATTGTCCAAGTCAATGGACTATTAGAAGCGGTTCATCTGCATTTACCTTGGCCACCTTTTACA
GTGTCCTCAGCGATAGATAATTTATCACGCCGAGTGGAACCAAGCATAACACTGAGATCCAGCTAATGCACAAAGAGGAG
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ATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAACTTAGTATGAGAT
GTGTTACATCC

LUPP2523

TTTAATGAAGAGTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAAC
TTGTGAGACAGTTGGGGAATTTATTTGTTATTTTTTGTAATAAAATGATAATAAATCATTGAACCCTTCTGTCTACTCAACTC
ATATAAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTC
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
CTTGCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCA

LUPP2524

TTAATGAAGAGTTTGGTTGTAGCTGGTCTATTTATTTAGGCATGTGCACACCTCCCTCTTTCATCCCACACACACCTGTGAAC
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ATATAAAATCAATTTATTTTAAATGAATGTAATGGATGTAACACATCTCATACTAAGTTTCAACAACGGATCTCTTGGCTCTC
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
CTTGCGCTCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTA
GTTTGATTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTCAGTGTTATGCTTGG
TTCCACTCAGCGTGATAAATTATCTATCGCTGAGGACACTG

A.2.2 Anastomosis Group (AG) identification of *Rhizoctonia solani* isolates, achieved by comparing their nucleotide sequences amplified using primers ITS4 and ITS5 with nucleotide sequences on NCBI databases.

Isolate	Sequence identity (%)	AG	GenBank accession number
LUPP2515	100	AG3-PT	MF070654.1
LUPP2516	100	AG3-PT	MF070654.1
LUPP2517	100	AG3-PT	MF070650.1
LUPP2518	100	AG3-PT	MF070654.1
LUPP2519	99	AG3-PT	MF070650.1
LUPP2520	100	AG3-PT	MF070647.1
LUPP2521	100	AG3-PT	KR006013.1
LUPP2522	100	AG2-1	KX118376.1
LUPP2523	99	AG3-PT	MF070650.1
LUPP2524	100	AG3-PT	MF070654.1

Analytical methods

B.2.1 Soil texture determination

Soil texture was determined by the method described by Miller and Miller (1987). Briefly, 50 mL tubes (3 replicates) containing 4 g of air-dried soil were added to 40 mL of a dispersing agent (10 mL of 1 N NaOH + 10 mL of 5 % sodium hexametaphosphate per litre). The tubes were put on an end-over-end shaker at 50 rpm for 12 hours. The tubes were removed from the lids and incubated at 30˚C for 1 hour 56 minutes 3 seconds. Then, 2.5 mL of each sample was pipetted at 2.5 cm depth from the surface in 5 seconds and put into a crucible for determining clay content. The rest of the sample was filtered through a No. 270 sieve (53 µm mesh), and the material remaining on the sieve was put into a crucible for sand determination. The samples for clay and sand determinations were oven-dried (Clayson Laboratory Apparatus Ltd, Upper Hutt, New Zealand) at 105℃ for 24 hours. The silt content was calculated based on the clay and sand contents. Upon the content of sand, silt, and clay in the soil sample, the soil texture was determined using the soil texture calculator from the United States Department of Agriculture (*https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167*).

B.2.2 Soil water holding capacity determination

The soil water holding capacity (WHC) of the air-dried soil was determined using the method of Rex *et al.* (2015). Briefly, WHC of the air-dried soil was determined by saturating 50 g air-dried soil (3 replicates) placed in polypropylene cylinders with the bottom closed with cheesecloth and filter paper for 24 hours, then the soil cylinders were left to drain for 24 hours. The weights of the drained saturated soil were recorded. Three replicates of air-dried soil samples were also oven-dried at 105˚C for 24 hours to determine the remaining water content in the air-dried soil. The WHC of soil was determined as follows:

WHC (%) = [(Wet soil – Dry soil)/Dry soil] × 100

Where: - WHC (%): Water holding capacity (%)

- Wet soil: Drained saturated soil (g)

- Dry soil: Oven-dried soil (g) at 105˚C

B.2.3 Soil organic matter determination

Soil organic matter was determined by the method of Rayment and Lyons (2011). Briefly, crucibles containing 10 g of air-dried soil were put into an oven at 105˚C for 24 hours. Then, the crucibles were put into a muffle furnace (CWF 110, Carbolite Gero Ltd, Hope Valley, UK) at 550˚C for 4 hours. The samples were then cooled in a desiccator. The organic matter was determined by the difference in weights between soil weight at 550˚C and initial air-dried soil weight at 105˚C.

B.2.4 Soil pH measurement

The soil pH was determined using the method of Rayment and Lyons (2011). Briefly, 7 g of air-dried soil was mixed with 35 mL reverse osmosis water (1:5 w:v) in a 50-mL plastic tube. The mixture was mixed vigorously in an end-over shaker for 1 hour, then left to stand overnight. The pH of the soil suspension was measured using a glass electrode (S20 SevenEasy™, Mettler Toledo, Columbus, Ohio, USA).

Statistical analyses

C.2.1 Analysis of variance of the effects of different concentrations of allyl isothiocyanate on mycelium growth of *Rhizoctonia solani* LUPP2522 (AG2-1) from agar plugs after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.001	0.0005	0.52	
Concentration	9	3.181	0.3534	380.48	<.001
Residual	18	0.017	0.0009		
Total	29	3.198			

C.2.2 Analysis of variance of correlation between allyl isothiocyanate concentrations and mycelium growth of *Rhizoctonia solani* LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	36077.70	36077.67	1461.63	<.001
Residual	28	691.10	24.68		
Total	29	36768.80	1267.89		

Percentage variance accounted for 98.1 (**R^2**)

C.2.3 Estimates of parameters of regression between allyl isothiocyanate concentrations and mycelium growth of *Rhizoctonia solani* LUPP2522 (AG2-1) after 3 days incubation.

Parameter	estimate	s.e.	t(28)	t pr.
Constant (**Intercept**)	0.58	1.30	0.44	0.661
Concentration (**Slope**)	10.49	0.27	38.23	<.001

C.2.4 Analysis of variance of the effects of different concentrations of 2-phenylethyl isothiocyanate on mycelium growth of *Rhizoctonia solani* LUPP2522 (AG2-1) after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.001	0.001	0.88	
Concentration	9	0.767	0.085	115.22	<.001
Residual	18	0.013	0.001		
Total	29	0.781			

C.2.5 Analysis of variance of the effects of volatiles from macerated tissue of ten *Brassica* plants on mycelium growth from agar plugs of ten *Rhizoctonia solani* isolates after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	478.78	159.59	4.31	
Treatment	11	202648.48	18422.59	497.43	<.001
Isolate	9	26972.99	2997.00	80.92	<.001
Treatment.Isolate	99	32492.20	328.20	8.86	<.001
Residual	357	13221.74	37.04		
Total	479	275814.19			

C.2.6 Analysis of variance of the effects of volatiles from macerated tissues from ten *Brassica* plants on sclerotium germination of ten *Rhizoctonia solani* isolates after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.004	0.001	1.58	
Treatment	11	0.035	0.003	3.88	<.001
Isolate	9	0.015	0.002	1.96	0.043
Treatment.Isolate	99	0.161	0.002	1.96	<.001
Residual	357	0.296	0.001		
Total	479	0.512			

C.2.7 Analysis of variance of the effects of volatiles from macerated tissue of ten *Brassica* plants on subsequent mycelium growth from sclerotia of ten *Rhizoctonia solani* isolates after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	19.41	6.47	1.13	
Treatment	10	185587.02	18558.70	3228.76	<.001
Isolate	9	4709.96	523.33	91.05	<.001
Treatment.Isolate	90	22677.51	251.97	43.84	<.001
Residual	327	1879.58	5.75		
Total	439	214873.47			

C.2.8 Analysis of variance of effect of volatiles from macerated tissue of ten *Brassica* plants to percentage of colonised barley grains of ten *Rhizoctonia solani* isolates having mycelium growth after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.007	0.002	1.43	
Treatment	11	0.142	0.013	7.74	<.001
Isolate	9	0.023	0.003	1.53	0.136
Treatment.Isolate	99	0.252	0.003	1.53	0.003
Residual	357	0.593	0.002		
Total	479	1.017			

C.2.9 Analysis of variance of effect of volatiles from macerated tissue of ten *Brassica* plants to subsequent mycelium growth of ten *Rhizoctonia solani* isolates from colonised barley grains after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	21.361	7.120	2.12	
Treatment	10	194381.929	19438.193	5796.44	<.001
Isolate	9	27614.579	3068.287	914.96	<.001
Treatment.Isolate	90	46422.552	515.806	153.81	<.001
Residual	327	1096.586	3.353		
Total	439	269537.008			

C.2.10 Analysis of variance of the effects of volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	10.222	3.407	2.93	0.038
Treatment	6	25819.177	4303.196	3695.82	<.001
Amount	2	61829.774	30914.887	26551.37	<.001
Isolate	1	8821.505	8821.505	7576.38	<.001
Treatment.Amount	8	2747.441	343.43	294.96	<.001
Treatment.Isolate	6	7798.659	1299.777	1116.32	<.001
Amount.Isolate	2	74.484	37.242	31.99	<.001
Treatment.Amount.Isolate	8	989.626	123.703	106.24	<.001
Residual	99	115.27	1.164		
Total	135	108206.158	801.527		

C.2.11 Analysis of variance of the effects of volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on sclerotium germination of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation. Data were sqrt(X+1) before analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.138	0.046	4.95	0.003
Treatment	7	0.124	0.018	1.90	0.076
Amount	2	0.080	0.040	4.30	0.016
Isolate	1	0.019	0.019	2.05	0.155
Treatment.Amount	8	0.063	0.008	0.85	0.565
Treatment.Isolate	7	0.110	0.016	1.68	0.121
Amount.Isolate	2	0.011	0.006	0.61	0.543
Treatment.Amount.Isolate	8	0.074	0.009	1.00	0.442
Residual	105	0.977	0.009		
Total	143	1.597	0.011		

C.2.12 Analysis of variance of the effects of volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	3.444	1.148	0.25	0.861
Treatment	6	35937.634	5989.606	1303.06	<.001
Amount	2	25594.64	12797.32	2784.11	<.001
Isolate	1	332.924	332.924	72.43	<.001
Treatment.Amount	8	2271.929	283.991	61.78	<.001
Treatment.Isolate	6	11027.149	1837.858	399.83	<.001
Amount.Isolate	2	97.812	48.906	10.64	<.001
Treatment.Amount.Isolate	8	765.198	95.65	20.81	<.001
Residual	99	455.059	4.597		
Total	135	76485.789	566.561		

C.2.13 Analysis of variance of the effects of volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on percentage of colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) having mycelium growth after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.029	0.010	2.50	0.064
Treatment	7	0.024	0.003	0.89	0.515
Amount	2	0.046	0.023	6.00	0.003
Isolate	1	0.000	0.000	0.00	1.000
Treatment.Amount	8	0.040	0.005	1.31	0.245
Biofumigant.Isolate	7	0.043	0.006	1.61	0.141
Amount.Isolate	2	0.000	0.000	0.00	1.000
Treatment.Amount.Isolate	8	0.086	0.011	2.81	0.007
Residual	105	0.400	0.004		
Total	143	0.667	0.005		

C.2.14 Analysis of variance of the effects of volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from barley grains of two *Rhizosctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	16.43	5.48	1.79	0.153
Treatment	6	15206.46	2534.41	830.01	<.001
Amount	2	75432.13	37716.06	12351.81	<.001
Ioslate	1	5921.88	5921.88	1939.38	<.001
Treatment.Amount	8	7993.32	999.16	327.22	<.001
Biofumigant.Isolate	6	11697.11	1949.52	638.46	<.001
Amount.Isolate	2	159.16	79.58	26.06	<.001
Treatment.Amount.Isolate	8	3281.60	410.20	134.34	<.001
Residual	99	302.30	3.05		
Total	135	120010.38	888.97		

C.2.15 Analysis of variance of the effects of volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	37.236	12.412	4.58	0.005
Treatment	6	18474.954	3079.159	1137.35	<.001
Tissue	2	3164.601	1582.3	584.46	<.001
Isolate	1	16329.837	16329.837	6031.78	<.001
Treatment.Tissue	8	9304.666	1163.083	429.61	<.001
Treatment.Isolate	6	8252.741	1375.457	508.05	<.001
Tissue.Isolate	2	2630.835	1315.418	485.88	<.001
Treatment.Tissue.Isolate	8	896.972	112.121	41.41	<.001
Residual	99	268.023	2.707		
Total	135	59359.864	439.703		

C.2.16 Analysis of variance of the effects of volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on sclerotium germination of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation. Data were sqrt(X+1) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.043	0.014	2.33	0.078
Treatment	7	0.186	0.027	4.33	<.001
Tissue	2	0.009	0.004	0.70	0.499
Isolate	1	0.019	0.019	3.11	0.081
Treatment.Tissue	8	0.020	0.003	0.41	0.914
Treatment.Isolate	7	0.038	0.005	0.89	0.518
Tissue.Isolate	2	0.009	0.004	0.70	0.499
Treatment.Tissue.Isolate	8	0.020	0.003	0.41	0.914
Residual	105	0.643	0.006		
Total	143	0.987	0.007		

C.2.17 Analysis of variance of the effects of volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	14.383	4.794	1.89	0.136
Treatment	6	21196.587	3532.764	1394.04	<.001
Tissue	2	570.908	285.454	112.64	<.001
Isolate	1	2883.379	2883.379	1137.79	<.001
Treatment.Tissue	8	12431.409	1553.926	613.18	<.001
Treatment.Isolate	6	2513.306	418.884	165.29	<.001
Tissue.Isolate	2	1279.695	639.847	252.49	<.001
Treatment.Tissue.Isolate	8	1673.87	209.234	82.56	<.001
Residual	99	250.885	2.534		
Total	135	42814.421	317.144		

C.2.18 Analysis of variance of the effects of volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on percentage of colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) having mycelium growth after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.004	0.001	1.00	0.396
Treatment	7	0.020	0.003	2.43	0.024
Tissue	2	0.000	0.000	0.00	1.000
Isolate	1	0.001	0.001	1.00	0.320
Treatment.Tissue	8	0.000	0.000	0.00	1.000
Treatment.Isolate	7	0.020	0.003	2.43	0.024
Tissue.Isolate	2	0.000	0.000	0.00	1.000
Treatment.Tissue.Isolate	8	0.000	0.000	0.00	1.000
Residual	105	0.125	0.001		
Total	143	0.170	0.001		

C.2.19 Analysis of variance of the effects of volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	5.292	1.764	0.5	0.683
Treatment	6	9215.908	1535.985	435.83	<.001
Tissue	2	3806.294	1903.147	540.01	<.001
Isolate	1	8736.612	8736.612	2478.96	<.001
Treatment.Tissue	8	11265.623	1408.203	399.57	<.001
Treatment.Isolate	6	4726.141	787.69	223.5	<.001
Tissue.Isolate	2	3302.072	1651.036	468.47	<.001
Treatment.Tissue.Isolate	8	2633.857	329.232	93.42	<.001
Residual	99	348.906	3.524		
Total	135	44040.704	326.227		

C.2.20 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	6.061	2.020	1.28	0.288
Treatment	4	20618.827	5154.707	3271.56	<.001
Flowering	2	483.766	241.883	153.52	<.001
Isolate	1	14706.656	14706.656	9333.93	<.001
Treatment.Flowering	4	2377.987	594.497	377.31	<.001
Treatment.Isolate	4	17179.325	4294.831	2725.82	<.001
Flowering.Isolate	2	36.700	18.350	11.65	<.001
Treatment.Flowering.Isolate	4	695.011	173.753	110.28	<.001
Residual	63	99.264	1.576		
Total	87	56203.598	646.018		

C.2.21 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on sclerotium germination of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.036	0.012	1.46	0.234
Treatment	5	0.064	0.013	1.57	0.179
Flowering	2	0.005	0.002	0.29	0.748
Isolate	1	0.007	0.007	0.87	0.353
Treatment.Flowering	4	0.081	0.020	2.47	0.052
Treatment.Isolate	5	0.122	0.024	2.97	0.017
Flowering.Isolate	2	0.043	0.021	2.62	0.080
Treatment.Flowering.Isolate	4	0.043	0.011	1.31	0.275
Residual	69	0.565	0.008		
Total	95	0.965	0.010		

C.2.22 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	17.24	5.747	1.2	0.316
Treatment	4	7843.42	1960.855	410.07	<.001
Flowering	2	27.946	13.973	2.92	0.061
Isolate	1	2954.838	2954.838	617.94	<.001
Treatment.Flowering	4	457.205	114.301	23.9	<.001
Treatment.Isolate	4	7963.834	1990.959	416.36	<.001
Flowering.Isolate	2	120.42	60.21	12.59	<.001
Treatment.Flowering.Isolate	4	77.789	19.447	4.07	0.005
Residual	63	301.252	4.782		
Total	87	19763.943	227.172		

C.2.23 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on percentage of colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) having mycelia after 3 days incubation. Data were sqrt(X/100) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.005	0.002	1	0.398
Treatment	5	0.005	0.001	0.60	0.700
Flowering	2	0.005	0.002	1.33	0.270
Isolate	1	0.002	0.002	1.00	0.321
Treatment.Flowering	4	0.010	0.002	1.33	0.266
Treatment.Isolate	5	0.005	0.001	0.60	0.700
Flowering.Isolate	2	0.005	0.002	1.33	0.270
Treatment.Flowering.Isolate	4	0.010	0.002	1.33	0.266
Residual	69	0.123	0.002		
Total	95	0.170	0.002		

C.2.24 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression on subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	1.455	0.485	0.18	0.908
Treatment	4	8427.665	2106.916	791	<.001
Flowering	2	490.648	245.324	92.1	<.001
Isolate	1	9889.029	9889.029	3712.64	<.001
Treatment.Flowering	4	1472.489	368.122	138.2	<.001
Treatment.Isolate	4	8394.222	2098.555	787.86	<.001
Flowering.Isolate	2	214.645	107.323	40.29	<.001
Treatment.Flowering.Isolate	4	851.726	212.931	79.94	<.001
Residual	63	167.807	2.664		
Total	87	29909.687	343.790		

C.2.25 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	23.299	7.766	2.62	
Biofumigant	2	4752.539	2376.269	801.88	<.001
Amount	2	17349.715	8674.858	2927.34	<.001
Isolate	1	3229.211	3229.211	1089.7	<.001
Biofumigant.Amount	4	210.456	52.614	17.75	<.001
Biofumigant.Isolate	2	719.284	359.642	121.36	<.001
Amount.Isolate	2	519.538	259.769	87.66	<.001
Biofumigant.Amount.Isolate	4	974.573	243.643	82.22	<.001
Residual	51	151.133	2.963		
Total	71	27929.749			

C.2.26 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	8.946	2.982	1.24	
Biofumigant	2	1358.101	679.051	282.89	<.001
Amount	2	4824.558	2412.279	1004.94	<.001
Isolate	1	29.609	29.609	12.33	<.001
Biofumigant.Amount	4	553.973	138.493	57.7	<.001
Biofumigant.Isolate	2	323.347	161.674	67.35	<.001
Amount.Isolate	2	125.102	62.551	26.06	<.001
Biofumigant.Amount.Isolate	4	944.806	236.202	98.4	<.001
Residual	51	122.421	2.4		
Total	71	8290.864			

C.2.27 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on subsequent mycelium growth from colonised barley grains two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	8.717	2.906	0.82	
Biofumigant	2	2738.012	1369.006	384.33	<.001
Amount	2	18506.218	9253.109	2597.66	<.001
Isolate	1	413.891	413.891	116.19	<.001
Biofumigant.Amount	4	2863.168	715.792	200.95	<.001
Biofumigant.Isolate	2	280.526	140.263	39.38	<.001
Amount.Isolate	2	215.957	107.978	30.31	<.001
Biofumigant.Amount.Isolate	4	327.83	81.957	23.01	<.001
Residual	51	181.667	3.562		
Total	71	25535.985			

C.2.28 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on sclerotium conversion of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) from retrieved agar plugs in nylon bags after 28 days incubation. Data were asin(sqrt(X/100)) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.002	0.001	0.80	0.457
Biofumigant	3	1.328	0.443	421.55	<.001
Amount	2	1.064	0.532	506.46	<.001
Isolate	1	0.025	0.025	24.23	<.001
Biofumigant.Amount	4	0.105	0.026	25.07	<.001
Biofumigant.Isolate	3	0.081	0.027	25.74	<.001
Amount.Isolate	2	0.170	0.085	80.87	<.001
Biofumigant.Amount.Isolate	4	0.281	0.070	66.84	<.001
Residual	38	0.040	0.001		
Total	59	3.096	0.052		

C.2.29 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on mycelium growth of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) from retrieved agar plugs in nylon bags after 28 days incubation. Data were asin(sqrt(X/100)) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.020	0.010	0.99	0.381
Biofumigant	3	0.836	0.279	28.05	<.001
Amount	2	3.009	1.505	151.4	<.001
Isolate	1	0.228	0.228	22.9	<.001
Biofumigant.Amount	4	0.434	0.108	10.92	<.001
Biofumigant.Isolate	3	0.023	0.008	0.76	0.524
Amount.Isolate	2	0.029	0.015	1.47	0.243
Biofumigant.Amount.Isolate	4	0.264	0.066	6.65	<.001
Residual	38	0.378	0.010		
Total	59	5.221	0.088		

C.2.30 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on germination of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) from retrieved sclerotia in nylon bags after 28 days incubation. Data were asin(sqrt(X/100)) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.0321	0.0161	1.95	0.156
Biofumigant	3	1.8558	0.6186	75.28	<.001
Amount	2	2.5920	1.2960	157.71	<.001
Isolate	1	1.5267	1.5267	185.78	<.001
Biofumigant.Amount	4	1.4507	0.3627	44.13	<.001
Biofumigant.Isolate	3	0.2508	0.0836	10.17	<.001
Amount.Isolate	2	0.0385	0.0192	2.34	0.11
Biofumigant.Amount.Isolate	4	0.7120	0.1780	21.66	<.001
Residual	38	0.3123	0.0082		
Total	59	8.7709	0.1487		

C.2.31 Analysis of variance of the effects of volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) on subsequent mycelium growth of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) from retrieved barley grains in nylon bags after 28 days incubation. Data were asin(sqrt(X/100)) transformed prior analysis.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.0145	0.0073	2.40	0.104
Biofumigant	3	0.6903	0.2301	76.20	<.001
Amount	2	2.9104	1.4552	481.93	<.001
Isolate	1	0.0101	0.0101	3.35	0.075
Biofumigant.Amount	4	0.0373	0.0093	3.09	0.027
Biofumigant.Isolate	3	0.0009	0.0003	0.10	0.958
Amount.Isolate	2	0.0057	0.0029	0.94	0.398
Biofumigant.Amount.Isolate	4	0.0127	0.0032	1.05	0.393
Residual	38	0.1147	0.0030		
Total	59	3.7967	0.0644		

C.2.32 Mean sclerotium germination (%) of ten *Rhizoctonia solani* isolates after 3 days exposure to volatile compounds from macerated tissues of ten biofumigant crops and allyl ITC at concentration to provide 100% (AITC100) suppression.

Treatment	Sclerotium germination percentage of sclerotia (%)[1]										Mean[2]
	LUPP2515	LUPP2516	LUPP2517	LUPP2518	LUPP2519	LUPP2520	LUPP2521	LUPP2522	LUPP2523	LUPP2524	
Control	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B [4]
AITC100	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	75 b	50 a	92.5 A
'Caliente' mustard	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
Brown mustard	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
'Nemat' arugula	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
'Corka' kale	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
'Pasja II' Leafy turnip	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
'Lunch' radish	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
Fodder radish	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
Rapeseed	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
White mustard	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
Forage rape	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 c	100 B
Mean [3]	100 X	100 X	100 X	100 X	100 X	100 X	100 X	100 X	97.7 XY	95.2 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

[1] Comparisons across interactions between biofumigant crops and *Rhizoctonia solani* isolates (a-c) were significant (P<0.001).
[2] Comparisons of overall effects of biofumigant crops (A-B) were significant (P<0.001).
[3] Comparisons of overall effects of *Rhizoctonia solani* isolates (X-Y) were significant (P=0.043).

C.2.33 Mean colonised barley grains having mycelium growth (%) of ten *Rhizoctonia solani* isolates after 3 days exposure to volatile compounds from macerated tissues of ten biofumigant crops and allyl ITC at concentration to provide 100% (Allyl ITC100) suppression.

Treatment	Proportion of colonised barley grains having mycelium growth (%)[1]										Mean[2]
	LUPP2515	LUPP2516	LUPP2517	LUPP2518	LUPP2519	LUPP2520	LUPP2521	LUPP2522	LUPP2523	LUPP2524	
Control	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
AITC100	45.7 b	71.9 ab	100 a	100 a	100 a	100 a	45.7 b	100 a	71.9 ab	100 a	82.8 B
Brown mustard	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
'Caliente' mustard	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
Fodder radish	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
Forage rape	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
'Corka' kale	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
'Pasja II' leafy turnip	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
Lunch radish	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
'Nemat' arugula	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
Rapeseed	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
White mustard	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 A
Mean [3]	95.2	97.7	100	100	100	100	95.2	100	97.7	100	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

[1] Comparisons across interactions between treatment and *Rhizoctonia solani* isolates (a-b) were significant (P=0.003).

[2] Comparisons of overall effects of treatment (A-B) were significant (P<0.001).

[3] Comparisons of overall effects of *Rhizoctonia solani* isolate were not significant (P=0.136).

C.2.34 Mean inhibition (%) in relation to the unamended control of mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean mycelium growth inhibition from agar plugs (%)[1]																		Mean across biofumigant[2]					Mean across amount[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip															
	AITC50	AITC100	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	AITC50	AITC100	1 g	5 g	10 g	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	68.4 h	100 a	66.9 h	100 a	100 a	60.2 j	100 a	100 a	63.7 i	100 a	100 a	16.7 o	80.3 e	86.5 d	21.7 n	80.4 e	88.4 c	68.4 F	100 A	45.8 H	92.1 C	95.0 B	68.4 T	100 N	89.0 O	86.7 Q	87.9 P	61.1 V	63.5 U	77.7 x
LUPP2522	52.3 k	100 a	51.5 k	100 a	100 a	40.7 m	87.7 cd	93.6 b	17.2 o	43.9 l	59.3 j	14.1 p	63.4 i	70.2 g	18.0 o	71.2 g	76.3 f	52.3 G	100 A	28.3 I	73.2 E	79.9 D	52.3 X	100 N	83.8 R	74.0 S	40.1 Z	49.2 Y	55.2 W	60.6 y
Mean across isolate[5]	60.3 I'	100 A'	59.2 J'	100 A'	100 A'	50.5 K'	93.9 C'	96.8 B'	40.5 L'	71.9 H'	79.6 E'	15.4 N'	71.8 H'	78.3 F'	19.9 M'	75.8 G'	82.4 D'														
Mean[6]																		60.3 Y'	100 V'	37.1 Z'	82.7 X'	87.4 W'								
Mean[7]	60.3 Q'	100 M'	86.4 N'			80.4 O'			64.0 P'			55.2 S'			59.3 R'																

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, amount and isolate (a-o) were significant (P<0.001).

[2]Interaction means between amount and isolate (data averaged across biofumigant) (A-I) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across amount) (N-Z) were significant (P<0.001).

[4]Means of overall isolate effect (x-y) were significant (P<0.001).

[5]Interaction means between treatment and amount (data averaged across isolate) (A'-N') were significant (P<0.001).

[6]Means of overall amount effect (W'-Z') were significant (P<0.001).

[7]Means of overall treatment effect (M'-S') were significant (P<0.001).

C.2.35 Mean sclerotium germination (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Sclerotia germination (%)[1]																		Mean across biofumigant[2]						Mean across amount[3]								Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip																	
		AITC50	AITC100	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	Control	AITC50	AITC100	1 g	5 g	10 g	Control	AITC50	AITC100	Caliente	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	100	100	45.7	100	100	71.8	100	71.8	71.8	100	100	45.7	100	100	100	100	100	100	100	100	45.7	100	94.3	77.2	100	100	45.7	90.4	80.9	80.9	100	100	90.2
LUPP2522	100	100	100	100	71.8	71.8	100	100	71.8	100	100	100	100	100	100	100	100	100	100	100	100	100	94.3	88.5	100	100	100	80.9	90.4	100	100	100	94.3
Mean across isolate[5]	100	100	71.8	100	85.6	71.8	100	85.6	71.8	100	100	71.8	100	100	100	100	100	100																
Mean[6]																			100 a	100 a	71.9 d	100 ab	94.3 abc	82.8 abcd									
Mean[7]	100	100	71.9		85.5			85.5			90.4			100			100																

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (*P*=0.05).
[1]Interaction means between treatment, amount and isolate were not significant (*P*=0.442)
[2]Interaction means between amount and isolate were not significant (*P*=0.543)
[3]Interaction means between treatment and isolate were not significant (*P*=0.121)
[4]Means of overall isolate effect were not significant (*P*=0.155)
[5]Interaction mean of treatment and amount were not significant (*P*=0.565)
[6]Mean of overall amount effect (a-d) were significant (*P*=0.016)
[7]Mean total effect of treatment were not significant (*P*=0.076)

C.2.36 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean inhibition of subsequent mycelium growth from sclerotia (%)[1]																Mean across biofumigant[2]					Mean across amount[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			Kale			'Pasja II' Leafy turnip														
	AITC50	AITC100	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	AITC50	AITC100	1 g	5 g	10 g	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	86.6 c	94.7 a	64.8 hi	97.2 a	96.0 a	56.1 lm	96.4 a	96.9 a	86.0 c	96.3 a	96.1 a	17.6 s	44.7 o	49.8 n	21.1 r	72.9 f	73.7 f	86.6 C	94.7 A	49.1 F	81.5 D	82.5 E	86.6 S	94.7 Q	86.0 S	83.2 T	92.8 QR	37.3 Z	55.9 W	71.2 x
LUPP2522	77.9 e	91.4 b	68.8 g	95.0 a	96.9 a	66.7 gh	94.3 ab	96.5 a	35.2 q	62.4 ij	60.9 jk	32.8 q	54.9 m	58.2 kl	41.1 p	81.8 d	79.0 de	77.9 E	91.4 B	48.9 F	77.7 E	78.3 E	77.9 U	91.4 R	86.9 S	85.9 S	52.8 X	48.7 Y	67.3 V	68.4 y
Mean across isolate[5]	82.2 C'	93.0 B'	66.8 F'	96.1 A'	96.4 A'	61.4 G	95.4 A'	96.7 A'	60.6 G'	79.4 D'	78.5 D'E'	25.2 K'	49.8 I'	54.0 H'	31.1 J'	77.3 D'E'	76.4 E'													
Mean[6]																		82.2 X'	93.0 W'	49.0 Z'	79.6 Y'	80.4 Y'								
Mean[7]	82.2 P'	93.0 M'	86.5 N'			84.5 O'			72.8 Q'			43.0 S'			61.6 R'															

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, amount and isolate (a-r) were significant (P<0.001).

[2]Interaction means between amount and isolate (data averaged across biofumigant) (A-E) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across amount) (Q-Z) were significant (P<0.001).

[4]Means of overall isolate effect (x-y) were significant (P<0.001).

[5]Interaction means between treatment and amount (data averaged across isolate) (A'-G') were significant (P<0.001).

[6]Means of overall amount effect (W'-Z') were significant (P<0.001).

[7]Means of overall treatment effect (M'-S') were significant (P<0.001).

C.2.37 Mean colonised barley grains having mycelium growth (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean mycelium growth inhibition (%)[1]																		Mean across biofumigant[2]						Mean across amount[3]								Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip																	
		AITC50	AITC100	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	Control	AITC50	AITC100	1 g	5 g	10 g	Control	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	45.7 c	100 a	100 a	100 a	100 a	100 a	100 a	100	100	100	100	100	88.5	100	100	100	100	100	80.9	100	100	96.3
LUPP2522	100 a	100 a	100 a	100 a	100 a	71.8 b	100 a	100 a	71.8 b	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100	100	100	100	100	88.5	100	100	100	90.4	90.4	100	100	100	96.3
Mean across isolate[5]	100	100	100	100	100	85.6	100	100	85.6	100	100	71.8	100	100	100	100	100	100																
Mean[6]																			100 a	100 a	100 a	100 a	100 a	88.5 b									
Mean[7]	100	100	100	95.2			95.2			90.4			100			100																	

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (*P*=0.05).

[1]Interaction means between treatment, amount and isolate were significant (*P*=0.007)

[2]Interaction means between amount and isolate were not significant (*P*=1.00)

[3]Interaction means between treatment and isolate were not significant (*P*=0.141)

[4]Means of overall isolate effect were not significant (*P*=1.00)

[5]Interaction mean of treatment and amount were not significant (*P*=0.245)

[6]Mean of overall amount effect were significant (*P*=0.003)

[7]Mean total effect of treatment were not significant (*P*=0.515)

C.2.38 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri plate, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean subsequent mycelium growth inhibition from colonised barley grains (%)[1]																		Mean across biofumigant[2]					Mean across amount[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip															
	AITC50	AITC100	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	AITC50	AITC100	1 g	5 g	10 g	AITC50	AITC100	Caliente mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	81.0 de	81.5 cde	3.4 o	79.5 e	83.7 abc	18.3 l	82.1 bcd	81.9 cde	83.4 abcd	85.8 a	83.4 abcd	6.9 n	63.7 i	71.5 g	11.7 m	52.5 j	68.5 h	81.0 A	81.5 A	24.7 G	72.7 C	77.8 B	81.0 R	81.5 R	55.5 U	60.8 T	84.2 Q	47.4 W	44.2 X	58.6 x
LUPP2522	52.3 j	76.1 f	9.6 m	76.3 f	83.7 abc	18.7 l	81.4 cde	84.5 ab	2.3 o	48.7 k	52.2 j	3.7 o	50.3 jk	49.5 k	9.6 m	50.6 jk	64.6 i	52.3 F	76.1 B	8.8 H	61.5 E	66.9 D	52.3 V	76.1 S	56.5 U	61.5 T	34.4 Z	34.5 Z	41.6 Y	45.9 y
Mean[5]	66.7 D'	78.8 C'	6.5 K'	77.9 C'	83.7 A'	18.5 I'	81.7 B'	83.2 A'B'	42.8 H'	67.2 D'	67.8 D'	5.3 K'	57.0 F'	60.5 E'	10.6 J'	51.6 G'	66.6 D'													
Mean[6]																		66.7 Y'	78.8 W'	16.7 Z'	67.1 Y'	72.4 X'								
Mean[7]	66.7 N'	78.8 M'	56.0 Q'			61.1 O'			59.3 P'			40.9 S'			42.9 R'															

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, amount and isolate (a-o) were significant (P<0.001).

[2]Interaction means between amount and isolate (data averaged across biofumigant) (A-H) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across amount) (Q-Z) were significant (P<0.001).

[4]Means of overall isolate effect (x-y) were significant (P<0.001).

[5]Interaction means between treatment and amount (data averaged across isolate) (A'-J') were significant (P<0.001).

[6]Means of overall amount effect (W'-Z') were significant (P<0.001).

[7]Means of overall treatment effect (M'-S') were significant (P<0.001).

C.2.39 Mean inhibition (%) in relation to the unamended control of mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean mycelium growth inhibition from agar plugs (%)[1]																		Mean across biofumigant[2]					Mean across tissue type[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			Kale			'Pasja II' Leafy turnip															
	AITC50	AITC100	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	AITC50	AITC100	R	S	R+S	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	69.2 h	100 a	89.9 cd	100 a	100 a	70.4 gh	100 a	91.9 c	100 a	100 a	100 a	94.6 b	60.7 j	71.5 g	86.5 e	78.5 f	89.4 d	69.2 E	100 A	88.3 C	87.8 C	90.6 B	69.2 V	100 P	96.6 Q	87.4 S	100 P	75.6 U	84.8 T	88.9 x
LUPP2522	53.2 l	100 a	69.0 h	100 a	100 a	56.1 k	91.1 cd	80.0 f	38.8 n	66.0 i	60.4 j	70.9 gh	48.9 m	59.7 j	21.9 o	52.5 l	61.6 j	53.2 F	100 A	51.3 G	71.7 D	72.3 D	53.2 Y	100 P	89.7 R	75.7 U	55.1 X	59.8 W	45.3 Z	65.2 y
Mean across isolate[5]	61.2 J'	100 A'	79.5 E'	100 A'	100 A'	63.3 I'	95.6 B'	85.9 C'	69.4 G'	83.0 D'	80.2 E'	82.7 D'	54.8 K'	65.6 H'	54.2 K'	65.5 H'	75.5 F'														
Mean[6]																		61.2 Z'	100 V'	69.8 Y'	79.8 X'	81.5 W'								
Mean[7]	61.2 S'	100 M'	93.2 N'			81.6 O'			77.5 P'			67.7 Q'			65.1 R'															

R: Roots; S: Shoots; R+S: Roots+Shoots

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, tissue type and isolate (a-o) were significant (P<0.001).

[2]Interaction means between tissue type and isolate (data averaged across biofumigant) (A-F) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across tissue type) (Q-Z) were significant (P<0.001).

[4]Means of overall isolate effect (x-y) were significant (P<0.001).

[5]Interaction means between treatment and tissue type (data averaged across isolate) (A'-K') were significant (P<0.001).

[6]Means of overall tissue type effect (W'-Z') were significant (P<0.001).

[7]Means of overall treatment effect (M'-S') were significant (P<0.001).

C.2.40 Mean sclerotium germination (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Sclerotium germination (%)[1]																		Mean across biofumigant[2]						Mean across tissue[3]							Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip			Control	AITC50	AITC100	R	S	R+S	Control	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
		AITC50	AITC100	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S															
LUPP2519	100	100	45.7	100	71.8	100	100	100	100	71.8	71.8	100	100	100	100	100	100	100	100	100	100	100	94.3	71.9	100	100	45.7	90.4	100	80.9	100	100	94.0
LUPP2522	100	100	71.8	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	88.5	45.7	100	100	71.9	100	100	100	100	100	99.9
Mean across isolate[5]	100	100	58.5	100	85.6	100	100	100	100	85.6	85.6	100	100	100	100	100	100	100															
Mean[6]																			100	100	58.5	97.1	94.3	100									
Mean[7]	100 a	100 a	58.5 b	95.2 a			100 a			90.4 a			100 a			100 a																

R: Roots; S: Shoots; R+S: Roots+Shoots

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, tissue and isolate were not significant (P=0.914)

[2]Interaction means between tissue and isolate were not significant (P=0.499)

[3]Interaction means between treatment and isolate were not significant (P=0.518)

[4]Means of overall isolate effect were not significant (P=0.081)

[5]Interaction mean of treatment and tissue were not significant (P=0.914)

[6]Mean of overall tissue effect were not significant (P=0.499)

[7]Mean total effect of treatment were significant (P<0.001)

C.2.41 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean inhibition of subsequent mycelium growth from sclerotia (%)[1]																		Mean across biofumigant[2]					Mean across tissue type[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip															
	AITC50	AITC100	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	AITC50	AITC100	R	S	R+S	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	89.2	94.0	82.1	97.4	96.0	80.7	92.4	94.9	97.4	96.4	97.1	81.9	42.2	51.9	86.8	75.9	90.6	89.2	94.0	85.8	80.9	86.1	89.2	94.0	91.8	89.3	97.0	58.7	84.4	84.3
	f	cd	h	a	abc	hi	de	bc	a	ab	ab	h	n	m	g	j	ef	B	A	C	D	C	S	Q	R	S	P	Y	U	x
LUPP2522	79.6	93.9	79.6	97.1	97.1	69.2	95.9	94.0	66.0	81.0	77.0	80.5	42.4	38.9	41.5	74.9	81.6	79.6	93.9	67.4	78.3	77.7	79.6	93.9	91.3	86.4	74.7	53.9	66.0	74.6
	i	cd	i	a	ab	k	abc	cd	l	hi	j	hi	n	o	n	j	hi	DE	A	G	EF	F	V	Q	R	T	W	Z	X	y
Mean across isolate[5]	84.4	93.9	80.9	97.3	96.5	75.0	94.2	94.5	81.7	88.7	87.0	81.2	42.3	45.4	64.1	75.4	86.1													
	E'	B'	F'	A'	A'	G'	B'	B'	F'	C'	D'	F'	J'	I'	H'	G'	D'													
Mean[6]																		84.4	93.9	76.6	79.6	81.9								
																		W'	V'	Z'	Y'	X'								
Mean[7]	84.4 Q'	93.9 M'	91.6 N'			87.9 O'			85.8 P'			56.3 S'			75.2 R'															

R: Roots; S: Shoots; R+S: Roots+Shoots

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, tissue type and isolate (a-o) were significant (P<0.001).

[2]Interaction means between tissue type and isolate (data averaged across biofumigant) (A-F) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across tissue type) (P-Z) were significant (P<0.001).

[4]Means of overall isolate effect were significant (P<0.001).

[5]Interaction means between treatment and tissue type (data averaged across isolate) were significant (P < 0.001).

[6]Means of overall tissue type effect were significant (P<0.001).

[7]Means of overall treatment effect were significant (P<0.001).

C.2.42 Mean proportion of colonised barley grains with mycelium growth (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots+shoots), and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean proportion of colonised barley grains with mycelium growth (%)[1]																		Mean across biofumigant[2]						Mean across tisue[3]								Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip																	
		AITC50	AITC100	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	Control	AITC50	AITC100	R	S	R+S	Control	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	100	100	71.8	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	71.9	100	100	100	100 a	100 a	71.9 b	100 a	100 a	100 a	100 a	100 a	100
LUPP2522	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100
Mean across isolate[5]	100	100	85.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100															
Mean[6]																			100	100	85.5	100	100	100									
Mean[7]	100 a	100 a	85.5 b	100 a			100 a			100 a			100 a			100 a																	

R: Roots; S: Shoots; R+S: Roots+Shoots

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, tissue and isolate were not significant (P=1.00)

[2]Interaction means between tissue and isolate were not significant (P=1.00)

[3]Interaction means between treatment and isolate were significant (P=0.024)

[4]Means of overall isolate effect were not significant (P=0.320)

[5]Interaction mean of treatment and tissue were not significant (P=1.00)

[6]Mean of overall tissue effect were not significant (P=1.00)

[7]Mean total effect of treatment were significant (P=0.024)

S

C.2.43 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots+shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean inhibition of subsequent mycelium growth from colonised barley grains (%)[1]																	Mean across biofumigant[2]					Mean across tissue type[3]							Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula			'Corka' kale			'Pasja II' Leafy turnip															
	AITC50	AITC100	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	R	S	R+S	AITC50	AITC100	R	S	R+S	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	'Corka' kale	'Pasja II' Leafy turnip	
LUPP2519	81.5	83.2	68.3	82.9	82.8	51.0	83.8	82.4	82.7	84.8	84.3	76.9	34.7	56.5	81.7	61.3	78.2	81.5	83.2	72.1	69.5	76.8	81.5	83.2	78.0	72.4	83.9	56.0	73.7	72.9
	b	ab	e	ab	ab	k	ab	ab	ab	a	a	c	m	h	b	g	c	A	A	C	D	B	S	RS	T	U	R	W	U	x
LUPP2522	53.7	68.0	53.4	83.9	72.6	52.3	83.2	81.5	28.2	65.5	56.1	57.8	37.2	42.2	21.9	56.9	70.0	53.7	68.0	42.7	65.3	64.5	53.7	68	70.0	72.4	49.9	45.7	49.6	57.6
	ij	ef	ijk	ab	d	jk	ab	b	n	f	hi	h	m	l	o	h	e	F	D	G	E	E	X	V	V	U	Y	Z	Y	y
Mean[5]	67.6	75.6	60.9	83.4	77.7	51.7	83.5	81.9	55.4	75.2	70.2	67.4	36.0	49.3	51.8	59.1	74.1													
	E'	C'	F'	A'	B'	H'	A'	A'	G'	C'	D'	E'	J'	I'	H'	F'	C'													
Mean[6]																		67.6	75.6	57.4	67.4	70.7								
																		Y'	W'	Z'	Y'	X'								
Mean[7]	67.6 P'	75.6 M'	74.0 N'			72.4 O'			66.9 P'			50.9 R'			61.7 Q'															

R: Roots; S: Shoots; R+S: Roots+Shoots

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, tissue type and isolate were significant (P<0.001).

[2]Interaction means between tissue type and isolate (data averaged across biofumigant) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across tissue type) were significant (P<0.001).

[4]Means of overall isolate effect were significant (P<0.001).

[5]Interaction means between treatment and tissue type (data averaged across isolate) were significant (P<0.001).

[6]Means of overall tissue type effect were significant (P<0.001).

[7]Means of overall treatment effect were significant (P < 0.001).

C.2.44 Mean inhibition (%) in relation to the unamended control of mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean mycelium growth inhibition from agar plugs (%)[1]											Mean across biofumigant[2]					Mean across flowering[3]					Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula													
	AITC50	AITC100	First	Mid	Full	First	Mid	Full	First	Mid	Full	AITC50	AITC100	First	Mid	Full	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	69.7 d	100 a	100 a	100 a	100 a	86.9 b	100 a	100 a	100 a	100 a	100 a	69.7 D	100 A	95.7 B	100 A	100 A	69.7 X	100 U	100 U	95.7 V	100 U	98.2 x
LUPP2522	53.6 e	100 a	100 a	100 a	100 a	55.3 e	83.9 c	87.9 b	38.1 f	32.5 g	20.9 h	53.6 F	100 A	64.5 E	72.2 C	69.6 D	53.6 Y	100 U	100 U	75.7 W	30.5 Z	68.9 y
Mean across isolate[5]	61.7 G'	100 A'	100 A'	100 A'	100 A'	71.1 D'	92.0 C'	93.9 B'	69.0 E'	66.3 F'	60.5 G'											
Mean[6]												61.7 Z'	100 V'	80.1 Y'	86.1 W'	84.8 X'						
Mean[7]	61.7 P'	100 M'		100 M'			85.7 N'			65.3 O'												

First: First flower emergence; Mid: Mid-anthesis; Full: Full anthesis

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, flowering time and isolate were significant (P<0.001).

[2]Interaction means between flowering time and isolate (data averaged across biofumigant) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across flowering time) were significant (P<0.001).

[4]Means of overall isolate effect were significant (P<0.001).

[5]Interaction means between treatment and flowering time (data averaged across isolate) were significant (P<0.001).

[6]Means of overall flowering time effect were significant (P<0.001).

[7]Means of overall treatment effect were significant (P<0.001).

C.2.45 Mean sclerotium germination (%) of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) affected after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean sclerotium germination (%)[1]												Mean across biofumigant[2]						Mean across flowering[3]						Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula															
		AITC50	AITC100	First	Mid	Full	First	Mid	Full	First	Mid	Full	Control	AITC50	AITC100	First	Mid	Full	Control	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	100	100	45.7	100	71.8	100	100	100	100	71.8	100	100	100	100	45.7	90.4	90.4	100	100 a	100 a	45.7 b	90.4 a	100 a	90.4 a	93.2
LUPP2522	100	100	100	100	100	100	100	100	45.7	100	100	100	100	100	100	100	100	80.9	100 a	100 a	100 a	100 a	80.9 a	100 a	93.8
Mean across isolate[5]	100 a'	100 a'	71.8 b'	100	85.6 a'b'	100 a'	100 a'	100 a'	71.8 b'	85.6 a'b'	100 a'	100 a'													
Mean[6]													100	100	71.9	95.2	95.2	90.4							
Mean[7]	100	100	71.9		95.2			90.4			95.2														

First: First flower emergence; Mid: Mid-anthesis; Full: Full anthesis

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, flowering and isolate were not significant (P=0.275)

[2]Interaction means between flowering and isolate were not significant (P=0.08)

[3]Interaction means between treatment and isolate were significant (P=0.017)

[4]Means of overall isolate effect were not significant (P=0.353)

[5]Interaction mean of treatment and flowering were significant (P=0.052)

[6]Mean of overall flowering effect were not significant (P=0.748)

[7]Mean total effect of biofumigant were not significant (P=0.179)

C.2.46 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean inhibition of subsequent mycelium growth from sclerotia (%)[1]												Mean across biofumigant[2]					Mean across flowering[3]					Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula														
	AITC50	AITC100	First	Mid	Full	First	Mid	Full	First	Mid	Full		AITC50	AITC100	First	Mid	Full	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	85.3 e	94.3 abc	94.6 abc	96.0 a	94.6 abc	84.8 e	90.0 d	96.3 a	94.6 abc	94.6 abc	91.6 cd		85.3 C	94.3 A	91.3 B	93.5 A	94.2 A	85.3 X	94.3 UV	95.1 U	90.4 W	93.6 UV	92.9 x
LUPP2522	77.3 f	92.5 bcd	95.2 ab	95.3 ab	95.3 ab	92.2 bcd	93.4 abc	94.3 abc	55.6 g	54.9 g	43.3 h		77.3 E	92.5 AB	81.0 D	81.2 D	77.6 E	77.3 Y	92.5 VW	95.3 U	93.3 V	51.3 Z	80.1 y
Mean across isolate[5]	81.3 E'	93.4 B'C'	94.9 A'B'	95.6 A'	95.0 A'B'	88.5 D'	91.7 C'	95.3 A'B'	75.1 F'	74.7 F'	67.4 G'												
Mean[6]													81.3	93.4	86.2	87.3	85.9						
Mean[7]	81.3 O'	93.4 M'N'		95.2 M'			91.8 N'			72.4 P'													

First: First flower emergence; Mid: Mid-anthesis; Full: Full anthesis

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, flowering time and isolate were significant (P=0.005).

[2]Interaction means between flowering time and isolate (data averaged across biofumigant) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across flowering time) were significant (P<0.001).

[4]Means of overall isolate effect were significant (P<0.001).

[5]Interaction means between treatment and flowering time (data averaged across isolate) were significant (P<0.001).

[6]Means of overall flowering time effect were not significant (P=0.061).

[7]Means of overall treatment effect were significant (P<0.001).

C.2.47 Mean colonised barley grains (%) having mycelium growth of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean proportion of colonised barley grains with mycelium growth (%)[1]												Mean across biofumigant[2]						Mean across flowering[3]						Mean[4]
	Control	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula															
		AITC50	AITC100	First	Mid	Full	First	Mid	Full	First	Mid	Full	Control	AITC50	AITC100	First	Mid	Full	Control	AITC50	AITC100	'Caliente mustard	Brown mustard	'Nemat' arugula	
LUPP2519	100	100	100	100	100	100	100	100	100	100	100	71.8	100	100	100	100	100	90.4	100	100	100	100	100	90.4	96.8
LUPP2522	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Mean across isolate[5]	100	100	100	100	100	100	100	100	100	100	100	85.6													
Mean[6]													100	100	100	100	100	100							
Mean[7]	100	100	100		100			100			95.2														

First: First flower emergence; Mid: Mid-anthesis; Full: Full anthesis

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (*P*=0.05).

[1]Interaction means between treatment, flowering and isolate were not significant (*P*=0.266)

[2]Interaction means between flowering and isolate were not significant (*P*=0.270)

[3]Interaction means between treatment and isolate were not significant (*P*=0.700)

[4]Means of overall isolate effect were not significant (*P*=0.321)

[5]Interaction mean of treatment and flowering were not significant (*P*=0.266)

[6]Mean of overall flowering effect were not significant (*P*=0.270)

[7]Mean total effect of treatment were not significant (*P*=0.700)

C.2.48 Mean inhibition (%) in relation to the unamended control of subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types of three biofumigant crops at first, mid and full flowering stages (5 g/Petri dish), and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression.

Isolate	Mean inhibition of subsequent mycelium growth from colonised barley grains (%)[1]											Mean across biofumigant[2]					Mean across flowering[3]					Mean[4]
	Positive control		'Caliente' mustard			Brown mustard			'Nemat' arugula													
	AITC50	AITC100	First	Mid	Full	First	Mid	Full	First	Mid	Full	AITC50	AITC100	First	Mid	Full	AITC50	AITC100	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	76.9 de	81.9 b	84.2 a	83.5 ab	84.5 a	77.9 cd	83.9 ab	83.1 ab	83.9 ab	83.9 ab	84.3 a	76.9 C	81.9 B	82.0 B	83.8 A	84.0 A	76.9 W	81.9 U	84.1 T	81.6 UV	84.0 T	83.2 x
LUPP2522	47.5 g	75.5 e	77.5 cde	79.3 c	84.1 ab	50.4 f	79.1 cd	82.7 ab	37.6 h	35.7 h	25.3 i	47.5 F	75.5 C	55.2 E	64.7 D	64.0 D	47.5 Y	75.5 W	80.3 U	70.7 X	32.9 Z	61.3 y
Mean across isolate[5]	62.2 F'	78.7 D'	80.9 C'	81.4 B'C'	84.3 A'	64.1 E'	81.5 B'C'	82.9 A'B'	60.8 F'G'	59.8 G'	54.8 H'											
Mean[6]												62.2 Z'	78.7 W'	68.6 Y'	74.2 X'	74.0 X'						
Mean[7]	62.2 P'	78.7 N'		82.2 M'			76.2 O'			58.5 Q'												

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between treatment, flowering time and isolate were significant (P<0.001).

[2]Interaction means between flowering time and isolate (data averaged across biofumigant) were significant (P<0.001).

[3]Interaction means between treatment and isolate (data averaged across flowering time) were significant (P<0.001).

[4]Means of overall isolate effect were significant (P<0.001).

[5]Interaction means between treatment and flowering time (data averaged across isolate) were significant (P<0.001).

[6]Means of overall flowering time effect were significant (P<0.001).

[7]Means of overall treatment effect were significant (P<0.001).

C.2.49 Mean inhibition (%) of mycelium growth from agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w).

Isolate	Mean mycelium growth inhibition from agar plugs (%)[1]									Mean across biofumigant[2]			Mean across amount[3]			Mean[4]
	'Caliente' mustard			Brown mustard			'Nemat' arugula									
	1%	5%	10%	1%	5%	10%	1%	5%	10%	1%	5%	10%	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	77.9 efg	100 a	100 a	54.8 i	77.2 fg	89.8 b	44.7 j	74.3 g	82.0 de	59.1 D	83.8 B	90.6 A	92.6 V	73.9 W	67.0 Y	77.9 x
LUPP2522	39.3 k	84.8 cd	87.3 bc	38.3 k	80.6 def	80.5 def	40.7 jk	66.4 h	62.3 h	39.4 E	77.3 C	76.7 C	70.5 X	66.4 Y	56.5 Z	64.5 y
Mean across isolate[5]	58.6 E'	92.4 A'	93.7 A'	46.5 F'	78.9 C'	85.2 B'	42.7 G'	70.3 D'	72.2 D'							
Mean[6]										49.3 Z'	80.5 Y'	83.7 X'				
Mean[7]		81.6 M'			70.2 N'			61.7 O'								

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between biofumigant, amount and isolate were significant (P<0.001)

[2]Interaction means between amount and isolate were significant (P<0.001)

[3]Interaction means between biofumigant and isolate were significant (P<0.001)

[4]Means of overall isolate effect were significant (P<0.001)

[5]Interaction mean of biofumigant and amount were significant (P<0.001)

[6]Mean of overall amount effect were significant (P<0.001)

[7]Mean total effect of biofumigant were significant (P<0.001)

C.2.50 Mean inhibition (%) of subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w).

Isolate	Mean inhibition of subsequent mycelium growthfrom sclerotia (%)[1]									Mean across biofumigant[2]			Mean across amount[3]			Mean[4]
	'Caliente' mustard			Brown mustard			'Nemat' arugula									
	1%	5%	10%	1%	5%	10%	1%	5%	10%	1%	5%	10%	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	73.7 e	90.5 ab	93.2 a	57.6 h	78.5 cd	81.2 c	62.4 g	81.1 c	73.4 e	64.6 E	83.4 B	82.6 B	85.8 V	72.4 Z	72.3 Z	76.9 y
LUPP2522	67.1 f	86.5 b	90.4 ab	74.6 de	82.5 c	68.1 f	61.2 gh	90.9 a	82.0 c	67.6 D	86.6 A	80.2 C	81.4 W	75.0 Y	78.0 X	78.1 x
Mean across isolate[5]	70.4 F'	88.5 B'	91.8 A'	66.1 G'	80.5 C'	74.6 E'	61.8 H'	86.0 B'	77.7 D'							
Mean[6]										66.1 Z'	85.0 X'	81.4 Y'				
Mean[7]		83.6 M'			73.7 O'			75.2 N'								

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between biofumigant, amount and isolate were significant (P<0.001)

[2]Interaction means between amount and isolate were significant (P<0.001)

[3]Interaction means between biofumigant and isolate were significant (P<0.001)

[4]Means of overall isolate effect were significant (P<0.001)

[5]Interaction mean of biofumigant and amount were significant (P<0.001)

[6]Mean of overall amount effect were significant (P<0.001)

[7]Mean total effect of biofumigant were significant (P<0.001)

C.2.51 Mean inhibition (%) of subsequent mycelium growth of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) from colonised barley grains after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w).

Isolate	Mean inhibition of subsequent mycelium growth from colonised barley grains (%)[1]									Mean across biofumigant[2]			Mean across amount[3]			Mean[4]
	'Caliente' mustard			Brown mustard			'Nemat' arugula									
	1%	5%	10%	1%	5%	10%	1%	5%	10%	1%	5%	10%	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	39.1 f	81.9 a	82.3 a	37.5 f	69.7 c	66.7 cd	55.3 e	67.4 cd	77.7 ab	44.0 D	73.0 B	75.5 A	67.8 W	58.0 Y	66.8 W	64.2 x
LUPP2522	31.8 g	78.0 ab	79.9 ab	14.7 h	66.2 cd	63.7 d	56.4 e	67.2 cd	76.5 b	34.3 E	70.4 C	73.4 AB	63.2 X	48.2 Z	66.7 W	59.4 y
Mean across isolate[5]	35.5 E'	79.9 A'B'	81.1 A'	26.1 F'	67.9 C'	65.2 C'	55.9 D'	67.3 C'	77.1 B'							
Mean[6]										39.2 Z'	71.7 Y'	74.4 X'				
Mean[7]		65.5 M'			53.1 N'			66.7 M'								

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05).

[1]Interaction means between biofumigant, amount and isolate were significant (P<0.001)

[2]Interaction means between amount and isolate were significant (P<0.001)

[3]Interaction means between biofumigant and isolate were significant (P<0.001)

[4]Means of overall isolate effect were significant (P<0.001)

[5]Interaction mean of biofumigant and amount were significant (P<0.001)

[6]Mean of overall amount effect were significant (P<0.001)

[7]Mean total effect of biofumigant were significant (P<0.001)

C.2.52 Mean proportion of retrieved agar plugs with sclerotia (%)of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) per nylon bag buried in soil amended with from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) after 28 days incubation.

Isolate	Proportion of retrieved agar plugs with sclerotia per nylon bag (%)[1]										Mean across biofumigant[2]				Mean across amount[3]				Mean[4]
	Control	'Caliente' mustard			Brown mustard			'Nemat' arugula											
		1%	5%	10%	1%	5%	10%	1%	5%	10%	Control	1%	5%	10%	Control	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	0 e	40.0 a	10.0 d	0 e	10.0 d	0 e	0 e	13.0 cd	0 e	0 e	0 E	19.6 A	1.1 D	0 E	0 Z	10.8 X	1.1 Y	1.5 Y	3.4 y
LUPP2522	0 e	16.8 c	26.5 b	23.2 b	10.0 d	0 e	0 e	10.0 d	0 e	0 e	0 E	12.1 B	3.2 C	2.8 C	0 Z	22.0 W	1.1 Y	1.1 Y	5.3 x
Mean across isolate[5]	0 E'	27.6 A'	17.5 B'	6.2 D'	10.0 C'	0 E'	0 E'	11.5 C'	0 E'	0 E'									
Mean[6]											0 Z'	15.7 W'	2.1 X'	0.7 Y'					
Mean[7]	0 O'		16.0 M'			1.1 N'			1.3 N'										

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05)

[1]Interaction means between biofumigant, amount and isolate (a-e) were significant (P<0.001)

[2]Interaction means between amount and isolate (A-E) were significant (P<0.001)

[3]Interaction means between biofumigant and isolate (W-Z) were significant (P<0.001)

[4]Means of overall isolate effect (x-y) were significant (P<0.001)

[5]Interaction mean of biofumigant and amount (A'-E') were significant (P<0.001)

[6]Mean of overall amount effect (W'-Z') were significant (P<0.001)

[7]Mean total effect of biofumigant (M'-O') were significant (P<0.001)

C.2.53 Mean proportion (%) of retrieved agar plugs of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) having mycelium growth per nylon mesh bag buried in soil amended withmacerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) after 28 days incubation.

Isolate	Proportion (%) of retrieved agar plugs having mycelium growth per nylon mesh bag[1]										Mean across biofumigant[2]				Mean across amount[3]				Mean[4]
	Control	'Caliente' mustard			Brown mustard			'Nemat' arugula											
		1%	5%	10%	1%	5%	10%	1%	5%	10%	Control	1%	5%	10%	Control	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	69.0 a	63.4 ab	43.3 c	10.0 fg	67.1 ab	23.2 de	36.6 cd	73.5 a	16.4 ef	10.0 fg	69.0	68.1	26.9	17.5	69.0	37.0	41.9	30.9	36.9 x
LUPP2522	60.0 ab	51.9 bc	43.3 c	4.5 g	70.3 a	23.2 de	4.5 g	43.3 c	4.5 g	10.0 fg	60.0	55.3	21.2	6.1	60.0	29.9	29.2	16.5	25.3 y
Mean across isolate[5]	64.5 A	57.7 A	43.3 B	7.0 E	68.7 A	23.2 C	17.5 CD	58.8 A	9.6 E	10.0 DE									
Mean[6]											64.5 X	61.8 X	24.0 Y	11.2 Z					
Mean[7]	64.5 M		35.4 N			33.4 N			23.3 O										

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05)

[1]Interaction means between biofumigant, amount and isolate (a-g) were significant (P<0.001)

[2]Interaction means between amount and isolate were not significant (P=0.243)

[3]Interaction means between biofumigant and isolate were not significant (P=0.524)

[4]Means of overall isolate effect (x-y) were significant (P<0.001)

[5]Interaction mean of biofumigant and amount (A-E) were significant (P<0.001)

[6]Mean of overall amount effect (X-Z) were significant (P<0.001)

[7]Mean total effect of biofumigant (M-O) were significant (P<0.001)

C.2.54 Mean germination proportion (%) of retrieved sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) per nylon mesh bag buried in soil amended with macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) after 28 days incubation.

Isolate	Germination proportion of retrieved sclerotia per nylon bag (%)[1]										Mean across biofumigant[2]				Mean across amount[3]				Mean[4]
	Control	'Caliente' mustard			Brown mustard			'Nemat' arugula											
		1%	5%	10%	1%	5%	10%	1%	5%	10%	Control	1%	5%	10%	Control	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	76.8 cde	93.3 b	100 a	63.4 e	100 a	100 a	98.9 a	100 a	87.0 bc	23.2 f	76.8	99.2	98.5	67.3	76.8 CD	91.1 B	99.9 A	78.8 C	92.6 x
LUPP2522	80.0 cd	63.4 e	76.8 cde	66.7 e	100 a	66.7 e	63.4 e	63.4 e	73.5 de	1.1 g	80.0	82.4	72.4	37.7	80.0 C	69.2 D	83.2 C	40.1 E	65.3 y
Mean across isolate[5]	78.4 D'	80.6 D'	93.8 B'	65.1 E'	100 A'	90.9 B'C'	86.3 C'D'	89.8 B'C'	80.7 D'	9.0 F'									
Mean[6]											78.4 Y	93.4 W	89.0 X	52.7 Z					
Mean[7]	78.4 N		81.3 N			94.9 M			60.3 O										

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05)

[1]Interaction means between biofumigant, amount and isolate (a-g) were significant (P<0.001)

[2]Interaction means between amount and isolate were not significant (P=0.11).

[3]Interaction means between biofumigant and isolate (A-E) were significant (P<0.001).

[4]Means of overall isolate effect (x-y) were significant (P<0.001).

[5]Interaction mean of biofumigant and amount (A'-F') were significant (P<0.001).

[6]Mean of overall amount effect (W-Z) were significant (P<0.001).

[7]Mean total effect of biofumigant (M-O) were significant (P<0.001).

C.2.55 Mean proportion (%) of retrieved barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) having mycelium growth per nylon mesh bag buried in soil amended with macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w) after 28 days incubation.

Isolate	Subsequent mycelium growth from retrieved colonised barley grains per nylon bag (%)[1]										Mean across biofumigant[2]				Mean across amount[3]				Mean[4]
	Control	'Caliente' mustard			Brown mustard			'Nemat' arugula											
		1%	5%	10%	1%	5%	10%	1%	5%	10%	Control	1%	5%	10%	Control	'Caliente' mustard	Brown mustard	'Nemat' arugula	
LUPP2519	40.0	23.2	20.7	0.0	36.6	16.4	0.0	26.5	13.0	0.0	40.0	28.6	16.6	0.0	40.0	10.2	12.1	8.9	10.6
LUPP2522	36.6	23.2	13.0	0.0	29.7	16.4	0.0	26.5	10.0	0.0	36.6	26.4	13.0	0.0	36.6	8.2	10.5	8.0	9.1
Mean across isolate[5]	38.3 a	23.2 b	16.7 c	0 e	33.1 a	16.4 c	0 e	26.5 b	11.5 d	0 e									
Mean[6]											38.3 W	27.5 X	14.8 Y	0 Z					
Mean[7]	38.3 M		9.2 NO			11.3 N			8.5 O										

Mean values within the rows or columns followed by the same letters are not significantly different based on Tukey's HSD test (P=0.05)

[1]Interaction means between biofumigant, amount and isolate were not significant (P<0.393).

[2]Interaction means between amount and isolate were not significant (P=0.398).

[3]Interaction means between biofumigant and isolate were not significant (P=0.958).

[4]Means of overall isolate effect were not significant (P=0.075).

[5]Interaction mean of biofumigant and amount (a-e) were significant (P=0.027).

[6]Mean of overall amount effect (W-Z) were significant (P<0.001).

[7]Mean total effect of biofumigant (M-O) were significant (P<0.001).

Additional results

D.2.1 Biofumigation effect of *Brassica* plants on subsequent mycelium growth of *Rhizoctonia solani* sclerotia

The statistical analyses for the inhibition of subsequent mycelium growth from sclerotia are presented in the Appendix C.2.7. There was a significant interaction (P<0.001) between treatment and isolate on the subsequent mycelium growth inhibition from sclerotia (Table D.2.1). AITC100 had the greatest inhibition of subsequent mycelium growth from sclerotia of all *R. solani* isolates (94.2-99.5% inhibition), followed by the volatiles from brown and 'Caliente' mustards. Inhibition of the subsequent mycelium growth from sclerotia of isolate LUPP2520 (85.2% inhibition) was significantly less by volatiles from brown mustard than that from 'Caliente' mustard (90.7% inhibition). However, in contrast for isolate LUPP2523 the inhibition was significantly greater with brown mustard (80.8% inhibition) than that from 'Caliente' mustard (76.1% inhibition). Volatiles from forage rape and fodder radish had the least effect on the subsequent mycelium growth from sclerotia of *R. solani* LUPP2521 (21.1% inhibition) and LUPP2524 (23.8% inhibition), respectively.

There was a significant effect (P<0.001) of treatment on subsequent mycelium growth from sclerotia (Figure D.2.1.1). AITC100 had the greatest inhibition of the subsequent mycelium growth (96.8% suppression), followed by volatiles from 'Caliente' and brown mustards (88.5-89.4% inhibition) which were not significantly different together. This was followed by volatiles from 'Nemat' arugula (83.6% inhibition) which was significantly different from all other treatments. Volatiles from fodder radish was the least effective at reducing subsequent mycelium growth (38.7% inhibition), being significantly different to all other biofumigant treatments.

There was a significant difference (P<0.001) in the susceptibility of *R. solani* isolates to volatiles from macerated biofumigants and pure allyl ITCs, with subsequent mycelium growth from sclerotia of *R. solani* LUPP2523 (61.1% inhibition) being significantly less sensitive to volatiles from treatments compared with all other isolates (63.6-72.0%) apart from isolate LUPP2521 (62.3%) (Figure D.2.1.2). The subsequent mycelia growth from sclerotia of *R. solani* LUPP2517 (72.0% inhibition) was the most sensitive to volatiles from treatments in comparison with the other isolates.

Table D.2.1 Mean inhibition (%) of subsequent mycelium growth from sclerotia in relation to the unamended control of ten *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissue of ten biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression.

Treatment	Inhibition of subsequent mycelium growth from sclerotia (%)									
	LUPP2515	LUPP2516	LUPP2517	LUPP2518	LUPP2519	LUPP2520	LUPP2521	LUPP2522	LUPP2523	LUPP2524
AITC100	96.4	96.0	97.6	97.3	97.5	94.8	98.7	96.2	94.2	99.5
Brown mustard	90.5	92.4	93.0	90.8	88.2	85.2	88.7	92.6	80.8	92.2
'Caliente' mustard	92.3	90.0	93.1	87.5	89.0	90.7	91.0	82.9	76.1	92.3
'Nemat' arugula	91.4	95.8	89.9	90.2	82.2	89.2	91.0	64.0	49.9	92.3
Leafy turnip	78.8	68.6	81.2	84.6	76.1	76.6	51.9	64.9	63.4	84.6
White mustard	67.0	69.2	69.3	72.3	68.9	66.4	60.2	72.7	73.7	71.7
'Corka' kale	47.6	59.0	58.2	60.8	47.4	46.8	44.5	60.5	61.8	51.3
'Lunch' radish	46.6	50.1	59.2	44.1	47.2	41.6	39.6	51.5	38.6	54.8
Rapeseed	43.8	35.2	57.0	47.1	40.1	37.7	47.3	54.1	49.1	44.0
Forage rape	68.5	36.5	53.9	40.5	39.2	32.9	21.1	40.9	40.7	39.7
Fodder radish	36.0	31.9	39.3	49.3	31.0	37.3	50.9	43.9	43.9	23.8
LSD (*P* = 0.05) of Biofumigant x *Rhizoctonia solani* isolate			3.3							

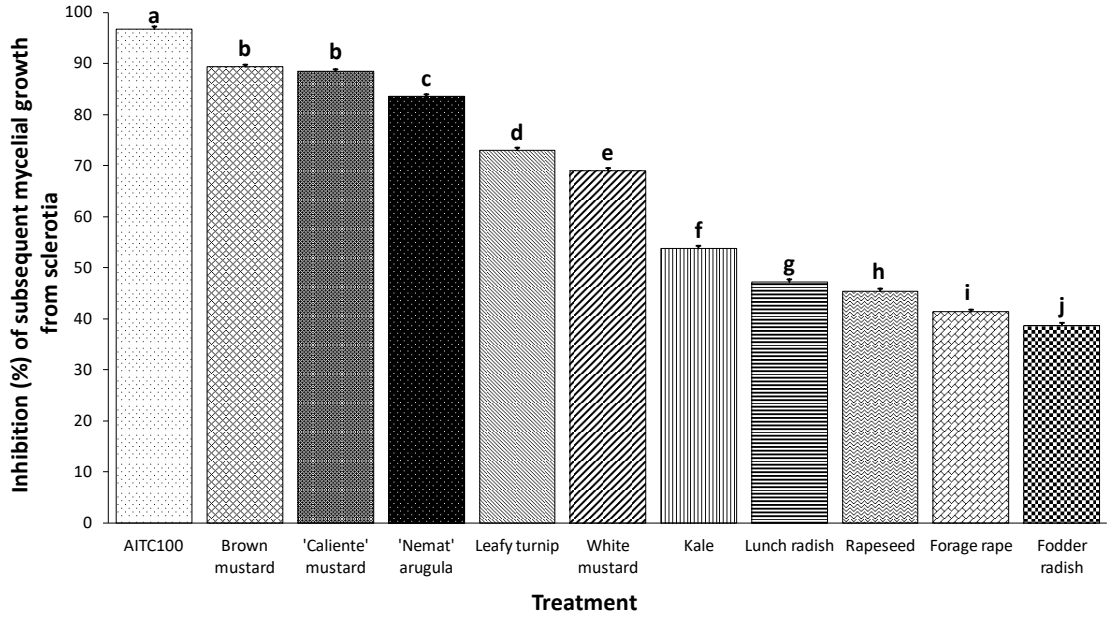


Figure D.2.1.1 Mean inhibition (%) subsequent mycelium growth from sclerotia in relation to the unamended control of ten *Rhizoctonia solani* isolates (data averaged across ten isolates) after 3 days exposure to volatiles from macerated tissue of ten biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 1.1.

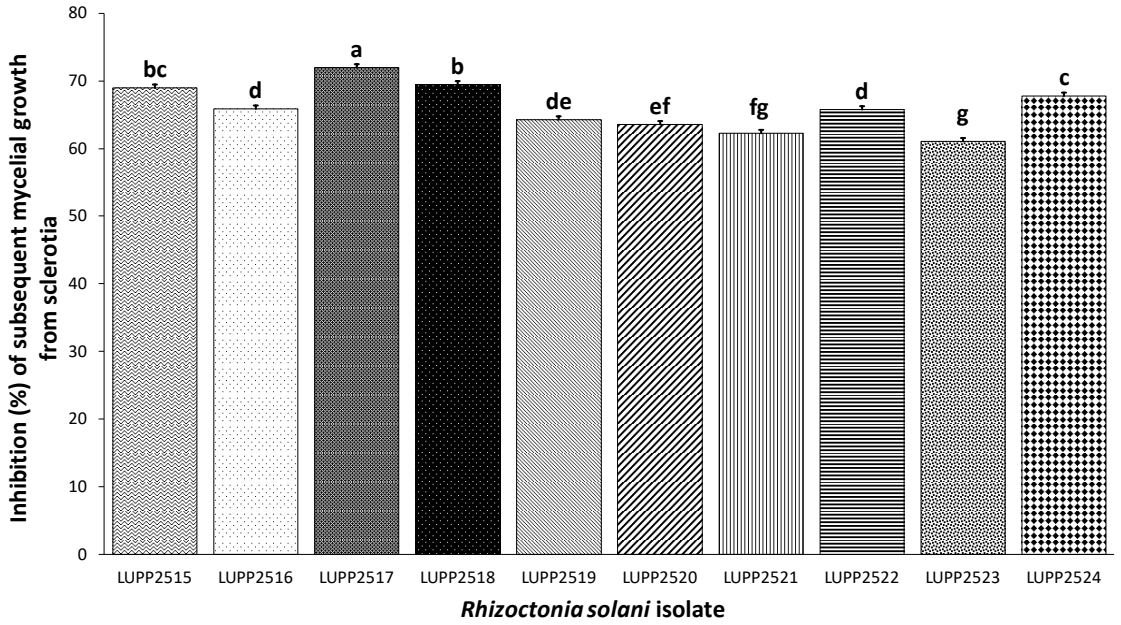


Figure D.2.1.2 Mean inhibition (%) subsequent mycelium growth from sclerotia in relation to the untreated control of ten different *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissue of biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression (data averaged across ten crops and AITC100). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 1.0.

D.2.2 Biofumigation effect of *Brassica* plants on subsequent mycelium growth of *Rhizoctonia solani* colonised barley grains

The statistical analyses for inhibition of subsequent mycelium growth from colonised barley grains are presented in Appendix C.2.9. There was a significant interaction (P<0.001) between treatment and isolate and on the subsequent mycelium growth from colonised barley grains, mainly as a result of difference between isolates in their relative response to the different biofumigant treatments (Table D.2.2). AITC100, 'Caliente' and brown mustards provided the greatest inhibition across all isolates, with no significant difference in the inhibition efficacy between these three treatments for isolates LU992515, LUPP2518 and LUPP2524, while for isolates LUPP2519, LUPP 2521 and LUPP2523 AITC100 resulted in significantly greater inhibition compared with both 'Caliente' and brown mustards. However, while for LUPP2517 and LUPP2522 both AITC100 and 'Caliente' mustard provided significantly greater inhibition than brown mustard. In contrast for isolate LUPP2519 AITC100 and brown mustard provided significantly greater inhibition than 'Caliente' mustard. There were no inhibition of the subsequent mycelium growth from colonised barley grains of some *R. solani* isolates by volatiles from forage rape (for isolates LUPP2515, LUPP2516 and LUPP2523) and fodder radish (for LUPP2523).

There was a significant effect (P<0.001) of treatment on the subsequent mycelium growth from the colonised barley grains (Figure D.2.2.1). AITC100 and 'Caliente' mustard (80.6 and 79.5% inhibition, respectively) significantly inhibited subsequent mycelium growth from colonised barley grains compared with all other biofumigant treatments (Figure C.2.2). This was followed by volatiles from brown mustard (71.1% inhibition) which significantly inhibited mycelium growth compared with the remaining biofumigant treatments. The next most effective treatment was volatiles from 'Nemat' arugula (54.9% inhibition) which was significantly different from all other treatments. Volatiles from fodder radish was the least effective at reducing mycelium growth (14.8% inhibition), being significantly less effective compared to all other biofumigant treatments.

There was a significant effect (P<0.001) of isolate on the subsequent mycelium growth from colonised barley grains (Figure D.2.2.2). The mycelium growth from barley grains colonised by *R. solani* isolate LUPP2523 was significantly less sensitive to biofumigants (29.3% inhibition) compared with all other isolates, followed by LUPP2522 (46.8% inhibition). By contrast, the mycelium growth from colonised barley grains of *R. solani* LUPP2521 was the most sensitive to the volatiles from biofumigants (60.9% inhibition).

Table D.2.2 Mean inhibition (%) of subsequent mycelium growth from colonised barley grains in relation to the untreated control of ten *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissues of ten biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression.

Treatment	Inhibition of subsequent mycelium growth from colonised barley grains (%)									
	LUPP2515	LUPP2516	LUPP2517	LUPP2518	LUPP2519	LUPP2520	LUPP2521	LUPP2522	LUPP2523	LUPP2524
AITC100	78.8	80.4	81.8	82.4	80.7	76.4	82.3	82.3	80.5	80.9
'Caliente' mustard	81.1	79.8	81.6	81.5	77.7	80.6	75.4	81.3	76.5	79.5
Brown mustard	81.0	78.3	77.4	81.4	81.5	77.7	68.2	52.5	31.7	81.7
'Nemat' arugula	57.0	41.8	42.1	68.3	62.2	62.9	62.3	47.3	21.6	83.6
'Corka' kale	55.9	56.1	54.1	54.2	53.8	55.1	64.9	46.4	19.3	60.7
Leafy turnip	52.6	51.9	54.8	48.9	50.8	52.6	73.5	47.1	21.6	55.5
White mustard	24.3	51.0	38.3	44.8	51.3	62.8	28.7	44.1	37.8	58.0
Rapeseed	44.9	46.8	36.3	33.9	41.2	22.5	46.9	43.9	23.0	43.3
'Lunch' radish	41.0	12.8	38.6	28.1	31.4	45.7	60.7	29.8	10.8	49.0
Forage rape	0	0	15.3	14.5	16.6	6.8	76.2	33.8	0	30.2
Fodder radish	14.6	36.2	18.3	7.9	5.7	6.6	31.0	6.0	0	21.7
LSD (*P*=0.05) of Biofumigant x *Rhizoctonia solani* isolate				2.6						

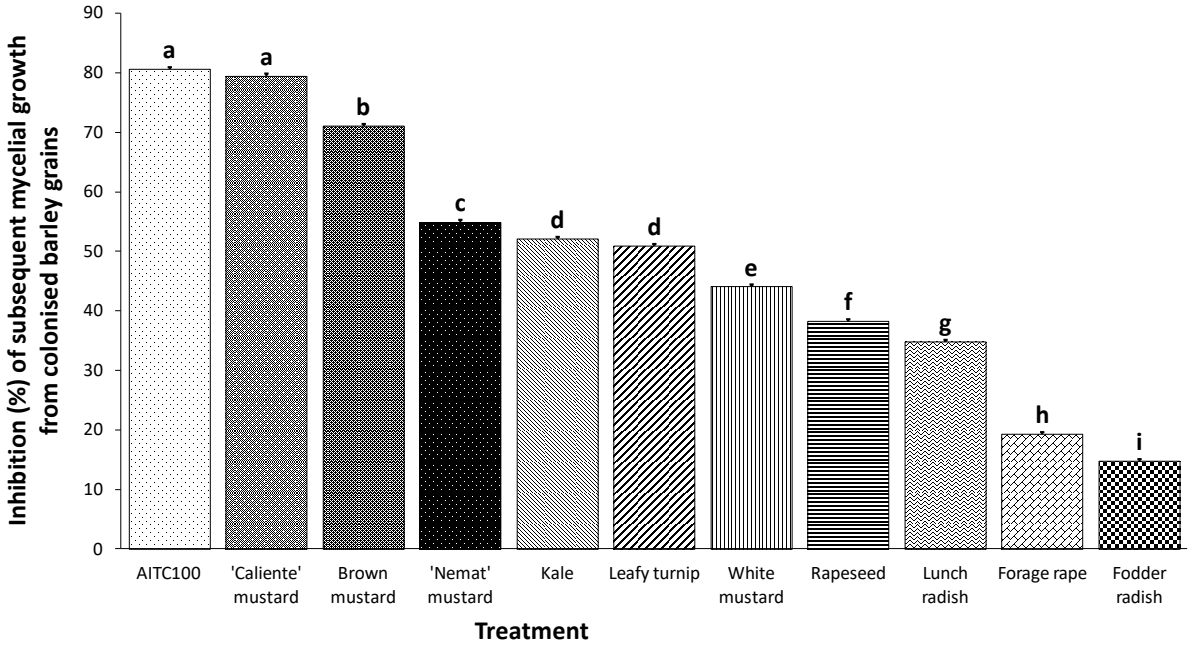


Figure D.2.2.1 Mean inhibition (%) of subsequent mycelium growth from colonised barley grains in relation to the untreated control of ten *Rhizoctonia solani* isolates (data averaged across ten isolates) after 3 days exposure to volatiles from macerated tissue of ten biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 0.8.

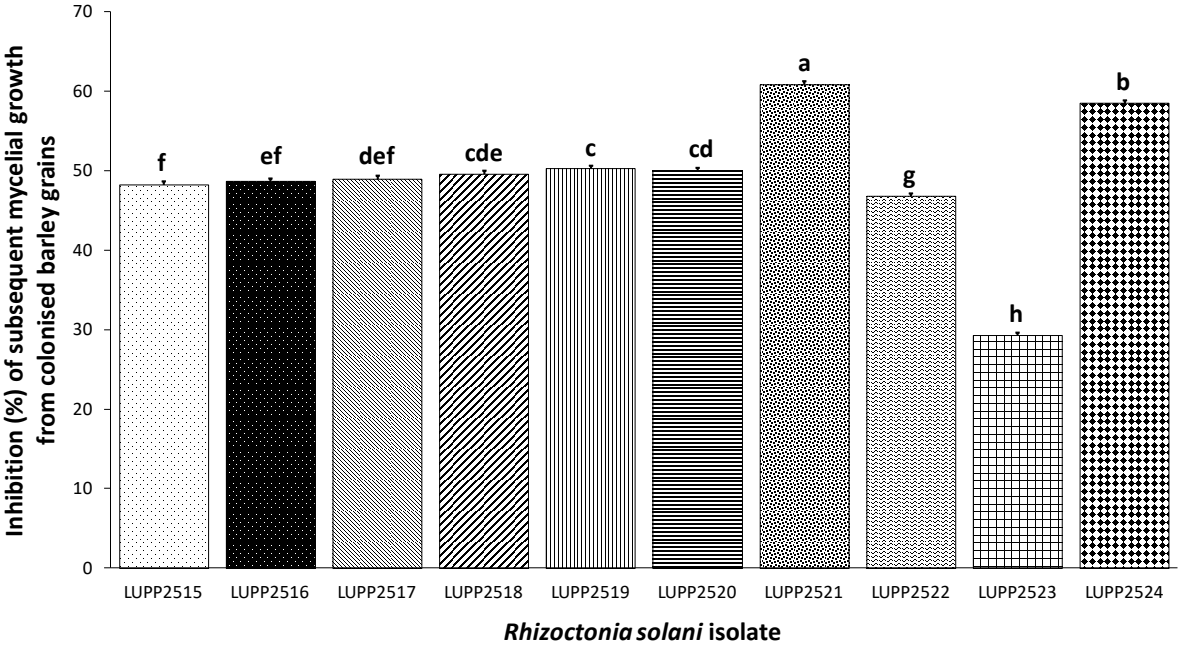


Figure D.2.2.2 Mean inhibition (%) subsequent mycelium growth from colonised barley grains in relation to the untreated control of ten different *Rhizoctonia solani* isolates after 3 days exposure to volatiles from macerated tissue of ten biofumigant crops (5 g/Petri dish) and allyl ITC at concentration to provide 100% (AITC100) suppression (data averaged across ten crops and AITC100). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 0.8.

D.2.3 Biofumigation effect of different amounts of *Brassica* plants on subsequent mycelium growth of *Rhizoctonia solani* sclerotia

The statistical analyses of the subsequent mycelium growth inhibition from sclerotia are presented in Appendix C.2.12, and the results are summarised in Appendix C.2.36. There was a significant interaction (P<0.001) between treatment, amount *and* isolate on the subsequent mycelium growth. Volatiles from 'Caliente' and brown mustards at 5 and 10 g had significantly greater inhibition of the subsequent mycelium growth from sclerotia of *R. solani* LUPP2519 and LUPP2522 (94.3-97.2% inhibition) compared with 1 g of both mustards and 'Corka' kale and 'Pasja II' leafy turnip at all amounts (Figure D.2.3). For LU2519, the inhibition by 'Caliente' and brown mustards at 5 and 10 g were not significantly different compared with 'Nemat' arugula at 5 and 10 g (96.1-96.3% inhibition) and AITC100 (94.7% inhibition). In contrast, for LUPP2522, the inhibition by 'Caliente' and brown mustards at 5 and 10 g was significantly greater than with 'Nemat' arugula at 5 and 10 g (78.5-79.4% inhibition), with the inhibition by 'Nemat' arugula at 5 and 10 g also being significantly less than that with volatiles from 'Pasja II' leafy turnip at 5 and 10 g (79.0-81.8% inhibition).

There was a significant interaction (P<0.001) between amount and isolate on the subsequent mycelium growth. For biofumigant crops at 1 g and 10 g there was no significant difference in the inhibition levels between the two *R. solani* isolates while biofumigant crops at 5 g, AITC50 and AITC100 provided significantly greater inhibition of the subsequent mycelium growth from sclerotia of *R. solani* LUPP2519 than for LUPP2522 (Appendix C.2.36). There was a significant interaction (P<0.001) between treatment and isolate on the subsequent mycelium growth. For 'Caliente' mustard, there was no significant difference in the inhibition levels between the two *R. solani* isolates, while 'Nemat' arugula, AITC50 and AITC100 provided significantly greater inhibition of the subsequent mycelium growth from sclerotia of *R. solani* LUPP2519 than for LUPP2522 (Appendix C.2.36). In contrast, volatiles from brown mustard, 'Corka' kale and 'Pasja II' Leafy turnip provided significantly greater inhibition of the subsequent mycelium growth from sclerotia of *R. solani* LUPP2519 than for LUPP2522 provided significantly greater inhibition of the subsequent mycelium growth from sclerotia of *R. solani* LUPP2522 and LUPP2519.

There was a significant interaction (P<0.001) between treatment and amount on the subsequent mycelium growth. For 'Caliente' and brown mustards, 'Nemat' arugula and 'Pasja II' leafy turnip there was no significant difference in the inhibition of the subsequent mycelium growth of *R. solani* sclerotia at 5 g and 10 g, which are significantly higher than that achieved with 1 g. In contrast for 'Corka' kale there was a significantly effect of amount on the subsequent mycelium growth from sclerotia, with 10 g providing significantly greater inhibition than 5 g which was significantly greater than 1 g. There was a significant effect (P<0.001) of biofumigant treatment on the subsequent mycelium growth. AITC100 had the greatest inhibition of the subsequent mycelium growth (93.0% inhibition), followed by 'Caliente' mustard (86.5% inhibition) and brown mustard (72.8% inhibition), while 'Corka' kale was the least effective at inhibiting the subsequent mycelium growth (43% inhibition).

There was a significant effect (P<0.001) of biofumigant amount on the subsequent mycelium growth, with 1 g of the biofumigant crops being significantly less effective at inhibiting the subsequent mycelium growth of sclerotia (49.0% inhibition) compared with both 5 g (79.6% inhibition) and 10 g (80.4% inhibition) with no significant difference between these. There was a significant effect (P<0.001) of *R. solani* isolate on the subsequent mycelium growth with LUPP2519 (71.2% inhibition) being more sensitive to volatiles from biofumigants than isolate LUPP2522 (68.4% inhibition).

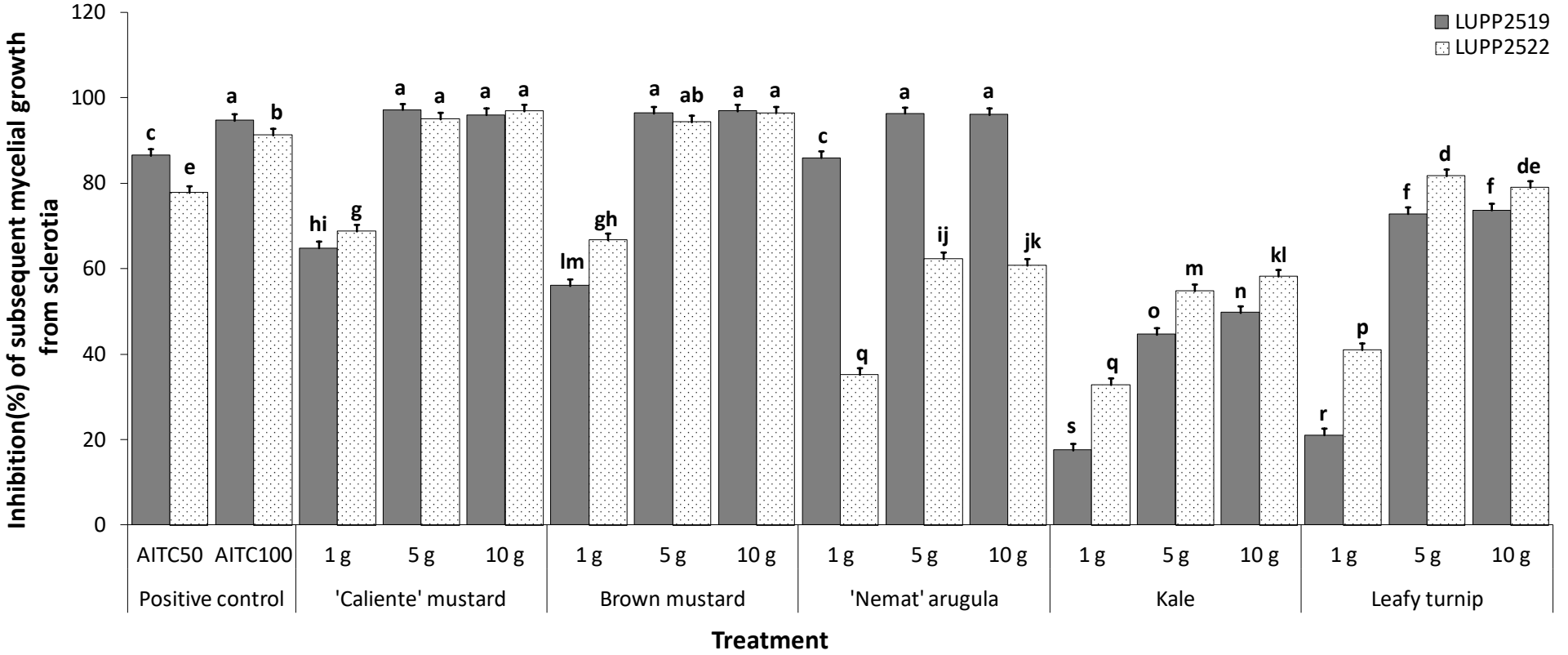


Figure D.2.3 Mean inhibition (%) of subsequent mycelium growth from sclerotia in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri dish, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 3.0.

D.2.4 Biofumigation effect of different amounts of *Brassica* plants on subsequent mycelium growth of *Rhizoctonia solani* colonised barley grains

The statistical analyses of the inhibition of the subsequent mycelium growth from colonised barley grains are presented in Appendix C.2.14, and the results are summarised in Appendix C.2.38. There was a significant interaction (P<0.001) between treatment, amount and isolate on the subsequent mycelium growth. Volatiles from 'Caliente' mustard at 10 g and brown mustard at 5 and 10 g has significantly greater inhibition of the subsequent mycelium growth of both *R. solani* isolates compared with 1 g of both mustards, Corka' kale and 'Pasja II' leafy turnip at all amounts (Figure D.2.4). For LU2519, the inhibition by 'Nemat' arugula at all amounts (83.4-85.8%) was not significantly different compared with 'Caliente' and brown mustards at 10 g. In contrast, for LUPP2522 the inhibition by 'Caliente' and brown mustard at 10 g was significantly greater than by 'Nemat' arugula at all amounts. There was a significant interaction (P<0.001) between amount and isolate on the subsequent mycelium growth, which was mostly due to the response of the two isolates to the two AITC concentrations, AITC50 and AITC100, where for *R. solani* LUPP2519 there was no significantly difference in the suppression of the mycelium growth, but for *R. solani* LUPP2522 suppression of the mycelium growth by AITC100 was significantly greatest than for AITC50. There was a significant interaction (P<0.001) between treatment and isolate on the subsequent mycelium growth. All biofumigant treatments, apart from 'Caliente' and brown mustards, had a significantly greater effect on the mycelium growth from LUPP2519 colonised barley grains compared with LUPP2522.

There was a significant interaction (P<0.001) of treatment and amount on the subsequent mycelium growth. For all biofumigant treatments, apart from brown mustard and 'Nemat' arugula, there was a significantly greater effect of volatiles from 10 g compared with from 5 g on the mycelium growth from colonised barley grains compared. There was a significant effect (P<0.001) of treatment on the subsequent mycelium growth. AITC100 had the greatest inhibition of the subsequent mycelium growth (78.8%), which was significantly greater than by AITC50 (66.7%). Of the biofumigant crops, volatiles from brown mustard had significantly greater inhibition of the subsequent mycelium growth (61.1%) compared with all other treatments, followed by 'Nemat' arugula (59.3%) and 'Caliente' mustard (56.0%).

There was a significant effect (P<0.001) of amount on the subsequent mycelium growth. Biofumigant crops at 10g (72.4%) significantly inhibited the subsequent mycelium growth followed by 5 g (67.1%) which was significantly greater than the inhibition by 1 g (16.7%). There was a significant effect (P<0.001) of *R. solani* isolate on the subsequent mycelium growth with isolate LUPP2519 significantly more sensitive to volatiles from biofumigants than LUPP2522.

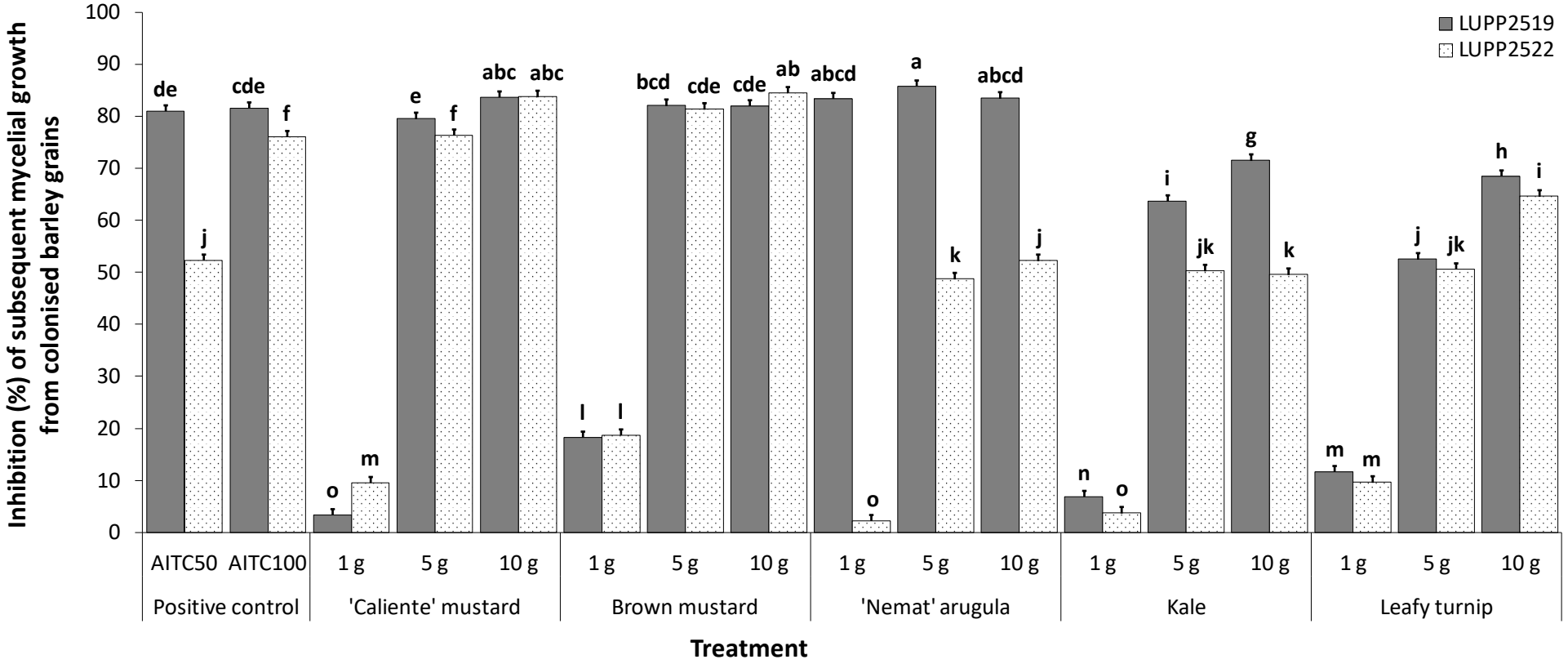


Figure D.2.4 Mean inhibition (%) of subsequent mycelium growth from colonised barley grains in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of five biofumigant crops at 1, 5 and 10 g per Petri dish, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.5.

D.2.5 Biofumigation effect of *Brassica* tissue types on subsequent mycelium growth of *Rhizoctonia solani* sclerotia

The statistical analyses of the inhibition of the subsequent mycelium from sclerotia are presented in Appendix C.2.17, and the results are summarised in Appendix C.2.41. There was a significant interaction (P<0.001) between treatment, tissue type and isolate on the subsequent mycelium growth from sclerotia. There was no significant difference between shoots or roots + shoots of 'Caliente' mustard on the subsequent mycelium growth of both *R. solani* isolates (96.0-97.4% inhibition) which was significantly greater than AITC100 for both isolates (93.9-94%), and was not significantly different from shoots of brown mustard for LUPP2522 (95.9% inhibition) and the three 'Nemat' arugula tissue types for LUPP2519 (96.4-97.4% inhibition). The combination of volatiles from roots and shoots of 'Pasja II' leafy turnip had the greater inhibition of the subsequent mycelium growth for both *R. solani* isolates (90.6% for LUPP2519 and 81.6% for LUPP2522) compared with roots or shoots alone. For both isolates, volatiles from roots of 'Corka' kale had the greater inhibition of the subsequent mycelium growth (80.5-81.9% inhibition) compared with those from roots or shoots (Figure D.2.5).

There was a significant interaction (P<0.001) between tissue type and isolate on the subsequent mycelium growth from sclerotia. There was no significant difference in the inhibition of mycelium growth of the two *R solani* isolates with AITC100. However, for all biofumigant tissue types, the mycelium growth of isolate LUPP2519 was inhibited to a significantly greater extent compared with isolate LUPP2252. Volatiles from the roots and roots + shoots had a similar effect on the subsequent mycelium growth of the isolate LUPP2519 (85.8-86.1% inhibition), and was significantly greater than that from shoots (80.9% inhibition). In contrast, for isolate LUPP2522 shoot tissue alone or in combination with root tissue (77.7-78.3%) had significantly greater inhibition of the mycelium growth compared with root tissue (67.4%). There was a significant interaction (P<0.001) between treatment and *R. solani* isolate on the subsequent mycelium growth from sclerotia. There was no significant difference in the inhibition of mycelium growth of the two *R. solani* isolates by the positive control AITC100 (94.0 and 93.9%, respectively for LUPP2519 and LUPP2522) or 'Caliente' mustard (91.8 and 91.3%, respectively for LUPP2519 and LUPP2522). In contrast for all other biofumigant tissue treatments, the mycelium growth of isolate LUPP2519 (58.7-97.0% inhibition) was significantly more inhibited compared with that of isolate LUPP2522 (53.9-86.4% inhibition).

There was a significant interaction (P<0.001) between treatment and tissue type on the subsequent mycelium growth from sclerotia. For both 'Caliente' and brown mustard, volatiles from both shoots alone, or roots + shoots had a significantly greater suppression of the subsequent mycelium growth compared with root tissue alone. In contrast, for 'Nemat' arugula shoot tissue significantly reduced mycelium growth compared with both root tissue alone, and shoot and root tissue together, and for 'Corka' kale root tissue was significantly more effective compared with shoot tissue alone or in combination with root tissue. However for 'Pasja II' leafy turnip the volatiles from the combination of roots and shoots were significantly more effective at inhibiting mycelium growth compared with either tissue alone (Appendix C.2.41).

There was a significant effect (P<0.001) of treatment on the subsequent mycelium growth from sclerotia. AITC100 had the greatest inhibition of the subsequent mycelium growth (93.9%), followed by volatiles from 'Caliente' mustard (91.6% inhibition), brown mustard (87.9%) and 'Nemat' arugula (85.8%). Volatiles from 'Corka' kale were the least effective at suppressing the subsequent mycelium growth (56.3% inhibition)

(Appendix C.2.41). There was a significant effect (P<0.001) of tissue type on the subsequent mycelium growth from sclerotia. The combination of roots and shoots had the greater inhibitory efficiency on the subsequent mycelium growth (81.9% inhibition) compared with shoots (79.6% inhibition) or roots (76.6% inhibition), but was significantly less than the positive controls AITC100 (93.9%) and AITC50 (84.4% inhibition) (Appendix C.2.41). There was a significant effect (P < 0.001) of isolate on the subsequent mycelium growth from sclerotia. *Rhizoctonia solani* LUPP2519 (AG3-PT) was more sensitive to volatiles from biofumigants compared with LUPP2522 (AG2-1) (Appendix B.1.41).

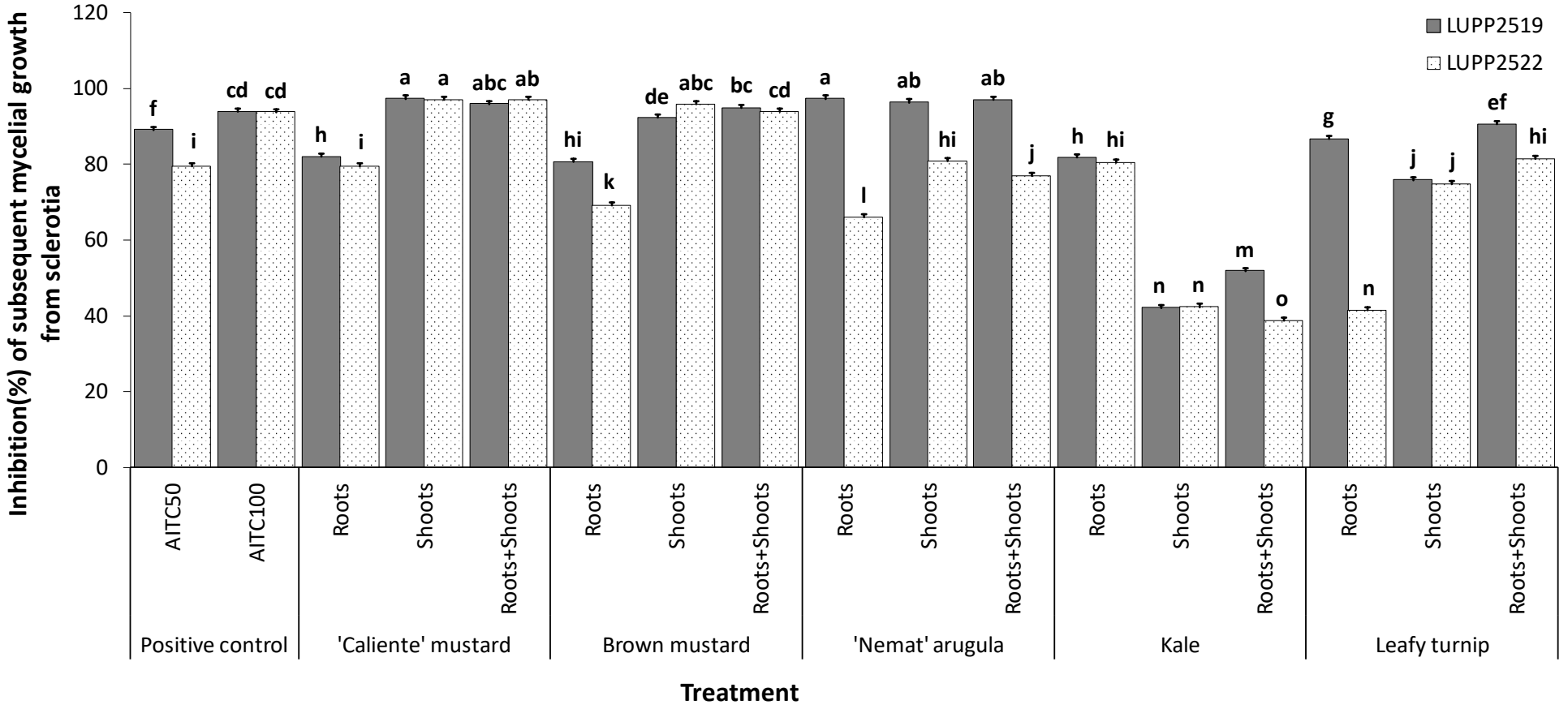


Figure D.2.5 Mean inhibition (%) of subsequent mycelium from sclerotia in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots + shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 1.6.

D.2.6 Biofumigation effect of *Brassica* tissue types on subsequent mycelium growth of *Rhizoctonia solani* colonised barley grains

The statistical analyses of the inhibition of the subsequent mycelium growth from colonised barley grains are presented in Appendix C.2.19 and the results are summarised in Appendix C.2.43. There was a significant interaction (P<0.001) between treatment, tissue type and isolate on the subsequent mycelium growth. For isolate LUPP2519 volatiles from all tissue types of 'Nemat' arugula, shoot alone or in combination with roots of 'Caliente' mustard and brown mustard 'Pasja II' leafy turnip roots and the positive control AITC100 significantly inhibited the subsequent mycelium growth from the colonised barley grains (81.5-84.8%) compared with all other treatments. In contrast for LU2522, volatiles from shoots of 'Caliente' mustard and shoots alone or in combination with roots of brown mustard significantly inhibited the subsequent mycelium growth compared with all other treatments including the positive control AITC100 (Figure D.2.6).

There was a significant interaction (P<0.001) between tissue type and isolate on the subsequent mycelium growth. For each tissue type the mycelium growth of LUPP2519 was significantly more inhibited compared to LUPP2522. For LU2522, shoots alone or the combination roots + shoots were significantly more effective at inhibiting the subsequent mycelium growth (65.3-64.5%) compared with roots alone (42.7%). In contrast for LUPP2519, roots and shoots combination resulted insignificantly greater reduction in mycelium growth (76.8) in comparison with either tissue alone (72.1 for roots and 69.5% for shoots), with roots alone being significantly more effective than shoots alone. There was a significant interaction (P<0.001) between treatment and *R. solani* isolate on the subsequent mycelium growth. There was no significant difference in the inhibition of mycelium growth of the two *R. solani* isolates by brown mustard (72.4%, for both. In contrast for all other biofumigant treatments, the mycelium growth of isolate LUPP2519 (56.0-83.9% inhibition) was significantly more inhibited compared with that of isolate LUPP2522 (53.7-86.4% inhibition).

There was a significant interaction (P<0.001) between treatment and tissue type on the subsequent mycelium growth. For brown mustard, volatiles from either shoots alone or roots + shoots had a significantly greater suppression of the subsequent mycelium growth compared with root tissue alone. In contrast, for both Caliente mustard and 'Nemat' arugula shoot tissue significantly reduced mycelium growth compared with both root tissue alone, and shoot and root tissue together, and for 'Corka' kale root tissue was significantly more effective compared with shoot tissue alone or in combination with root tissue. However for 'Pasja II' leafy turnip the volatiles from the combination of roots and shoots were significantly more effective at inhibiting mycelium growth compared with either tissue alone.

There was significant effect (P<0.001) of treatment on the subsequent mycelium growth. Allyl ITC at the highest concentration (AITC100) had the greatest inhibition of the subsequent mycelium growth (75.6%), followed by 'Caliente' mustard (74.0%), brown mustard (72.4%). 'Corka' kale had the least effective to the subsequent mycelium growth (50.9%). There was significant effect (P<0.001) of tissue type on the subsequent mycelium growth. The combination of roots and shoots was significantly more effective at inhibiting the mycelium growth (70.7% inhibition) compared with shoots (67.4% inhibition) or roots (57.4% inhibition) alone, but was significantly less than AITC100 (75.6% inhibition). There was significant effect (P<0.001) of isolate on the subsequent mycelium growth, with LUPP2519 more sensitive to volatiles from biofumigant crops compared with LUPP2522.

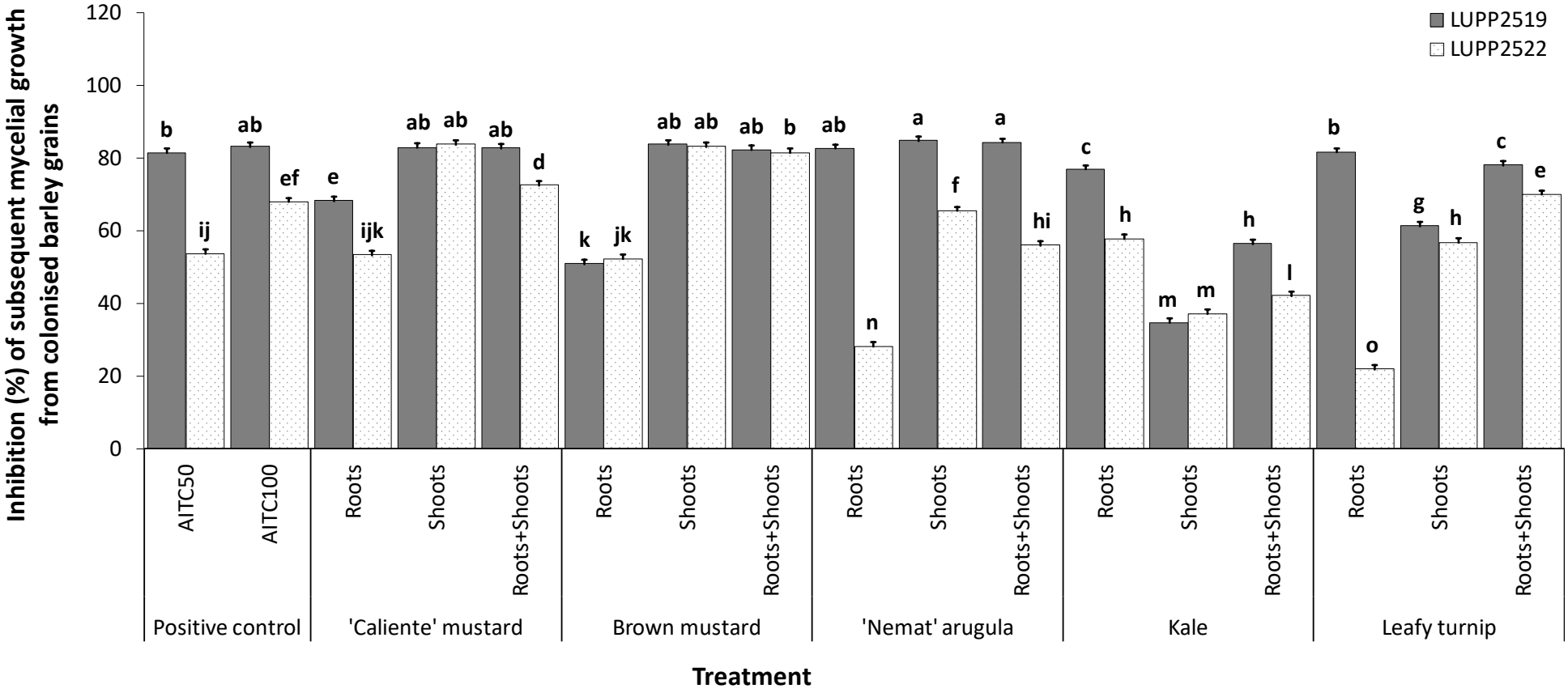


Figure D.2.6 Mean inhibition (%) of subsequent mycelium from colonised barley grains in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue types (roots, shoots, roots + shoots) of five biofumigant crops, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.5.

D.2.7 Biofumigation effect of different flowering times of *Brassica* plants on *Rhizoctonia solani* sclerotia

The statistical analyses of the inhibition of mycelium growth from sclerotia are presented in Appendix C.2.22, and the results are summarised in Appendix C.2.46. There was a significant interaction (P=0.005) between treatment, flowering time and isolate on the subsequent mycelium growth from sclerotia. For 'Caliente' mustard there was no significant difference in the mycelium growth at three flowering stages for both *R. solani* isolates. In contrast, 'Nemat' arugula harvested at the three flowering times had significantly greater suppression of the subsequent mycelium growth of LUPP2519 (91.6-94.6% inhibition) compared with LUPP2522 (43.3-55.6% inhibition). For brown mustard harvested at flowering times had significantly greater suppression of the subsequent mycelium growth for LUPP2522 (92.2-93.4% inhibition) compared with that for LUPP2519 (84.8-90% inhibition), with no significant difference in the mycelium growth inhibition between the two isolates for brown mustard harvested at full flowering (Figure D.2.7).

There was a significant interaction (P<0.001) between flowering time and isolate on the subsequent mycelium growth from sclerotia. For LU2519, biofumigant flowering time had no significant difference on the level of inhibition of mycelium growth. In contrast for LU2522, biofumigants harvested at the first and mid flowering times had significantly greater inhibition of mycelium growth compared with the biofumigants harvested at full flowering (77.6%). There was a significant interaction (P<0.001) between treatment and isolate on the subsequent mycelium growth from sclerotia. There was no significant difference between the level of mycelium growth inhibition by 'Caliente' mustard and the positive control AITC100 between the two *R. solani* isolates. However, both brown mustard and 'Nemat' arugula were significantly more effective at inhibiting the mycelium growth of LUPP2519 (95.7-100%) compared with LUPP2522 (30.5-75.7%).

There was a significant interaction (P<0.001) between treatment and flowering time on the subsequent mycelium growth from sclerotia. For 'Caliente' mustard there was no significant difference in the level of inhibition of mycelium growth when harvested at the three anthesis stages which were not significantly different to the positive control AITC100 (100% for all). In contrast, for brown mustard was significantly more effective at inhibiting mycelium growth when harvested at mid and full flowering times (95.3 and 91.7% inhibition, respectively) compared with at first flowering (88.5%), while 'Nemat' arugula at first and mid flowering stages had significantly greater inhibition of the mycelium growth (75.1 and 74.7% inhibition, respectively) compared with full anthesis stage (67.4% inhibition). There was a significant effect (P<0.001) of treatment on the subsequent mycelium growth from sclerotia. The subsequent mycelium growth inhibition by 'Caliente' mustard (95.2% inhibition) was not significantly different from by AITC100 (93.4%), but was significantly different from other treatments.

There was a significant effect (P<0.001) of isolate on the subsequent mycelium growth from sclerotia, with LUPP2519 having the greater sensitivity to volatiles from biofumigants (92.9% inhibition) compared with LUPP25922 (80.1% inhibition). There was no significant effect (P=0.061) of flowering time on the subsequent mycelium growth from sclerotia, with inhibition ranging from 81.3 to 93.4%.

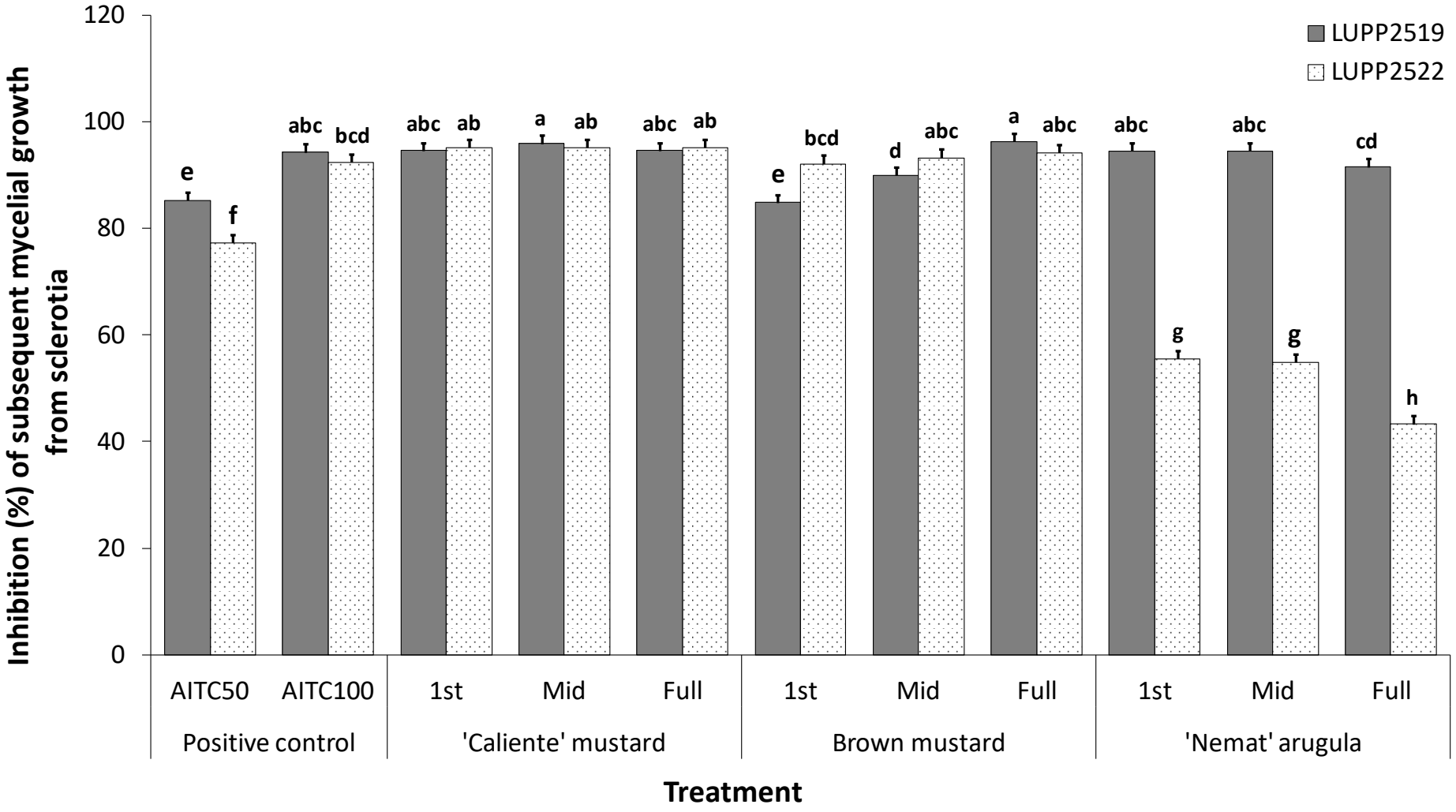


Figure D.2.7 Mean inhibition (%) of subsequent mycelium from sclerotia in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 3.1.

D.2.8 Biofumigation effect of different flowering times of *Brassica* plants on subsequent mycelium growth of *Rhizoctonia solani* colonised barley grains

The statistical analyses of the inhibition of subsequent mycelium growth from colonised barley grains are presented in Appendix C.2.24, and the results are summarised in Appendix C.2.48. There was a significant interaction (P<0.001) between treatment, flowering time and isolate on the subsequent mycelium growth. 'Caliente' mustard at all three flowering stages for LUPP2519 and full flowering time for LUPP2522, brown mustard at the mid and full flowering stage for LUPP2519 and full flowering time for LUPP2522, 'Nemat' arugula for LUPP2519, and AITC100 for LUPP2519 resulted in significantly greater inhibition of subsequent mycelium growth (81.9-84.5% inhibition) compared with all other biofumigant treatments. 'Caliente' and brown mustards at first and mid flowering times and 'Nemat' arugula at all three flowering stages had significantly greater inhibition of the subsequent mycelium growth for *R. solani* isolate LUPP2519 compared with that for LUPP2522 (Figure D.2.8).

There was a significant interaction (P<0.001) between flowering time and isolate on the subsequent mycelium growth. For isolate LUPP 2519, the inhibition of mycelium growth inhibition by biofumigant crops at mid and full flowering times (83.8-84.0%) was significantly greater compared with the biofumigant crop harvested at first flowering (82.0%) , or the positive control AITC100 (81.9%). In contrast, for isolate LUPP2522 inhibition of mycelium growth inhibition by AITC100 (75.5%) was significantly greater than for the biofumigant crop harvested at all three flowering times (55.2-64.7%). There was a significant interaction (P<0.001) between treatment and isolate on the subsequent mycelium growth. For isolate LUPP2519, 'Caliente' mustard and "Nemat' arugula had significantly greater inhibition of the subsequent mycelium growth (84-84.1%) compared with all other treatments including the positive control AITC100. For isolate LUPP2522 only 'Caliente' mustard (80.3% inhibition) significantly inhibited mycelium growth compared with the positive control AITC100 and all other treatments.

There was a significant interaction (P<0.001) between treatment and flowering time on the subsequent mycelium growth. For 'Caliente' mustard, harvesting at full flowering resulted in significantly greater inhibition of mycelium growth compared with first and mid flowering. In contrast, for brown mustard at both mid and full flowering time resulted in significantly greater inhibition of mycelium growth compared with first flowering, and for 'Nemat' arugula first flowering resulted in significantly greater inhibition of mycelium growth compared with mid and full flowering.

There was a significant effect (P<0.001) of treatment on the subsequent mycelium growth, with 'Caliente' mustard having the greatest suppression of the subsequent mycelium growth (82.2%), followed by AITC100 (78.7%), and brown mustard (76.2%). There was a significant effect (P<0.001) of flowering time on the subsequent mycelium growth. Biofumigants at mid and full flowering times had significantly greater inhibition of the subsequent mycelium growth (74-74.2%) than at first flowering time (68.6%), but was significantly less than AITC100 (78.7%). There was significant effect (P<0.001) of isolate on the subsequent mycelium growth, with *R. solani* LUPP2522 being less sensitive to volatiles from biofumigants (61.3% inhibition) compared with LUPP2519 (83.2% inhibition).

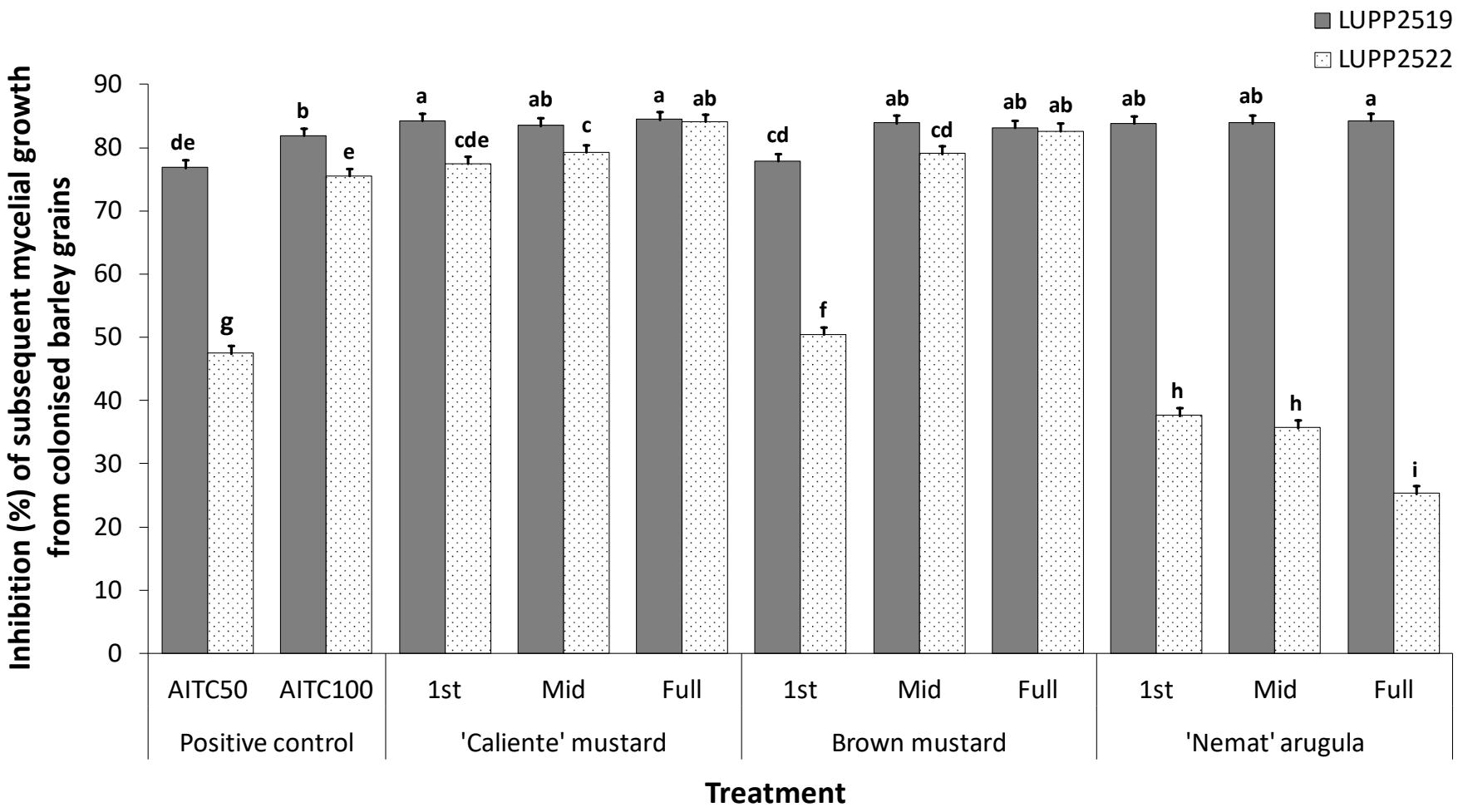


Figure D.2.8 Mean inhibition (%) of subsequent mycelium from barley grains in relation to the unamended control of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 3 days exposure to volatiles from macerated tissue of three biofumigant crops at first, mid and full flowering stages, and allyl ITC at concentrations to provide 50% (AITC50) and 100% (AITC100) suppression. Bars with the same letter are not significantly different based on Tukey's HSD test (P=0.05). Error bars indicate LSD (P=0.05) = 2.3.

D.2.9 Biofumigation effect of different amount of *Brassica* plants amended in soil on *Rhizoctonia solani* sclerotia

The statistical analyses of the inhibition of subsequent mycelium growth from sclerotia are presented in Appendix C.2.26, and the results are summarised in Appendix C.2.50. There was a significant interaction (P<0.001) between biofumigant crop, biofumigant amount and *R. solani* isolate on the subsequent mycelium growth from sclerotia. Three biofumigants at 1% were less effective at inhibiting the subsequent mycelium growth from sclerotia compared to higher concentrations. For both isolates, there was no significantly difference in the mycelium growth inhibition between 'Caliente' mustard at 10% and 5%, with both concentrations significantly reducing mycelium growth compared with at 1%. For LUPP2519, brown mustard at 5 and 10% had significantly greater inhibition of mycelium growth compared with that at 1% (57.6%), while for LUPP2522 brown mustard at 5% for LUPP2522 had the significantly greater inhibition (82.5%) compared with that at 1% and 10% (74.6 and 68.1%, respectively). In contrast, 'Nemat' arugula at 5% was significantly more effective at reducing the mycelium growth of both *R. solani* isolates compared with that at 1% and 10% (Figure D.2.9).

There was a significant interaction (P<0.001) between biofumigant amount and *R. solani* isolate on the subsequent mycelium growth from sclerotia. Biofumigants at 5 and 10% for LUPP2519 resulted in the greater subsequent mycelium growth suppression (82.6-83.4%) compared with that at 1% (64.6%), while biofumigants at 5% had the greatest suppression of LUPP2522 (86.6%), followed by that at 10% (80.2%). There was a significant interaction (P<0.001) between biofumigant crop and *R. solani* isolate on the subsequent mycelium growth from sclerotia. The subsequent mycelium growth inhibition by 'Caliente' mustard was significantly greater for LUPP2519 (85.8%) than that for LUPP2522 (81.4%), whereas brown mustard and 'Nemat' arugula had the greater subsequent mycelium growth reduction for LUPP2522 (75% from brown mustard and 78% from 'Nemat' arugula) compared with that for LUPP2519 (72.3-72.4%). There was a significant interaction (P<0.001) between biofumigant crop and biofumigant amount on the subsequent mycelium growth from sclerotia. 'Caliente' mustard at 10% significantly reduced mycelium growth (91.87% inhibition) compared with that achieved with either 5% or 1% (88.5% and 70.4%, respectively). In contrast for both brown mustard and 'Nemat' arugula 5% resulted in significantly greater growth inhibition (80.5% and 86.0%, respectively) compared with both 1% (66.1% and 61.8%, respectively) and 10% (74.6% and 77.7%, respectively).

There was a significant effect (P<0.001) of biofumigant crop on the subsequent mycelium growth from sclerotia, with 'Caliente' mustard having the greatest inhibition (83.6%), followed by 'Nemat' arugula (75.2%) and brown mustard (73.7%). There was a significant effect (P<0.001) of biofumigant amount on the subsequent mycelium growth from sclerotia, with biofumigants at 5% having the greatest inhibition (85.0%), followed by that at 10% (81.4%). There was a significant effect (P<0.001) of *R. solani* isolate on the subsequent mycelium growth from sclerotia, with LUPP2522 being more sensitive to volatiles from biofumigants (78.1% inhibition) compared with LUPP2519 (76.9% inhibition).

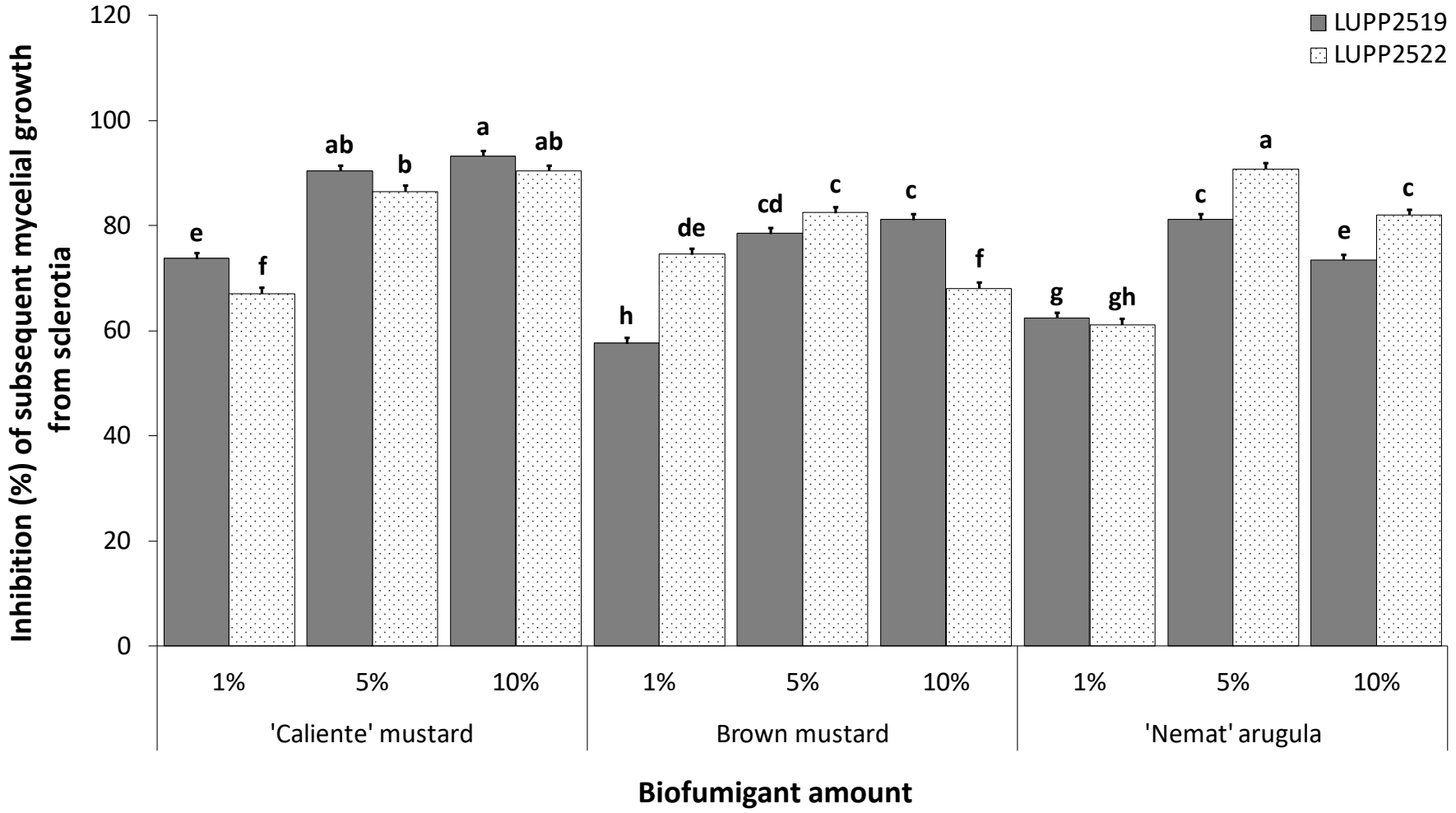


Figure D.2.9 Mean inhibition (%) of subsequent mycelium growth from sclerotia of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.2.

D.2.10 Biofumigation effect of different amount of *Brassica* plants amended in soil on subsequent mycelium growth of *Rhizoctonia solani* colonised barley grains

The statistical analyses of the inhibition of subsequent mycelium growth from colonised barley grains are presented in Appendix C.2.27, and the results are summarised in Appendix C.2.51. There was a significant interaction (P<0.001) between biofumigant crop, biofumigant amount and *R. solani* isolate on the subsequent mycelium growth from colonised barley grains. 'Caliente' mustard at 5 and 10% significantly suppressed the subsequent mycelium growth of both *R. solani* isolates (78.0-82.3% inhibition) compared with that at 1% (31.8-39.1% inhibition). Brown mustard at 5 and 10% (66.7-69.7% inhibition for LUPP2519 and 63.7-66.2% inhibition for LUPP2522) significantly inhibited the subsequent mycelium growth of both *R. solani* isolates compared to that with 1% incorporation (37.5% inhibition for LUPP2519 and 14.7% inhibition for LUPP2522). However, for' Nemat' arugula there was significant difference in the inhibition of subsequent mycelium growth for both *R. solani* isolates at 10% (76.5-77.7% inhibition) in comparison with that at 5% (67.2-67.4% inhibition) and 1% (55.3-56.4% inhibition) (Figure D.2.10).

There was a significant interaction (P<0.001) between biofumigant amount and *R. solani* isolate on the subsequent mycelium growth from colonised barley grains. There was no significant difference in the mycelium growth inhibition for the two *R. solani* isolates by biofumigants at 10% (75.5% and 73.4, respectively for LUPP2419 and LUPP2522) however at 5% and 1% LUPP2519 (73.0% and 44.0%, respectively) was significantly more inhibited compared with LUPP2522 (70.4% and 34.3%, respectively). There was a significant interaction (P<0.001) between biofumigant crop and *R. solani* isolate on the subsequent mycelium growth from colonised barley grains. Both 'Caliente' and brown mustards resulted in significantly greater inhibition of the mycelium growth of LUPP2519 compared with LUPP2522. In contrast, with 'Caliente' mustard, there was no significant difference in the inhibition of the mycelium growth of LUPP2519 compared with LUPP2522. There was a significant interaction (P<0.001) between biofumigant crop and biofumigant amount on the subsequent mycelium growth from colonised barley grains. For both 'Caliente' and brown mustards at 5 and 10% both significantly reduced the mycelium growth, and mycelium growth (79.9 and 81.1%, respectively for 'Caliente' mustard and 65.2% and 67.9%, respectively for brown mustard) compared with 1% (35.5% and 26.1%, respectively for 'Caliente' and brown mustards). In contrast for 'Nemat' arugula 10% resulted in significantly greater growth inhibition (77.1%) compared with both 5% (67.3%) and 1% (55.9%).

There was a significant effect (P<0.001) of biofumigant crop on the subsequent mycelium growth from colonised barley grains. Both 'Caliente' mustard and 'Nemat' arugula significantly reduced mycelium growth (65.5-66.7% inhibition) compared with brown mustard (53.1% inhibition). There was a significant effect (P<0.001) of biofumigant amount on the subsequent mycelium growth from colonised barley grains, with biofumigants at 10% having the greatest inhibition (74.4%). There was a significant effect (P<0.001) of *R. solani* isolate on the subsequent mycelium growth from colonised barley grains, with LUPP2519 being more sensitive to volatiles from the biofumigants (64.2% inhibition) compared with LUPP2522 (59.4% inhibition).

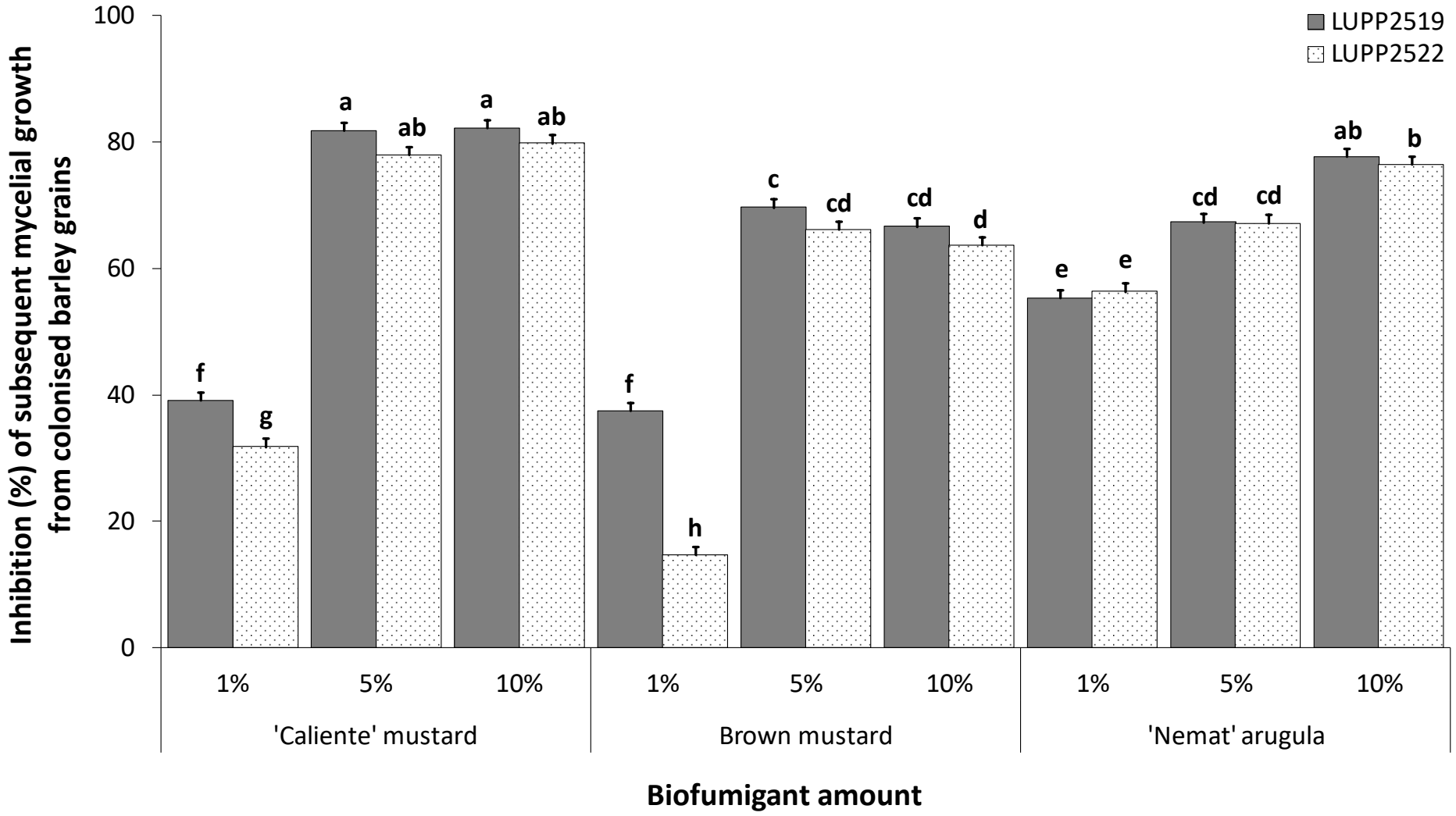


Figure D.2.10 Mean inhibition (%) of subsequent mycelium growth from colonised barley grains of two *Rhizoctonia solani* isolates LUPP2519 (AG3-PT) and LUPP2522 (AG2-1) after 7 days exposure to volatiles from macerated tissue of three biofumigant crops incorporated into soil at 1, 5 and 10% (w:w). Bars with the same letter are not significantly different based on Tukey's HSD test (*P*=0.05). Error bars indicate LSD (*P*=0.05) = 2.7

Appendices for chapter 3

A.3.1 Soil pH titration curve constructions

The initial soil pH (5.9) was determined as described in Appendix B.2.4 (Chapter 2)**.** The required soil pH levels of 4.5, 5.5, and 6.5 were produced by adjusting the soil pH value with either $Ca(OH)_2$ (to increase pH), and H_2SO_4 (to decrease pH) depending on the initial pH value of the soil as described by Watanabe *et al.* (2011). Separate samples of 100 g of air-dried soil were thoroughly mixed with $Ca(OH)_2$ solution at a range of concentrations being 0, 2, 6, 12, and 20 mmol, or with H_2SO_4 at concentrations of 0, 10, 30, 40, 60 mmol, in 250-mL plastic pots. Millipore water was added to each soil sample to achieve 100% WHC. The soil samples in the plastic pots were left at room temperature for 7 days to equilibrate. Then, subsamples from each soil (7 g) were taken and the pH measured. Based on the measured pH values, soil titration curves were calculated and used to estimate the amount of $Ca(OH)_2$ or H_2SO_4 to adjust the soil pH to the required value (Watanabe *et al.*, 2011). The treated soils with the desired pH values were incubated at 22°C for 4 weeks, with the pH measured weekly. Then the soils were air-dried and passed through a 2-mm mesh sieve, and stored in the dark at 4°C until used (Rooney *et al.*, 2007). Three final soil pH levels, 4.8, 5.8, and 6.6, were used for the experiment (Figure A.3.1).

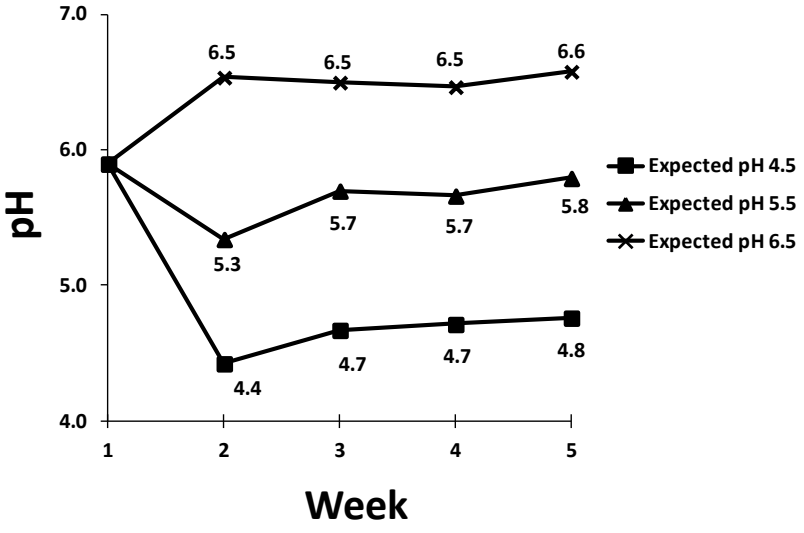
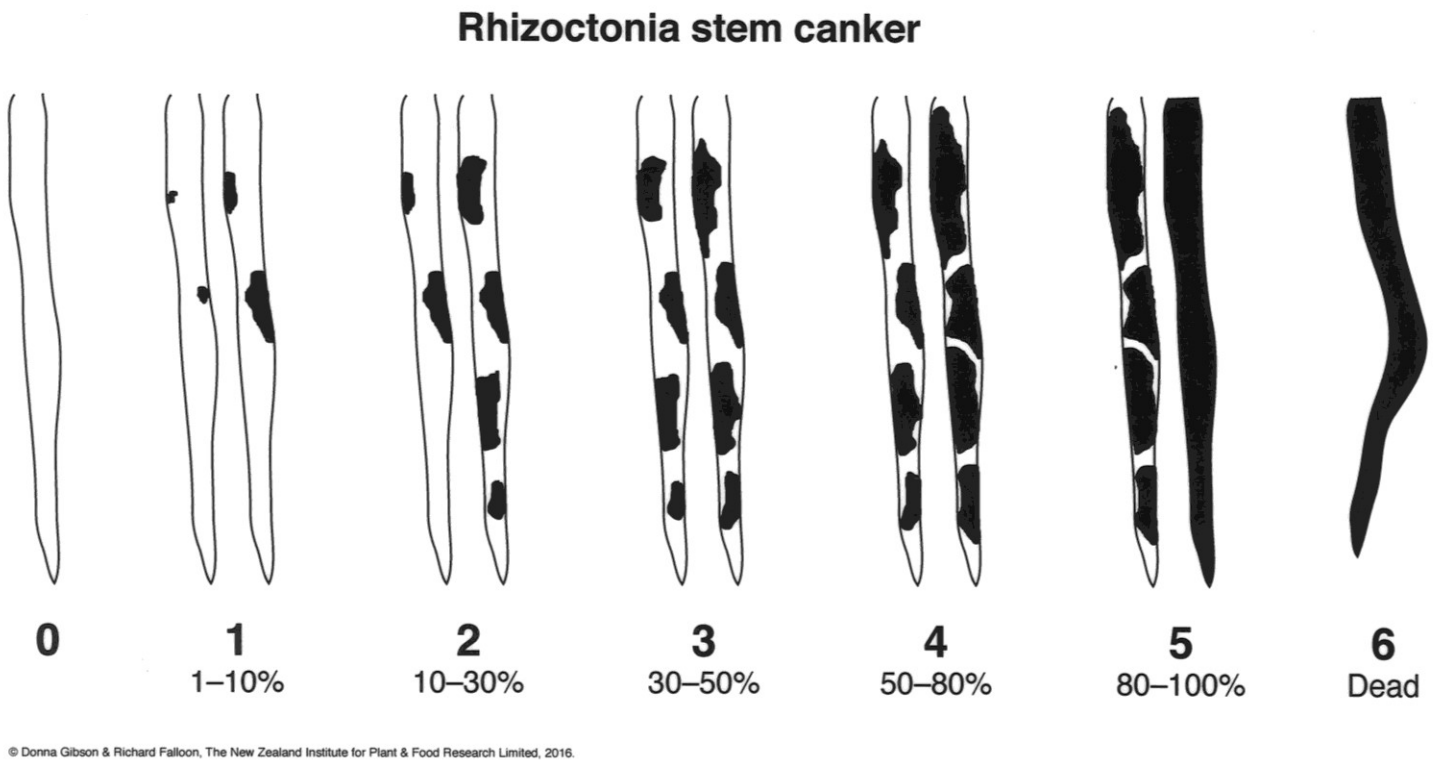
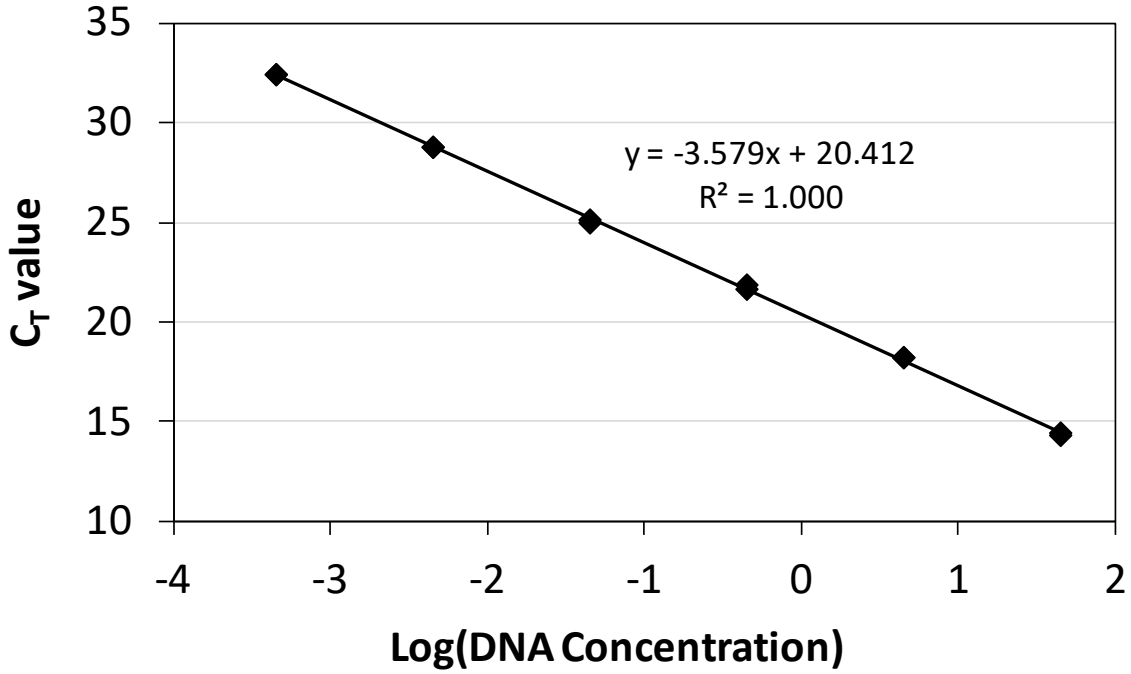


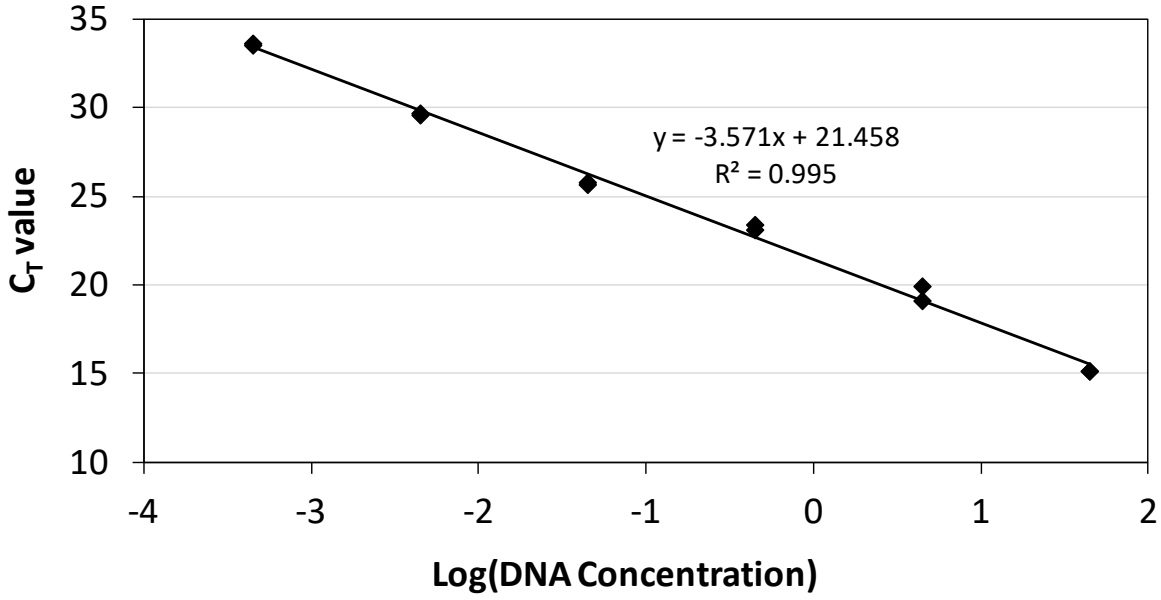
Figure A.3.1 Changes in soil pH during 4 weeks of measurements (week 1: starting point of soil pH; week 1-5 relative to 4 week measurements).

A.3.2 *Rhizoctonia solani* stem canker scores for potato plants (Gibson and Falloon, 2016).

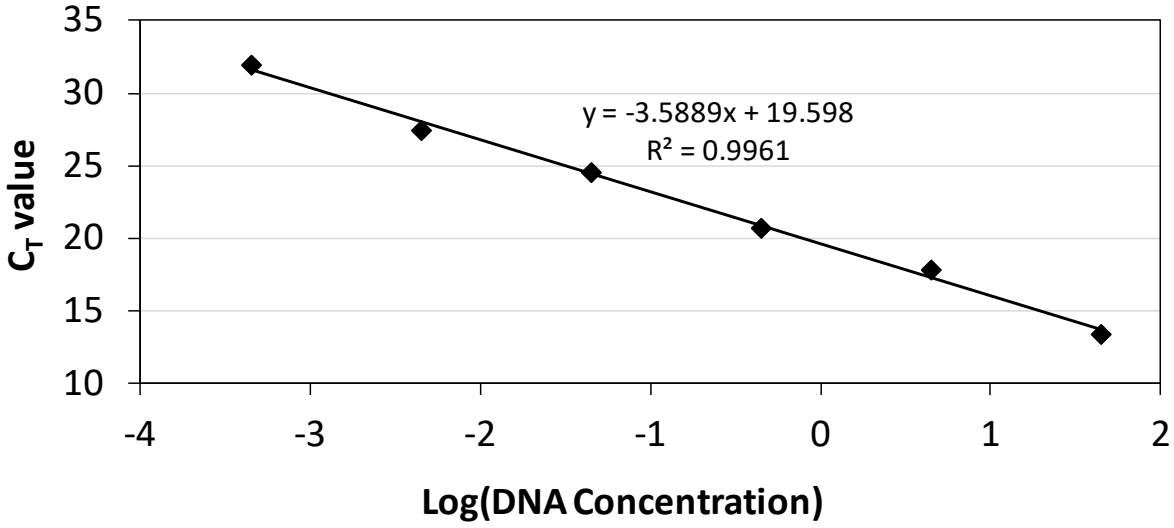




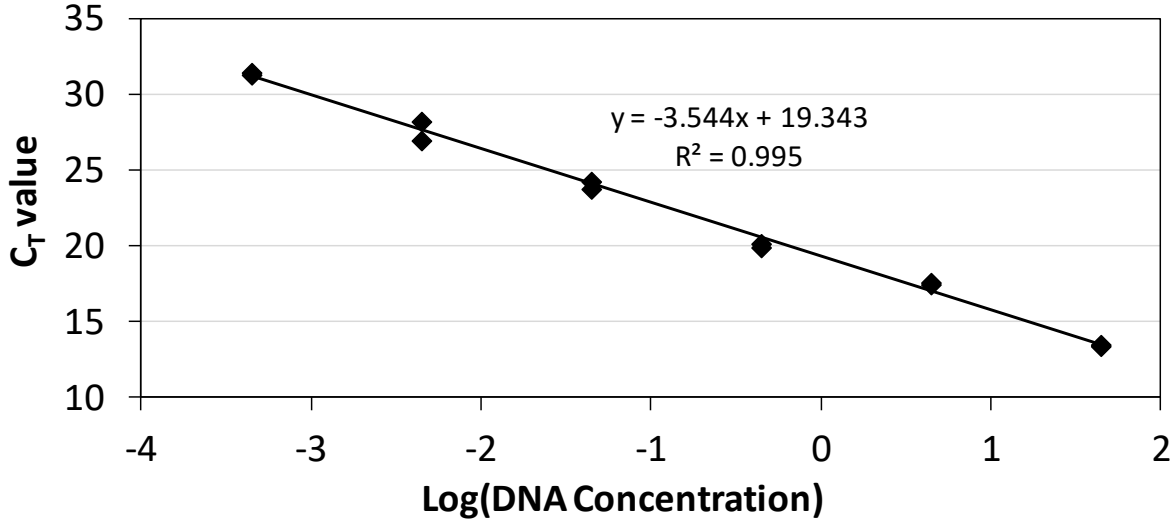
A.3.3 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 44.40, 4.44, 4.44 x 10^{-1}, 4.44 x 10^{-2}, 4.44 x 10^{-3} or 4.44 x 10^{-4} ng per well used with samples in Section 3.2.4.1. The DNA concentrations were log(X) transformed before being plotted against the cycle threshold values to generate the standard curve. PCR amplification efficiency = 90.3%.



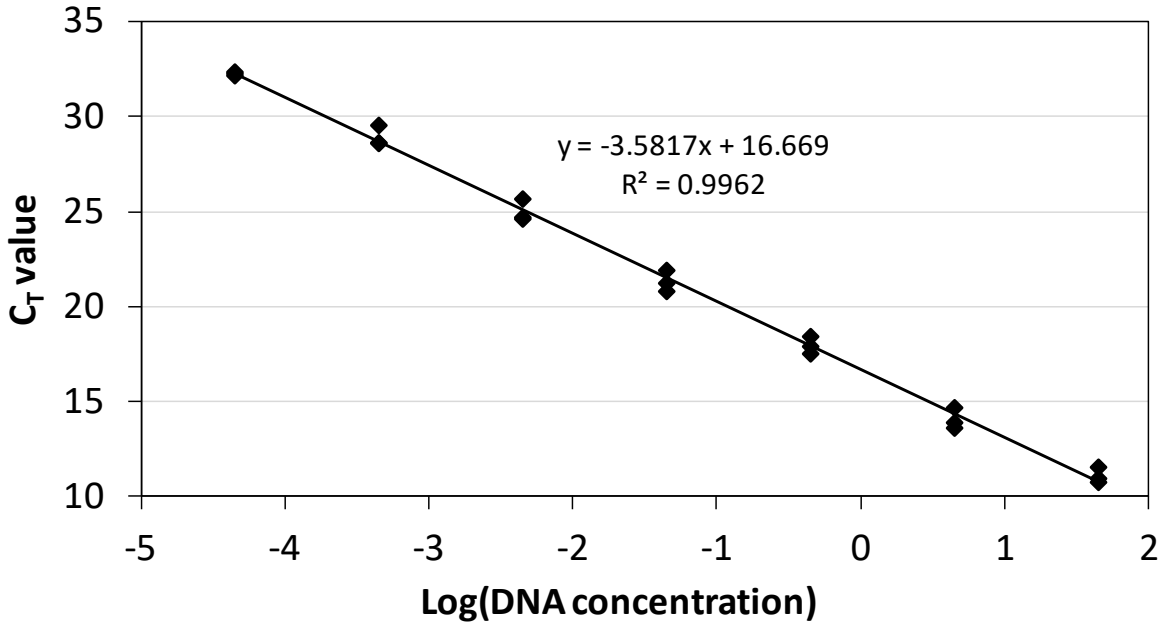
A.3.4 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 44.40, 4.44, 4.44 x 10^{-1}, 4.44 x 10^{-2}, 4.44 x 10^{-3} or 4.44 x 10^{-4} ng per well used with samples in Section 3.2.4.2. The DNA concentrations were log(X) transformed before being plotted against with the cycle threshold values to generate the standard curve. PCR amplification efficiency = 90.6%.



A.3.5 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 44.40, 4.44, 4.44 x 10^{-1}, 4.44 x 10^{-2}, 4.44 x 10^{-3} or 4.44 x 10^{-4} ng per well used with samples in Section 3.2.4.3. The DNA concentrations were log(X) transformed before being plotted against the cycle threshold values to generate the standard curve. PCR amplification efficiency = 90.0%.



A.3.6 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 44.40, 4.44, 4.44 x 10^{-1}, 4.44 x 10^{-2}, 4.44 x 10^{-3} or 4.44 x 10^{-4} ng per well used with samples in Section 3.2.4.4. The DNA concentrations were log(X) transformed before being plotted against the cycle threshold values to generate the standard curve. PCR amplification efficiency = 91.5%.



A.3.7 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 45.40, 4.54, 4.54 x 10^{-1}, 4.54 x 10^{-2}, 4.54 x 10^{-3}, 4.54 x 10^{-4} or 4.54 x 10^{-5} ng per well used with samples in Section 3.2.4.5. The DNA concentrations were log(X) transformed before being plotted against the cycle threshold values to generate the standard curve. PCR amplification efficiency = 90.2%.

A.3.8 Soil microbial activity (Soil dehydrogenase activity)

The activity of soil microorganisms was determined by measuring dehydrogenase activity as described by (Cresswell and Hassall, 2015). In principal, under soil anaerobic conditions (without O_2), 2,3,5-triphenyltetrazolium chloride (TTC) is an H receptor for dehydrogenase systems, and TTC (colourless) will be reduced to triphenyltetrazoliumformazan (TPF, red colour) (Benefield *et al.*, 1977). The intensity of the colour due to the concentration of TPF in the soil samples was read at 485 nm in a spectrophotometer (Cresswell and Hassall, 2015).

Briefly, soil samples were sieved through a 2 mm mesh, mixed thoroughly, and stored at 4°C prior to being processed. Subsamples of soil were firstly taken for moisture (dried at 105°C for 24 hours) and pH (Appendix B.2.4, Chapter 2) determinations. Samples (2 g) of each soil sample were placed into a test tube (duplicate for each sample). Then, 2 mL of 1% TTC (dissolved in AR grade tris(hydroxymethyl)aminomethane (Tris) with the solution pH adjusted based on the pH of the soil samples measured initially) was added to each test tube. The test tubes were covered tightly and vortex thoroughly to mix the soil sample with the 1% TTC solution. The test tubes were then incubated at 25°C for 24 hours. Ten millilitres of methanol was added to each test tube and the test tubes were shaken by hand. The upper liquid in each test tube was carefully poured off into a new 15 mL centrifuge tube. The 15 mL tubes were then centrifuged (Kubota 8420, Kubota Corporation, Japan) at 1,880 *g* for 10 minutes. The liquid from the top layer in the centrifuge tubes was removed and the absorbance at 485 nm was measured in a UV-VIS spectrophotometer (UVmini-1240, Shimadzu, Japan) connected with a sipper unit.

The TPF standard curve was created using a TPF concentration series (dissolved in methanol) of0, 5, 10, 20, 30, and 40 μg/mL (Cresswell and Hassall, 2015). Then based on the standard curve the dehydrogenase activity (DHA) was calculated using the following formula (Cresswell and Hassall, 2015):

DHA (μg/g dried soil.hr^{-1}) = [(a-b) x 1200]/[24 x m x DM]

Where: a = TPF concentration of soil sample (μg/mL)

b = average of blanks (Tris buffer, μg/mL)

m = weight of sample (g)

DM = soil dried matter content (%).

A.3.9 Analysis of variance of the influence of soil type on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00006	0.00003	3.0	
Treatment	3	1.96662	0.65554	64567.5	<.001
Soil texture	2	5.49423	2.74712	270600.0	<.001
Treatment.Soil texture	6	0.68210	0.11368	11197.2	<.001
Residual	22	0.00022	0.00001		
Total	35	8.14323			

A.3.10 Analysis of variance of the influence of soil pH on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00008	0.00004	1.5	
Treatment	3	2.61100	0.87040	32938.8	<.001
Soil pH	2	8.39300	4.19700	158800.0	<.001
Treatment.Soil pH	6	2.37100	0.39510	14952.8	<.001
Residual	22	0.00058	0.00003		
Total	35	13.38000			

A.3.11 Analysis of variance of the influence of soil temperature on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 28 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00005	0.00003	0.8	
Treatment	3	0.34290	0.11430	3405.9	<.001
Temperature (Temp)	2	0.49602	0.24801	7390.1	<.001
Treatment.Temp	6	0.28672	0.04779	1423.9	<.001
Residual	22	0.00074	0.00003		
Total	35	1.12643			

A.3.12 Analysis of variance of the influence of soil water holding capacity on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00001	0.000005	0.7	
Treatment	3	4.43100	1.477000	201500.0	<.001
Water holding capacity (WHC)	2	0.01162	0.005810	792.5	<.001
Treatment.WHC	6	0.71760	0.119600	16314.1	<.001
Residual	22	0.00016	0.000007		
Total	35	5.16100			

A.3.13 Analysis of variance of the influence of soil water holding capacities (40 or 70% WHC) and temperatures (15 or 22°C) on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 14 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0502	0.025	5.3	
Treatment	1	1.0271	1.027	216.4	<.001
Temperature (Temp)	1	1.4731	1.473	310.4	<.001
Water holding capacity (WHC)	1	0.0496	0.050	10.5	0.006
Treatment.Temp	1	0.0607	0.061	12.8	0.003
Treatment.WHC	1	0.1746	0.175	36.8	<.001
Temp.WHC	1	0.0673	0.067	14.2	0.002
Treatment.Temp.WHC	1	0.0646	0.065	13.6	0.002
Residual	14	0.0664	0.005		
Total	23	3.0337			

A.3.14 Analysis of variance of the influence of soil water holding capacities (40 or 70% WHC) and temperatures (15 or 22°C) on biofumigation effects on *Rhizoctonia solani* AG3-PT DNA amounts after 28 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.004	0.002	0.7	
Treatment	1	1.657	1.657	546.8	<.001
Temperature (Temp)	1	0.829	0.829	273.5	<.001
Water holding capacity (WHC)	1	0.001	0.001	0.3	0.578
Treatment.Temp	1	0.119	0.119	39.3	<.001
Treatment.WHC	1	0.082	0.082	27.1	<.001
Temp.WHC	1	0.057	0.057	18.7	<.001
Treatment.Temp.WHC	1	0.001	0.001	0.5	0.495
Residual	14	0.042	0.003		
Total	23	2.792			

A.3.15 Analysis of variance of the influence of soil type on biofumigation effects on soil microbial activity after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.0007	0.0004	3.4	
Treatment	4	2.8802	0.7200	6597.4	<.001
Texture	2	11.8055	5.9028	54083.7	<.001
Treatment.Texture	8	3.4240	0.4280	3921.6	<.001
Residual	28	0.0031	0.0001		
Total	44	18.1136			

A.3.16 Analysis of variance of influence of soil pH on biofumigation effects on soil microbial activity after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00002	0.000009	0.04	
Treatment	4	1.42667	0.356667	1688.94	<.001
pH	2	2.63186	1.315932	6231.37	<.001
Treatment.pH	8	0.49105	0.061381	290.66	<.001
Residual	28	0.00591	0.000211		
Total	44	4.55551			

A.3.17 Analysis of variance of influence of soil temperature on biofumigation effects on soil microbial activity after 28 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.002	0.0008	0.5	
Treatment	4	10.974	2.7434	1770.3	<.001
Temperature (Temp)	2	2.555	1.2777	824.5	<.001
Treatment.Temp	8	2.268	0.2835	183.0	<.001
Residual	28	0.043	0.0016		
Total	44	15.842			

A.3.18 Analysis of variance of the influence of soil water holding capacity on biofumigation effects on microbial activity after 28 days incubation at 22°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00003	0.00002	0.2	
Treatment	4	0.14265	0.03566	504.9	<.001
Water holding capacity (WHC)	2	1.33664	0.66832	9461.8	<.001
Treatment.WHC	8	0.18504	0.02313	327.5	<.001
Residual	28	0.00198	0.00007		
Total	44	1.66634			

A.3.19 Analysis of variance of the influence of soil water holding capacities (40 or 70% WHC) and temperatures (15 or 22°C) on biofumigation effects on soil microbial activity after 14 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.00019	0.00009	0.2	
Treatment	3	0.30556	0.10185	244.2	<.001
Temperature (Temp)	1	0.45518	0.45518	1091.2	<.001
Water holding capacity (WHC)	1	0.13581	0.13581	325.6	<.001
Treatment.Temp	3	0.07964	0.02655	63.6	<.001
Treatment.WHC	3	0.22229	0.07410	177.6	<.001
Temp.WHC	1	0.18575	0.18575	445.3	<.001
Treatment.Temp.WHC	3	0.07275	0.02425	58.1	<.001
Residual	30	0.01251	0.00042		
Total	47	1.46968			

A.3.20 Analysis of variance of the influence of soil water holding capacities (40 or 70% WHC) and temperatures (15 or 22°C) on biofumigation effects on soil microbial activity after 28 days incubation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.0007	0.0003	0.6	
Treatment	3	1.0118	0.3373	622.9	<.001
Temperature (Temp)	1	4.1212	4.1212	7611.3	<.001
Water holding capacity (WHC)	1	0.1354	0.1354	250.1	<.001
Treatment.Temp	3	1.3183	0.4394	811.6	<.001
Treatment.WHC	3	0.0747	0.0249	46.0	<.001
Temp.WHC	1	0.1121	0.1121	207.1	<.001
Treatment.Temp.WHC	3	0.1012	0.0337	62.3	<.001
Residual	30	0.0162	0.0005		
Total	47	6.8916			

A.3.21 Analysis of variance of potato stem severity (%) assessed on potato plants at 35 days after planting in soil previously inoculated with *Rhizoctonia solani* AG3-PT colonised grain fragments (inoculated control) or heat treated colonised grain fragments (heat treated inoculum), amended with 'Caliente' mustard and incubated at two different temperatures (15 or 22°C) in shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.003	0.002	0.4	
Treatment	1	0.070	0.070	17.9	<.001
Temperature (Temp)	1	0.043	0.043	10.9	0.005
Water holding capacity (WHC)	1	0.003	0.003	0.7	0.411
Treatment.Temp	1	0.026	0.026	6.6	0.022
Treatment.WHC	1	0.005	0.005	1.3	0.283
Temp.WHC	1	0.001	0.001	0.3	0.595
Treatment.Temp.WHC	1	0.010	0.010	2.5	0.134
Residual	14	0.055	0.004		
Total	23	0.215			

A.3.22 Analysis of variance of potato plant height (cm) assessed 35 days after planting in soil previously inoculated with *Rhizoctonia solani* AG3-PT colonised grain fragments (inoculated control) or heat treated colonised grain fragments (heat treated inoculum), amended with 'Caliente' mustard and incubated at two temperatures (15 or 22°C) in shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.4861	0.243	0.9	
Treatment	3	9.296	3.0987	11.54	<.001
Temperature (Temp)	1	4.9565	4.9565	18.45	<.001
Water holding capacity (WHC)	1	0	0	0	0.993
Treatment.Temp	3	6.4868	2.1623	8.05	<.001
Treatment.WHC	3	1.976	0.6587	2.45	0.083
Temp.WHC	1	2.2314	2.2314	8.31	0.007
Treatment.Temp.WHC	3	0.6315	0.2105	0.78	0.512
Residual	30	8.058	0.2686		
Total	47	34.1224			

A.3.23 Analysis of variance of potato plant dry biomass (g) assessed 35 days after planting in soil previously inoculated with *Rhizoctonia solani* and incubated at two different temperatures (15 or 22°C) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.1192	0.0596	2.4	
Treatment	3	7.7212	2.5737	101.9	<.001
Temperature (Temp)	1	2.1172	2.1172	83.8	<.001
Water holding capacity (WHC)	1	0.2219	0.2219	8.8	0.006
Treatment.Temp	3	1.4803	0.4934	19.5	<.001
Treatment.WHC	3	0.1485	0.0495	2.0	0.141
Temp.WHC	1	0.0127	0.0127	0.5	0.484
Treatment.Temp.WHC	3	0.0658	0.0219	0.9	0.468
Residual	30	0.7577	0.0253		
Total	47	12.6444			

A.3.24 Representative results of qPCR measurements in soil type affected to biofumigation.

Treatment	Soil type	Rep	CT values
Uninoculated	Loam	1	Undetermined
Nil	Loam	1	Undetermined
Nil	Loam	2	Undetermined
Nil	Loam	2	Undetermined
Nil	Loam	3	Undetermined
Nil	Loam	3	Undetermined
Nil	Loamy sand	1	Undetermined
Nil	Loamy sand	1	Undetermined
Nil	Loamy sand	2	Undetermined
Nil	Loamy sand	2	Undetermined
Nil	Loamy sand	3	Undetermined
Nil	Loamy sand	3	Undetermined
Nil	Clay loam	1	Undetermined
Nil	Clay loam	1	Undetermined
Nil	Clay loam	2	Undetermined
Nil	Clay loam	2	Undetermined
Nil	Clay loam	3	Undetermined
Nil	Clay loam	3	Undetermined
RS	Loam	1	16.6230
RS	Loam	1	16.6437
RS	Loam	2	16.6536
RS	Loam	2	16.5624
RS	Loam	3	16.5730
RS	Loam	3	16.5632
RS	Loamy sand	1	16.6521
RS	Loamy sand	1	16.6515
RS	Loamy sand	2	16.6693
RS	Loamy sand	2	16.6517
RS	Loamy sand	3	16.7068
RS	Loamy sand	3	16.6159
RS	Clay loam	1	16.5836
RS	Clay loam	1	16.6853
RS	Clay loam	2	16.6141
RS	Clay loam	2	16.6527
RS	Clay loam	3	16.5101
RS	Clay loam	3	16.6629
Nil	Loam	1	37.2984
Nil	Loam	1	37.1934
Nil	Loam	2	37.2371
Nil	Loam	2	37.2527
Nil	Loam	3	37.2407

A.3.24 continued

Treatment	Soil type	Rep	CT values
Nil	Loam	3	37.2347
Nil	Loamy sand	1	37.6853
Nil	Loamy sand	1	37.2420
Nil	Loamy sand	2	37.4937
Nil	Loamy sand	2	37.4585
Nil	Loamy sand	3	37.4825
Nil	Loamy sand	3	37.4384
Nil	Clay loam	1	37.8837
Nil	Clay loam	1	37.6926
Nil	Clay loam	2	37.7540
Nil	Clay loam	2	37.8822
Nil	Clay loam	3	37.7663
Nil	Clay loam	3	37.8787
RS	Loam	1	24.3867
RS	Loam	1	24.3569
RS	Loam	2	24.3561
RS	Loam	2	24.3513
RS	Loam	3	24.3276
RS	Loam	3	24.3658
RS	Loamy sand	1	23.2470
RS	Loamy sand	1	23.2655
RS	Loamy sand	2	23.2623
RS	Loamy sand	2	23.2566
RS	Loamy sand	3	23.2387
RS	Loamy sand	3	23.2806
RS	Clay loam	1	22.6948
RS	Clay loam	1	22.6962
RS	Clay loam	2	22.6989
RS	Clay loam	2	22.6986
RS	Clay loam	3	22.6989
RS	Clay loam	3	22.6955
Caliente	Loam	1	28.5877
Caliente	Loam	1	28.5027
Caliente	Loam	2	28.5331
Caliente	Loam	2	28.5409
Caliente	Loam	3	28.5430
Caliente	Loam	3	28.5376
Caliente	Loamy sand	1	25.7622
Caliente	Loamy sand	1	25.8227
Caliente	Loamy sand	2	25.7635

A.3.24 continued

Treatment	Soil type	Rep	CT values
Caliente	Loamy sand	2	25.8243
Caliente	Loamy sand	3	25.7635
Caliente	Loamy sand	3	25.8196
Caliente	Clay loam	1	23.0468
Caliente	Clay loam	1	23.0344
Caliente	Clay loam	2	23.0230
Caliente	Clay loam	2	23.0581
Caliente	Clay loam	3	23.0652
Caliente	Clay loam	3	23.0163
Brown mustard	Loam	1	27.6492
Brown mustard	Loam	1	27.8381
Brown mustard	Loam	2	27.7359
Brown mustard	Loam	2	27.7573
Brown mustard	Loam	3	27.7306
Brown mustard	Loam	3	27.7650
Brown mustard	Loamy sand	1	24.9261
Brown mustard	Loamy sand	1	24.9204
Brown mustard	Loamy sand	2	24.8963
Brown mustard	Loamy sand	2	24.9162
Brown mustard	Loamy sand	3	24.9882
Brown mustard	Loamy sand	3	24.8541
Brown mustard	Clay loam	1	23.6488
Brown mustard	Clay loam	1	23.5594
Brown mustard	Clay loam	2	23.5872
Brown mustard	Clay loam	2	23.5897
Brown mustard	Clay loam	3	23.5501
Brown mustard	Clay loam	3	23.6551
Nemat	Loam	1	29.2661
Nemat	Loam	1	29.2718
Nemat	Loam	2	29.2671
Nemat	Loam	2	29.2556
Nemat	Loam	3	29.2322
Nemat	Loam	3	29.3290
Nemat	Loamy sand	1	25.5858
Nemat	Loamy sand	1	25.6560
Nemat	Loamy sand	2	25.6987
Nemat	Loamy sand	2	25.5867
Nemat	Loamy sand	3	25.7446
Nemat	Loamy sand	3	25.5283
Nemat	Clay loam	1	23.4529
Nemat	Clay loam	1	23.6930

A.3.24 continued

Treatment	Soil type	Rep	CT values
Nemat	Clay loam	2	23.6112
Nemat	Clay loam	2	23.5536
Nemat	Clay loam	3	23.6056
Nemat	Clay loam	3	23.5329
AG2-1			Undetermined
AG2-1			Undetermined
PCR water			Undetermined
PCR water			Undetermined

Appendices for chapter 4

A.4.1 The 10-year cropping history of the potato field (Pendarves, Ashburton, Canterbury, New Zealand) from which field soil was obtained for the shadehouse experiment (information provided by Sarah Sinton, Plant & Food Research, Lincoln).

Year	Crop	Soil collected
2016-2017	Winter wheat	October 2016
2015-2016	'Innovator' potatoes	
2014-2015	Winter wheat	
2013-2014	Canola	
2012-2013	Winter Wheat	
2011-2012	Ryegrass	
2010-2011	Wheat	
2009-2010	Potatoes	
2008-2009	Wheat	
2007-2008	Ryegrass	

A.4.2 Analysis of variance of DNA amounts of *Rhizoctonia solani* in soil before potato planting (T1), 35 days after potato planting (T2) and at harvest (134 days after potato planting, T3) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.001	0.000	1.1	
Treatment	6	38.667	6.445	16016.5	<.001
Samping time	2	0.282	0.141	350.1	<.001
Treatment.Samping time	12	1.321	0.110	273.7	<.001
Residual	60	0.024	0.000		
Total	83	40.296			

A.4.3 Analysis of variance of dehydrogenase activity (DHA) in soil before biofumigant planting (T0), before potato planting (T1), 35 days after potato planting (T2) and at harvest (134 days after potato planting, T3) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.0001	0.00003	0.04	
Treatment	6	1.3127	0.21878	275.85	<.001
Sampling time	3	8.1929	2.73098	3443.41	<.001
Treatment x Sampling time	18	0.9951	0.05529	69.71	<.001
Residual	81	0.0642	0.00079		
Total	111	10.5651			

A.4.4 Analysis of variance of stem canker of potato caused by *Rhizoctonia solani* at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.002	0.0007	1.2	
Treatment	6	0.162	0.0271	46.3	<.001
Residual	18	0.011	0.0006		
Total	27	0.175			

A.4.5 Analysis of variance of potato stolon disease caused by *Rhizoctonia solani* at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.003	0.0009	1.6	
Treatment	6	0.403	0.0671	123.9	<.001
Residual	18	0.010	0.0005		
Total	27	0.415			

A.4.6 Analysis of variance of potato root disease caused by *Rhizoctonia solani* at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.065	0.022	3.3	
Treatment	6	0.158	0.026	4.0	0.01
Residual	18	0.119	0.007		
Total	27	0.342			

A.4.7 Analysis of variance of stem canker of potato caused by *Rhizoctonia solani* at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.002	0.0006	0.9	
Treatment	6	0.356	0.0593	94.8	<.001
Residual	18	0.011	0.0006		
Total	27	0.369			

A.4.8 Analysis of variance of black scurf on potato tubers caused by *Rhizoctonia solani* at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.0008	0.0003	0.9	
Treatment	6	0.1345	0.0224	74.3	<.001
Residual	18	0.0054	0.0003		
Total	27	0.1407			

A.4.9 Analysis of variance of powdery scab on potato tubers caused by *Spongospora subterranea* at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.003	0.001	1.5	
Treatment	6	0.206	0.034	45.5	<.001
Residual	18	0.014	0.001		
Total	27	0.223			

A.4.10 Analysis of variance of potato root galls caused by *Spongospora subterranea* at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.011	0.004	0.2	
Treatment	6	0.296	0.049	2.4	0.069
Residual	18	0.368	0.020		
Total	27	0.675			

A.4.11 Analysis of variance of potato stem death incidence caused by *Colletotrichum coccodes* at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.006	0.002	2.7	
Treatment	6	0.112	0.019	23.9	<.001
Residual	18	0.014	0.001		
Total	27	0.133			

A.4.12 Analysis of variance of potato emergence day under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	7	0.726	0.104	1.2	
Treatment	6	0.892	0.149	1.7	0.156
Residual	42	3.773	0.090		
Total	55	5.391			

A.4.13 Analysis of variance of potato plant height at 35 days after planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.146	0.049	0.8	
Treatment	6	3.932	0.655	10.3	<.001
Residual	18	1.144	0.064		
Total	27	5.222			

A.4.14 Analysis of variance of potato plant height at harvest under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.079	0.026	0.5	
Treatment	6	5.751	0.958	16.9	<.001
Residual	18	1.023	0.057		
Total	27	6.852			

A.4.15 Analysis of variance of number of stems at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.007	0.002	0.1	
Treatment	6	0.372	0.062	1.4	0.283
Residual	18	0.820	0.046		
Total	27	1.198			

A.4.16 Analysis of variance of number of stems at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.546	0.182	1.2	
Treatment	6	1.095	0.183	1.2	0.372
Residual	18	2.844	0.158		
Total	27	4.485			

A.4.17 Analysis of variance of number of stolons at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.043	0.014	3.1	
Treatment	6	0.010	0.002	0.4	0.897
Residual	18	0.083	0.005		
Total	27	0.136			

A.4.18 Analysis of variance of root dry weight at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.077	0.026	2.0	
Treatment	6	0.245	0.041	3.1	0.028
Residual	18	0.235	0.013		
Total	27	0.557			

A.4.19 Analysis of variance of shoot dry weight at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.058	0.019	0.7	
Treatment	6	5.133	0.856	28.8	<.001
Residual	18	0.534	0.030		
Total	27	5.725			

A.4.20 Analysis of variance of total dry biomass (root + shoot) at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.061	0.020	0.7	
Treatment	6	5.213	0.869	27.7	<.001
Residual	18	0.564	0.031		
Total	27	5.839			

A.4.21 Analysis of variance of root : shoot ratio at 35 days after potato planting under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0023	0.0008	2.4	
Treatment	6	0.0038	0.0006	2.0	0.122
Residual	18	0.0057	0.0003		
Total	27	0.0117			

A.4.22 Analysis of variance of root dry weight at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.014	0.005	0.8	
Treatment	6	0.151	0.025	4.2	0.008
Residual	18	0.107	0.006		
Total	27	0.272			

A.4.23 Analysis of variance of shoot dry weight at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.053	0.018	0.3	
Treatment	6	10.687	1.781	31.7	<.001
Residual	18	1.012	0.056		
Total	27	11.752			

A.4.24 Analysis of variance of total dry biomass (root + shoot) at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	0.059	0.020	0.3	
Treatment	6	10.551	1.759	29.8	<.001
Residual	18	1.061	0.059		
Total	27	11.672			

A.4.25 Analysis of variance of root : shoot ratio at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	3	1.601E-05	5.338E-06	1.0	
Treatment	6	5.126E-04	8.543E-05	15.4	<.001
Residual	18	9.967E-05	5.537E-06		
Total	27	6.282E-04			

A.4.26 Analysis of variance of number of initials at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.061	0.020	0.2	
Treatment	6	11.769	1.961	22.3	<.001
Residual	18	1.587	0.088		
Total	27	13.417			

A.4.27 Analysis of variance of number of tubers at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.230	0.077	2.4	
Treatment	6	2.853	0.476	14.8	<.001
Residual	18	0.577	0.032		
Total	27	3.660			

A.4.28 Analysis of variance of total tuber weight at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.085	0.028	0.1	
Treatment	6	47.634	7.939	40.5	<.001
Residual	18	3.533	0.196		
Total	27	51.252			

A.4.29 Analysis of variance of weight of a tuber at harvest (134 days after potato planting) under shadehouse conditions.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.501	0.167	3.5	
Treatment	6	8.336	1.389	28.7	<.001
Residual	18	0.873	0.048		
Total	27	9.710			

A.4.30 The results of analyses of the field soil used in the shadehouse experiment.

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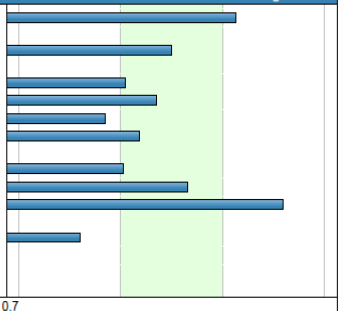
ANALYSIS REPORT Page 4 of 11

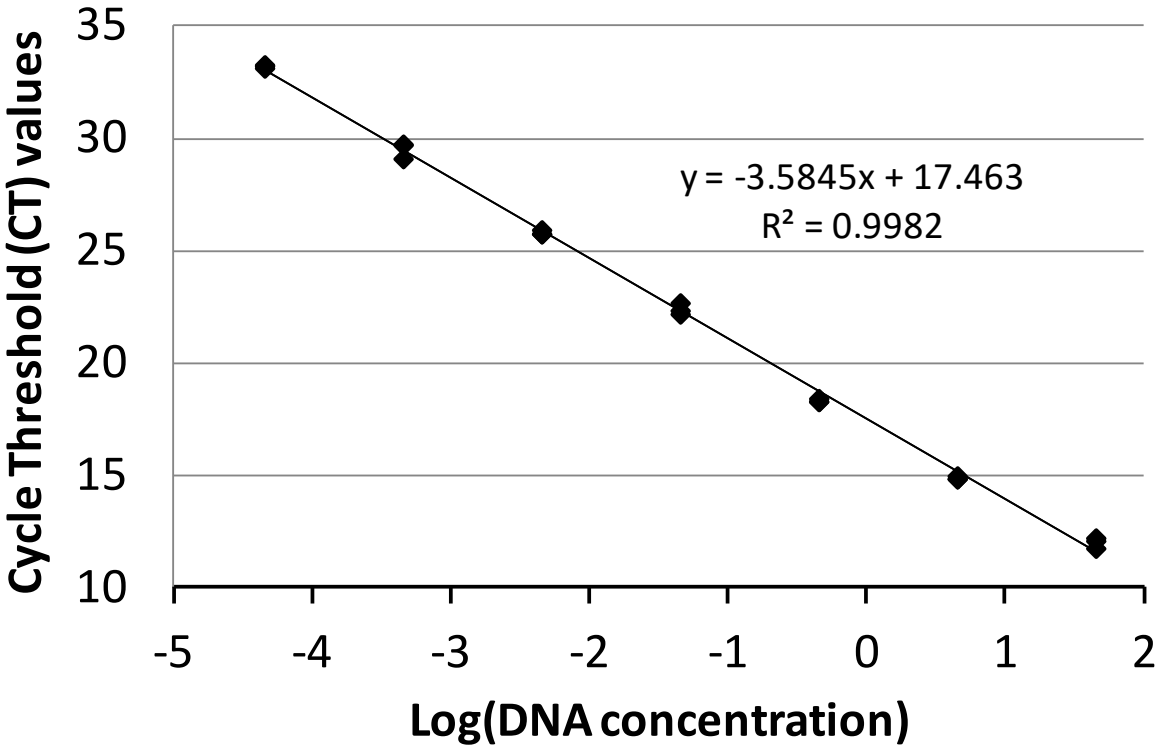
Client:	Lincoln University	**Lab No:**	1678543	shpv1
Address:	PO Box 85084	**Date Received:**	10-Nov-2016	
	Lincoln	**Date Reported:**	18-Nov-2016	
	Christchurch 7647	**Quote No:**		
		Order No:	LU439208	
		Client Reference:		
Phone:	03 325 2811 ext 8386	**Submitted By:**	Thanh Le	

Sample Name: GH **Lab Number:** 1678543.4
Sample Type: SOIL Potato (S64)

Analysis		Level Found	Medium Range
pH	pH Units	5.9	5.4 - 5.8
Olsen Phosphorus	mg/L	45	30 - 60
Potassium	me/100g	0.52	0.50 - 1.00
Calcium	me/100g	6.1	4.0 - 10.0
Magnesium	me/100g	0.89	1.00 - 3.00
Sodium	me/100g	0.09	0.00 - 0.50
CEC	me/100g	12	12 - 25
Total Base Saturation	%	61	35 - 75
Volume Weight	g/mL	1.15	0.60 - 1.00
Organic Matter*	%	4.2	7.0 - 17.0
Total Carbon*	%	2.5	
Soil Sample Depth*	mm	0-200	
Base Saturation %		K 4.2	Ca 49 Mg 7.2 Na 0.7
MAF Units		K 12	Ca 9 Mg 23 Na 5

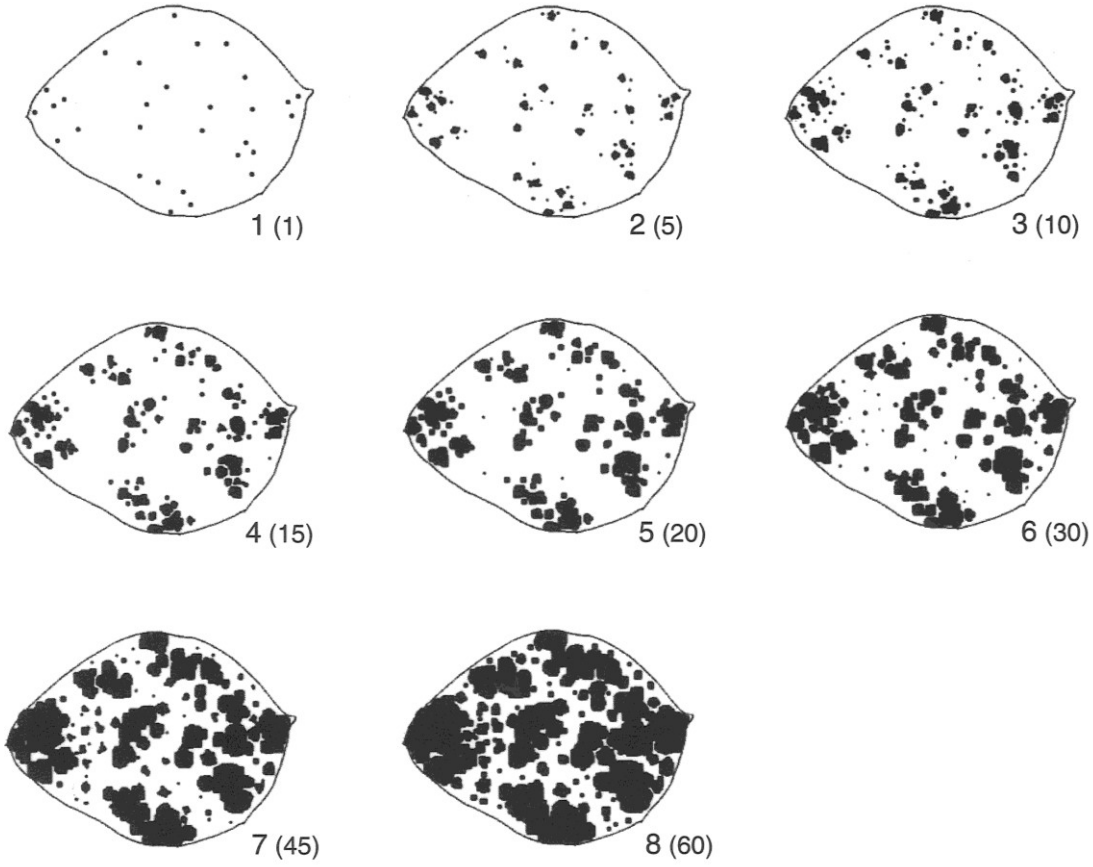


The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.

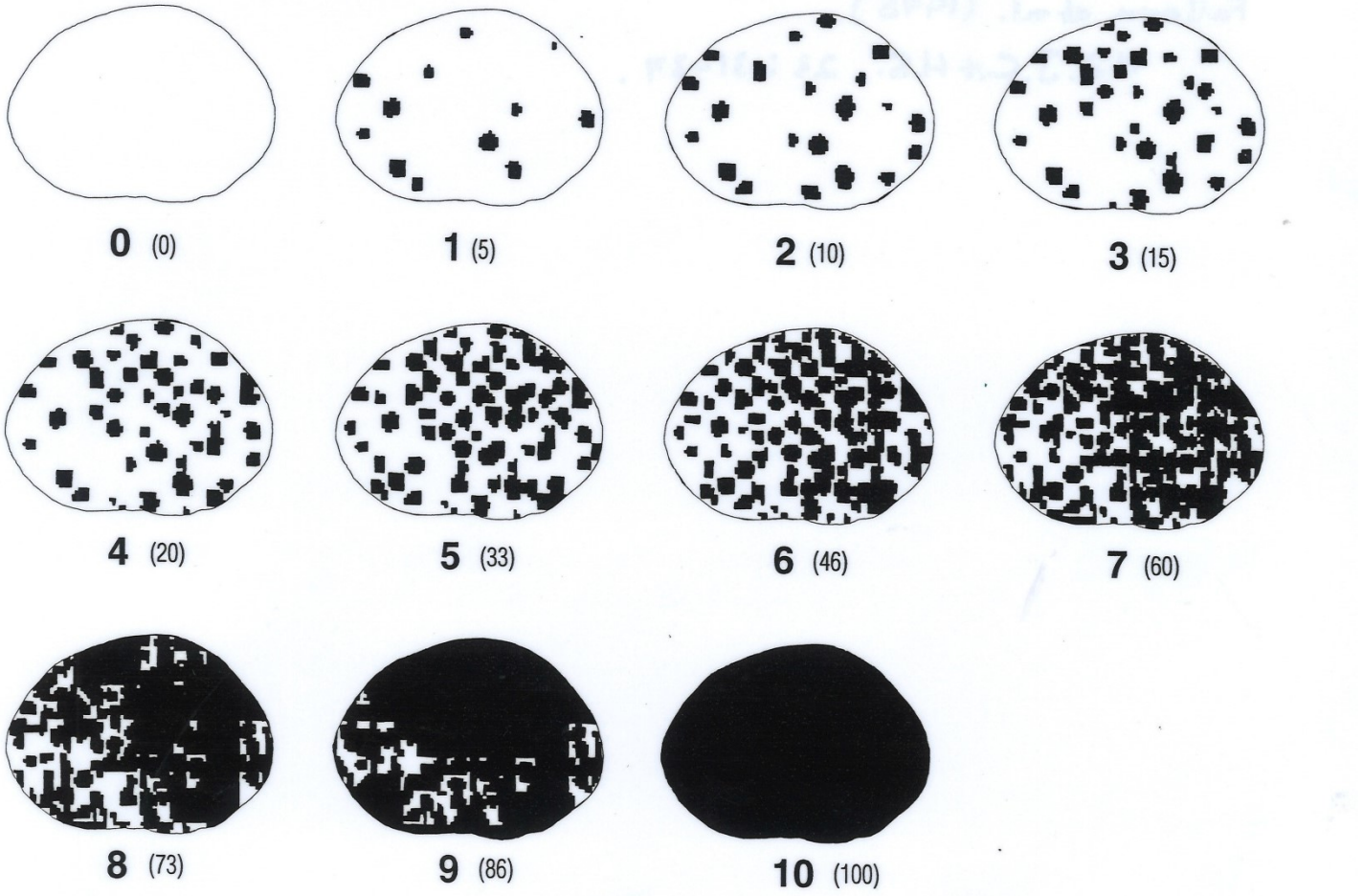


A.4.31 Standard curve constructed from pure *Rhizoctonia solani* AG3-PT DNA concentrations 45.40, 4.54, 4.54 x 10^{-1}, 4.54 x 10^{-2}, 4.54 x 10^{-3}, 4.54 x 10^{-4} or 4.54 x 10^{-5} ng. The DNA concentrations were log(X) transformed before being plotted with the cycle threshold values to generate the standard curve. PCR amplification efficiency = 90.1%.

A.4.32 *Rhizoctonia solani* black scurf severity scores for potato tubers. The Ascochyta blight of pea leaf severity diagrams were used for black scurf assessments (severity key of S. L. H Viljanen, Plant & Food Research, personal communication).



A.4.33 *Spongospora subterranea* powdery scab severity key for potato tubers (Falloon *et al.*, 1995). Numbers in parentheses are percentages of tuber surface area affected.



Appendices for Chapter 5

A.5.1 The 10-year cropping history of the Timaru field trial site (2015/6) (Canterbury, New Zealand) (provided by Steven Dellow and Sarah Sinton, Plant & Food Research, Lincoln).

Year	Mustard block	Fallow (Unplanted) block
Spring 2015	'Innovator' potatoes	'Innovator' potatoes
Winter 2015	'Caliente' mustard	Fallow (Unplanted)
2014-2015	Wheat	Wheat
2013-2014	Linseed	Linseed
2012-2013	Ryegrass seed	Ryegrass seed
2011-2012	Ryegrass seed	Ryegrass seed
2010-2011	Wheat	Wheat
2009-2010	Kale seed	Kale seed
2008-2009	Process peas	Process peas
2007-2008	Wheat	Wheat
2006-2007	Potatoes	Potatoes
2005-2006	Barley	Barley

A.5.2 The 10-year cropping history of the Ashburton field trial site (2015/16) (Canterbury, New Zealand) (provided by Steven Dellow and Sarah Sinton, Plant & Food Research, Lincoln.

Year	Mustard block	Oat block	Fallow (Untreated) block
Spring 2015	'Russet Burbank' potatoes	'Russet Burbank' potatoes	'Russet Burbank' potatoes
Winter 2015	'Caliente' mustard	Oat	Fallow (Unplanted)
2014-2015	Barley (feed grain)	Barley (feed grain)	Barley (feed grain)
2013-2014	Grass seed	Grass seed	Grass seed
2012-2013	Wheat	Wheat	Wheat
2011-2012	Maize	Maize	Radish (Northeast), maize (Southwest)
2010-2011	Grass seed	Grass seed	Grass seed
2009-2010	Wheat	Wheat	Wheat
2008-2009	Potatoes	Potatoes	Potatoes
2007-2008	Wheat	Wheat	Wheat
2006-2007	Clover	Clover	Clover
2005-2006	Wheat	Wheat	Wheat

A.5.3 The 10-year cropping history of the Timaru field trial (201617) (Canterbury, New Zealand) (provided by Steven Dellow and Sarah Sinton, Plant & Food Research, Lincoln).

Year	Fallow (Untreated) block	Oat block	Radish block	Mustard block
Spring 2016	'Russet Burbank' potatoes	'Russet Burbank' potatoes	'Russet Burbank' potatoes	'Russet Burbank' potatoes
Winter 2016	Fallow (Untreated)	Oat (Unknown cultivar)	'Graza' Radish	'Caliente' mustard
2015-2016	Wheat	Wheat	Wheat	Wheat
2014-2015	Radish	Radish	Radish	Radish
2013-2014	Ryegrass	Ryegrass	Ryegrass	Ryegrass
2012-2013	Ryegrass	Ryegrass	Ryegrass	Ryegrass
2011-2012	Wheat	Wheat	Wheat	Wheat
2010-2011	Red beet	Red beet	Red beet	Red beet
2009-2010	Wheat	Wheat	Wheat	Wheat
2008-2009	Potatoes	Potatoes	Potatoes	Potatoes
2007-2008	Ryegrass	Ryegrass	Ryegrass	Ryegrass
2006-2007	Ryegrass	Ryegrass	Ryegrass	Ryegrass

A.5.4 0% and 100% denaturing polyacrylamide (PA) 8% used for α-proteobacteria, β-proteobacteria, total fungi, and AMF

0% denaturing PA 8%	
Component	Volume
40% Acrylamide:Bisacrylamide (37.5:1) (Bio-Rad, USA)	20 mL
50x TAE	1 mL
100% glycerol	2 mL
Millipore water	to 100 mL
100% denaturing PA 8%	
Component	Volume/Weight
40% Acrylamide:Bisacrylamide (37.5:1) (Bio-Rad, USA)	20 mL
Urea (Sigma-Aldrich, USA)	42 g
Formamide (Sigma-Aldrich, USA)	40 mL
50x TAE	1 mL
100% glycerol	2 mL
Millipore water	to 100 mL

Store in the dark at room temperature, low heat (≤37°C) to dissolve.

A.5.5 0% and 100% denaturing polyacrylamide (PA) 7% (used for γ-proteobacteria)

0% denaturing PA 7%	
Component	Volume
40% Acrylamide:Bisacrylamide (37.5:1) (Bio-Rad, USA)	20 mL
50× TAE	1 mL
100% glycerol	2 mL
Millipore water	to 100 mL
100% denaturing PA 7%	
Component	Volume/Weight
40% Acrylamide:Bisacrylamide (37.5:1) (Bio-Rad, USA)	17.5 mL
Urea	42 g
Formamide	40 mL
50xTAE	1 mL
100% glycerol	2 mL
Millipore water	to 100 mL

Store in the dark at room temperature, low heat (≤37°C) to dissolve.

A.5.6 50x TAE

Component	Volume/Weight
Tris Base	242 g
Millipore water	500 mL
Glacial acetic acid	57.1 mL
0.5 M EDTA (pH 8)	100 mL
Millipore water	to 1000 mL

A.5.7 10% ammonium persulfate (APS)

Component	Volume/Weight
APS	1 g
Millipore water	10 mL

Aliquot 200 µL into tubes and stored at -20°C in a freezer.

A.5.8 2x DGGE gel Loading dye

Component	Volume	Final concentration
2% bromophenol blue	0.25 mL	0.05%
2% xylene cyanol	0.25 mL	0.05%
100% glycerol	7.0 mL	70%
Millipore water	2.5 mL	
Total	10 mL	

A.5.9 8x fixative solution

Component	Volume
96% ethanol	800 mL
Acetic acid	40 mL
Millipore water	160 mL

A.5.10 1x fixative solution

Component	Volume
8x fixative solution	250 mL
Millipore water	to 2000 mL

A.5.11 Silver stain (for 2 gels, to prepare fresh just before staining)

Component	Volume/Weight
1x fixative solution	500 mL
Silver nitrate	1 g

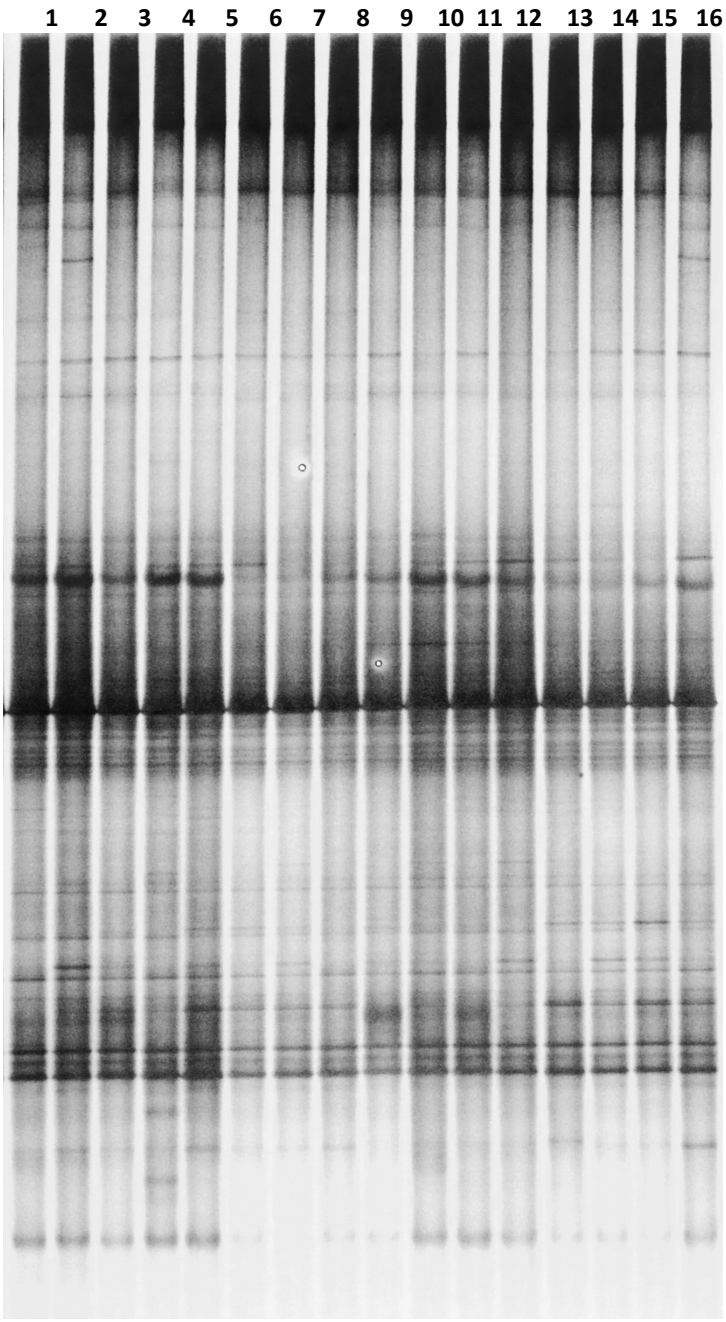
A.5.12 Developer (for 2 gels)

Component	Volume
3% NaOH	250 mL
Millipore water	250 mL
Formaldehyde	1 mL

A.5.13 Cairn's preservation solution

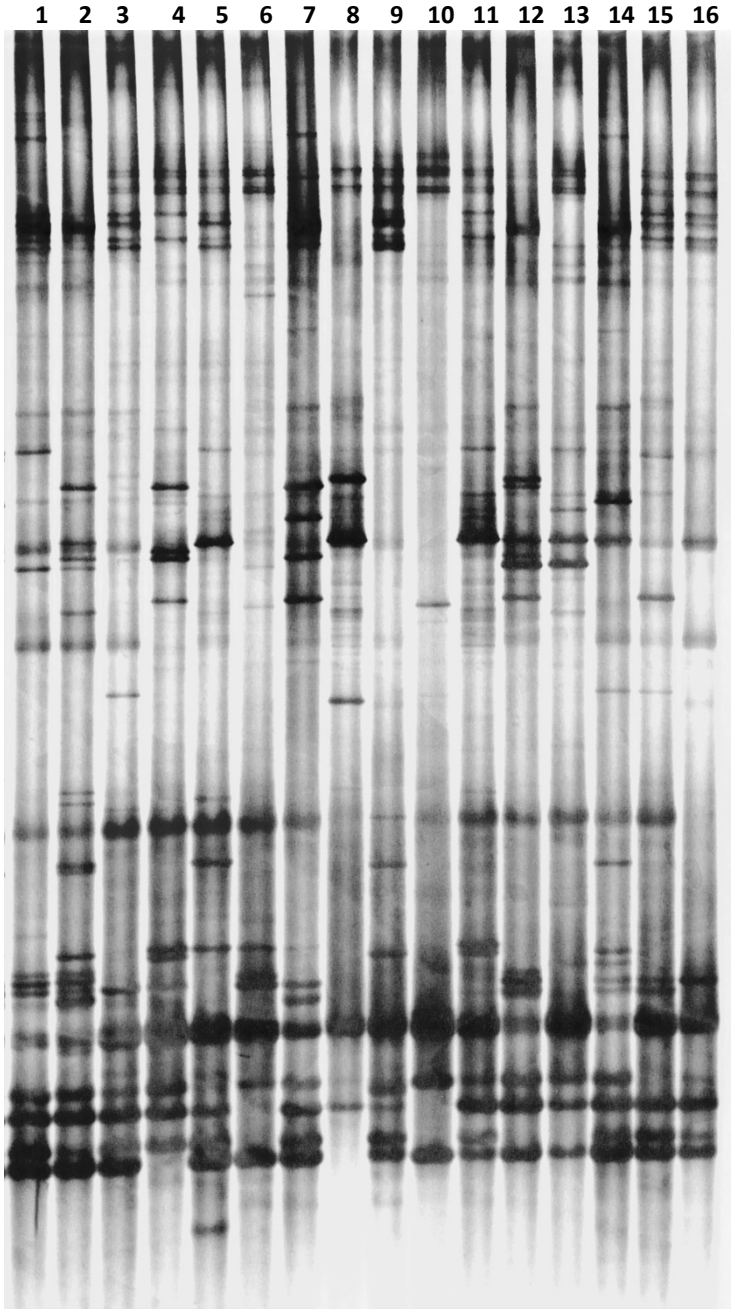
Component	Volume
96% ethanol	250 mL
Glycerol	100 mL
Millipore water	650 mL

A.5.14 A DGGE gel showing the total soil fungi community in treatments at T3 (Before potato planting).



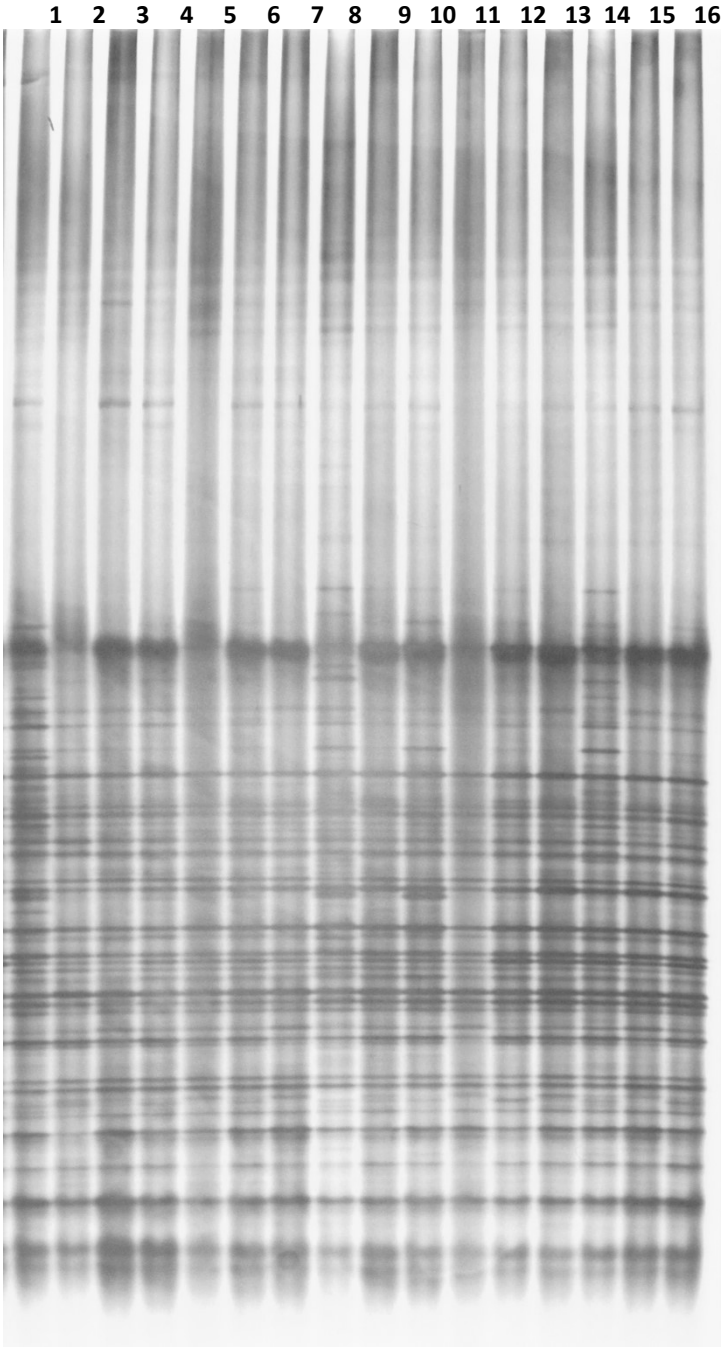
Lane 1-4: Unplanted treatment
Lane 5-8: 'Caliente' mustard treatment
Lane 9-12: Oat treatment
Lane 13-16: 'Graza' radish treatment

A.5.15 A DGGE gel showing the soil AMF community in treatments at T3 (Before potato planting).



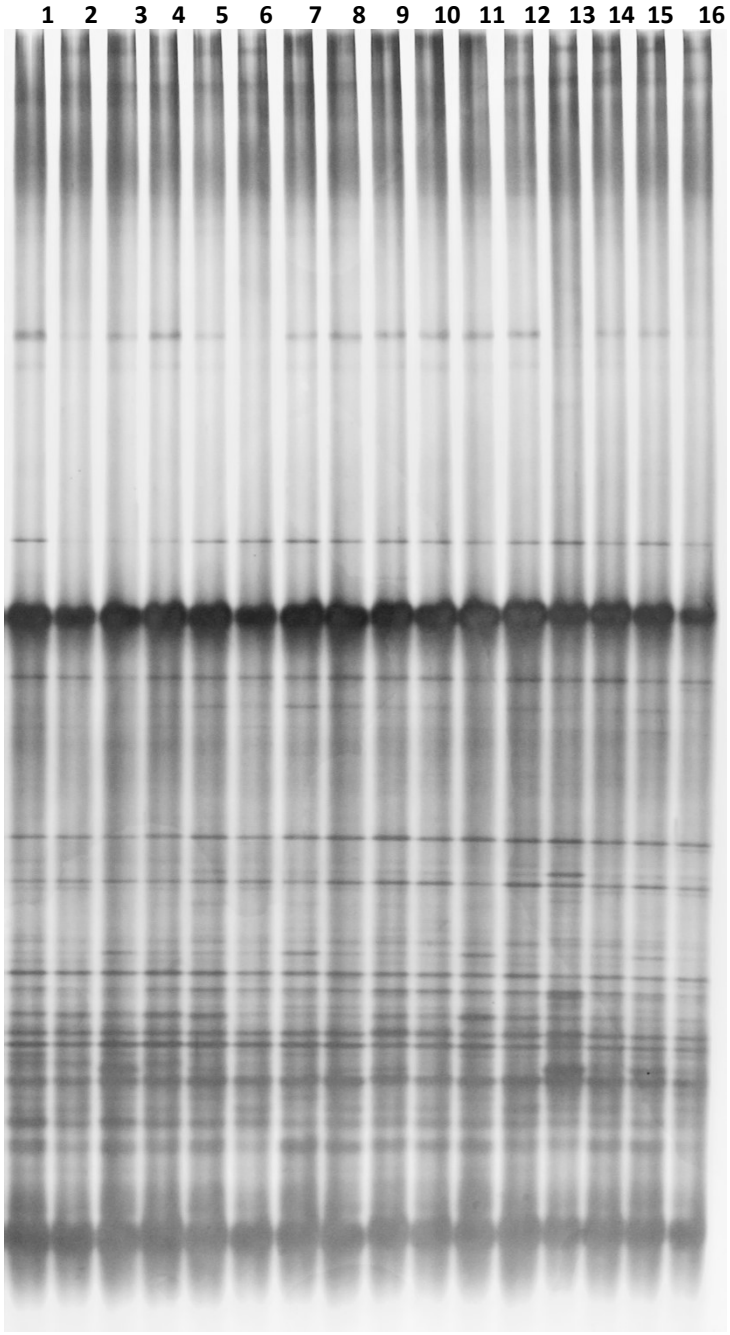
Lane 1-4: Unplanted treatment
Lane 5-8: 'Caliente' mustard treatment
Lane 9-12: Oat treatment
Lane 13-16: 'Graza' radish treatment

A.5.16 A DGGE gel showing the soil α-proteobacteria community in treatments at T3 (Before potato planting).



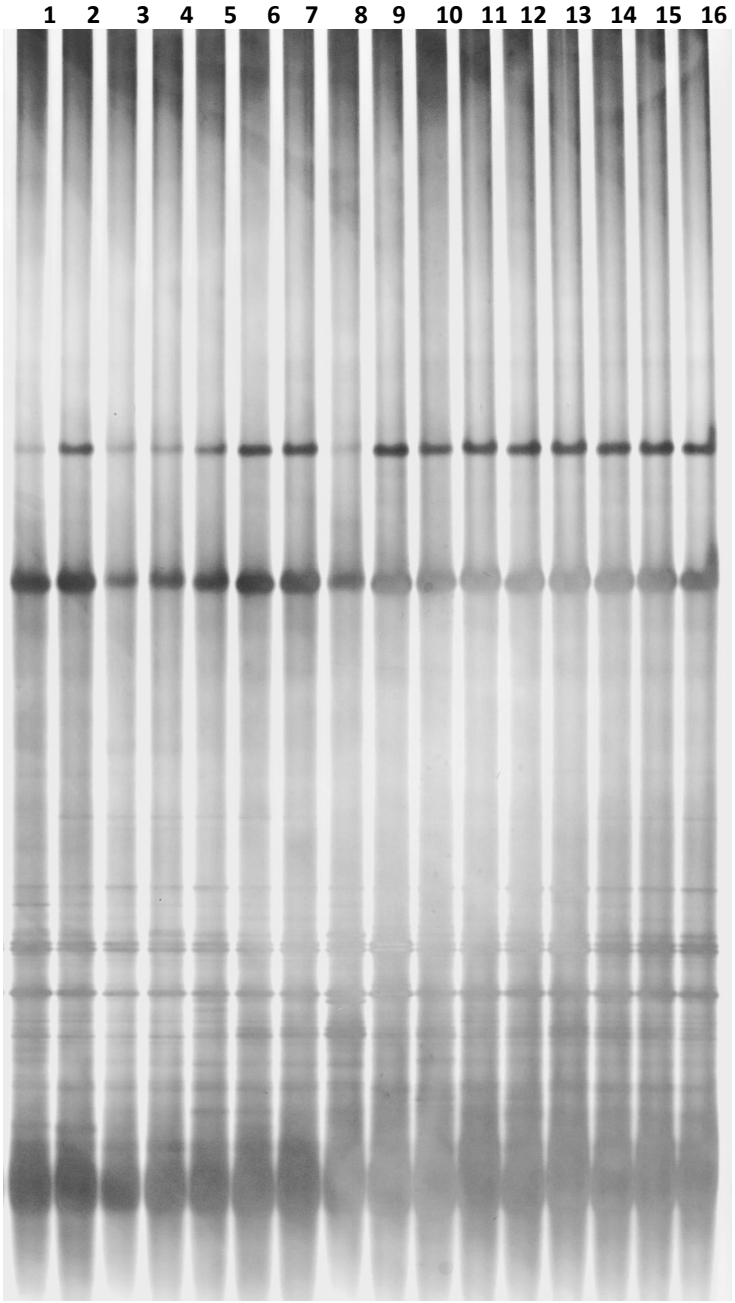
Lane 1-4: Unplanted treatment
Lane 5-8: 'Caliente' mustard treatment
Lane 9-12: Oat treatment
Lane 13-16: 'Graza' radish treatment

A.5.17 DGGE gel showing the soil β-proteobacteria community in treatments at T3 (Before potato planting).



Lane 1-4: Unplanted treatment
Lane 5-8: 'Caliente' mustard treatment
Lane 9-12: Oat treatment
Lane 13-16: 'Graza' radish treatment

A.5.18 A DGGE gel showing the soil γ-proteobacteria community in treatments at T3 (Before potato planting).



Lane 1-4: Unplanted treatment
Lane 5-8: 'Caliente' mustard treatment
Lane 9-12: Oat treatment
Lane 13-16: 'Graza' radish treatment

A.5.19 Analysis of variance of dehydrogenase activity of the soil microbial communities after incorporation of 'Caliente' mustard or unplanted and assessed before potato planting (T0) and 90 days after potato planting (T1) in the Timaru field trial (2015/16).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.0354	0.0088	1.11	
Treatment	1	0.1225	0.1225	15.39	0.002
Sampling time	1	0.0001	0.0001	0.01	0.915
Treatment.Time	1	0.0031	0.0031	0.39	0.543
Residual	12	0.0955	0.0080		
Total	19	0.2566			

A.5.20 Analysis of variance of dehydrogenase activity) of the soil microbial communities after incorporation of 'Caliente' mustard, oat or unplanted and assessed before potato planting (T0) and 90 days after potato planting (T1) in the in the Ashburton field trial (2015/16).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.020	0.005	2.4	
Treatment	2	0.026	0.013	6.1	0.009
Time	1	0.008	0.008	3.7	0.069
Treatment.Time	2	0.014	0.007	3.4	0.054
Residual	20	0.043	0.002		
Total	29	0.111			

A.5.21 Analysis of variance of dehydrogenase activity of the soil microbial communities associated with incorporation of 'Caliente' mustard, oat, 'Graza' radish or unplanted and assessed at 6 sampling times (T0: Before cover crop planting; T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/Before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) in the Timaru field trial (2016/17).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	7	0.129	0.018	2.4	
Treatment	3	0.607	0.202	26.6	<.001
Sampling time	4	1.096	0.274	36.1	<.001
Treatment. Sampling time	12	0.366	0.031	4.0	<.001
Residual	133	1.010	0.008		
Total	159	3.208			

A.5.22 Pearson correlation analysis using Primer6 of 22 carbon sources used to determine the microbial functionality in the soil collected from the Ashburton field trial (2015/16).

Carbon substrate	Pearson's regression coefficient (R)	
	MDS1	MDS2
Arginine	0.15	0.69
Water	0.65	0.55
Glycolic	0.60	0.52
Glucosamine hydrochloride	0.81	0.37
Triton x-100	0.68	0.34
Tartaric	0.69	0.30
Tyrosine	0.66	0.28
Proline	0.72	0.09
Sucrose	0.56	0.02
Citric acid	0.85	-0.07
Glycine	0.71	-0.11
Xylose	0.77	-0.20
Alanine	0.65	-0.20
Galactose	0.83	-0.21
Maltose	0.77	-0.34
Raffinose	0.67	-0.36
Serine	0.58	-0.37
Cysteine	0.54	-0.39
Arabinose	0.83	-0.44
Glucose	0.72	-0.45
Urea	0.69	-0.49
Fructose	0.78	-0.51
Glycerol 50%	0.63	-0.56

A.5.23 Pearson correlation analysis using Primer6 of 22 carbon sources used to determine the microbial functionality in the soil collected from the Timaru field trial (2016/17).

Carbon substrate	Pearson's regression coefficient (R)	
	MDS1	MDS2
Arginine	-0.49	0.23
Glycolic	-0.74	0.23
Urea	-0.85	-0.21
Raffinose	-0.86	0.34
Citric	-0.89	0.20
Water	-0.91	0.09
Serine	-0.92	-0.03
Tartaric	-0.92	0.11
Cysteine	-0.92	-0.10
Sucrose	-0.92	-0.21
Triton X-100	-0.93	-0.14
Proline	-0.93	0.14
Glucosamine	-0.94	0.01
Fructose	-0.94	-0.07
Tyrosine	-0.94	-0.06
Maltose	-0.95	-0.18
Xylose	-0.95	-0.16
Glucose	-0.95	-0.02
Alanine	-0.96	-0.02
Arabinose	-0.96	0.06
Galactose	-0.97	0.00
Glycerol 50%	-0.97	-0.05
Glycine	-0.97	0.03

A.5.24 The richness of the total fungi community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	52.0	41.0	46.1 A
Caliente' mustard	43.7	34.2	38.6 B
Mean of sampling time	47.6 X	37.4 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.25 The richness of the Betaproteobacteria community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	29.5	34.7	31.6 B
Caliente' mustard	38.9	37.2	38.0 A
Mean of sampling time	33.9	35.5	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.26 The richness of the Gammaproteobacteria community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	16.6 b	22.9 a	19.5
Caliente' mustard	23.4 a	18.6 b	20.9
Mean of sampling time	19.5	20.4	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.27 The diversity index of the total fungi community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.57	1.44	1.51 A
Caliente' mustard	1.47	1.40	1.45 B
Mean of sampling time	1.51 X	1.41 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.28 The diversity index of the Betaproteobacteria community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.45	1.38	1.41 A
Caliente' mustard	1.29	1.32	1.32 B
Mean of sampling time	1.35	1.35	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.29 The diversity index of the Gammaproteobacteria community after incorporation with 'Caliente' mustard or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Timaru field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.20 a	1.12 ab	1.15
Caliente' mustard	1.07 b	1.17 ab	1.12
Mean of sampling time	1.12	1.15	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.30 The richness of the total fungi community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	47.9	44.7	45.7
Caliente' mustard	46.8	42.7	44.7
Oat	50.1	43.7	46.8
Mean of sampling time	47.9 X	43.7 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.31 The richness of the arbuscular mycorrhizal fungi community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	31.6	20.4	25.1
Caliente' mustard	37.2	24.0	30.2
Oat	38.0	26.9	31.6
Mean of sampling time	35.5 X	23.4 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.32 The richness of the Betaproteobacteria community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	32.4 a	22.4 b	26.9
Caliente' mustard	28.2 a	31.6 a	30.2
Oat	28.2 a	30.2 a	28.8
Mean of sampling time	29.5	27.5	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.33 The richness of the Gammaproteobacteria community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	11.0 ab	9.3 b	10.0
Caliente' mustard	9.3 b	14.8 a	11.7
Oat	12.0 ab	12.6 ab	12.3
Mean of sampling time	10.70	12.00	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.34 The diversity index of the total fungi community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.50 abc	1.51 abc	1.51
Caliente' mustard	1.53 ab	1.46 bc	1.50
Oat	1.55 a	1.45 c	1.50
Mean of sampling time	1.51 X	1.48 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.35 The diversity index of the arbuscular mycorrhizal fungi community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.2	1	1.10
Caliente' mustard	1.3	1.1	1.20
Oat	1.2	1.1	1.20
Mean of sampling time	1.24 X	1.04 Y	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.36 The diversity index of the Alphaproteobacteria community after incorporation with 'Caliente' mustard, oat or unplanted assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

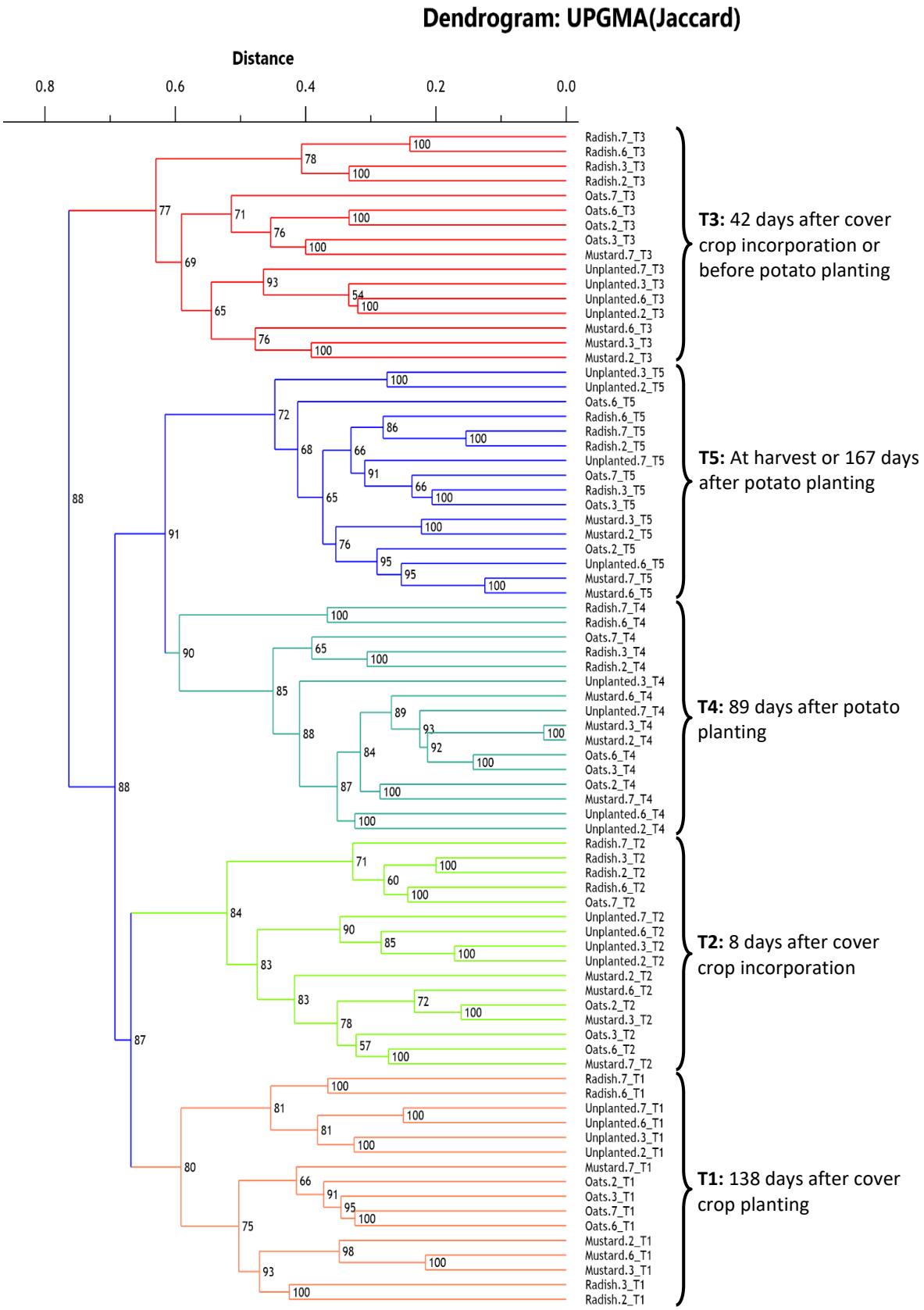
Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.64 a	1.65 a	1.65 A
Caliente' mustard	1.64 a	1.57 b	1.61 B
Oat	1.64 ab	1.63 ab	1.63 AB
Mean of sampling time	1.66	1.62	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

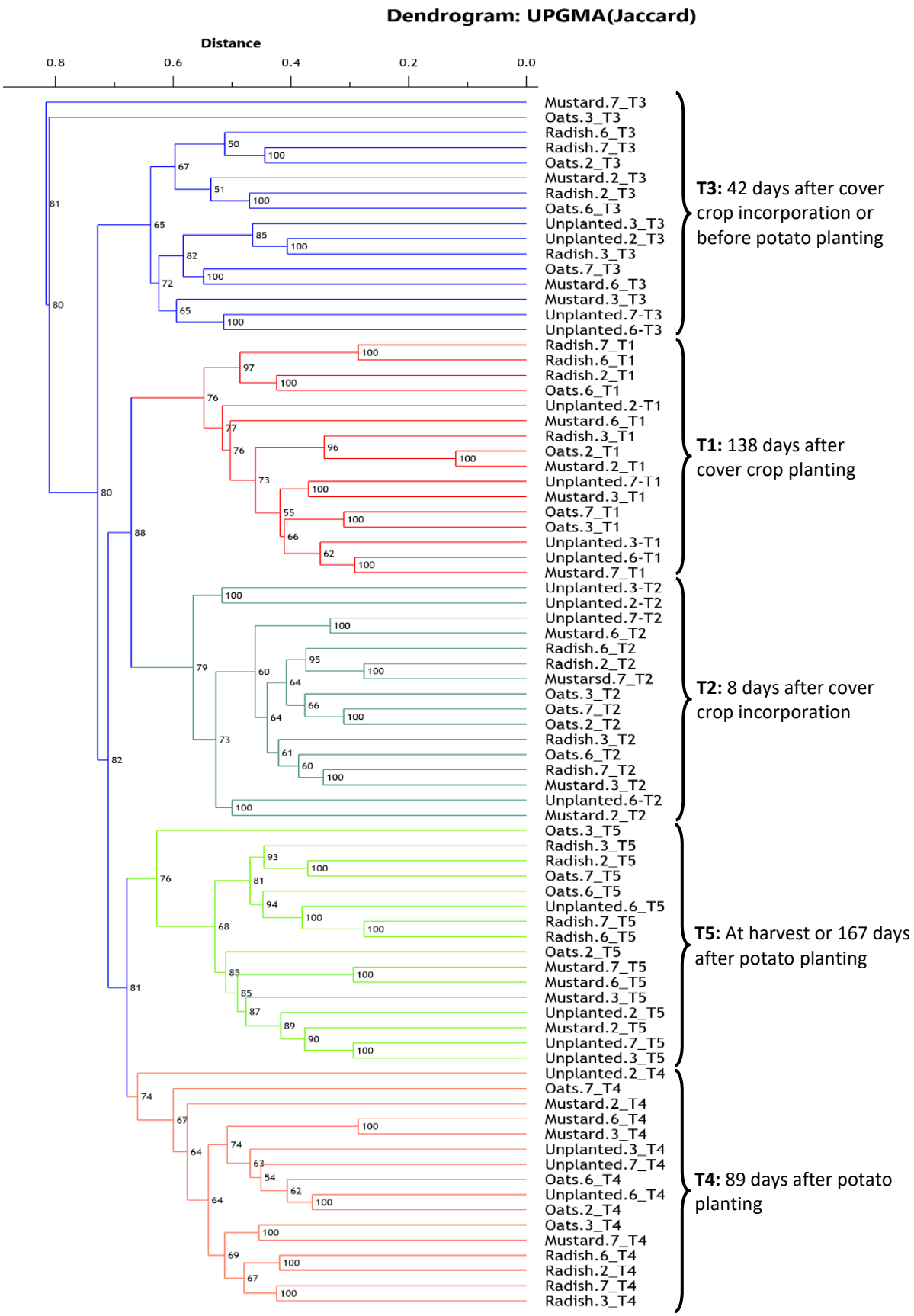
A.5.37 The diversity index of the Betaproteobacteria after incorporation with 'Caliente' mustard, oat or unplanted on assessed before (T0) and 90 days after potato planting (T1) using PCR-DGGE in the Ashburton field trial (2015/16).

Treatment	Sampling time		Mean of treatment
	T0	T1	
Unplanted	1.38 a	1.23 b	1.33
Caliente' mustard	1.33 ab	1.34 ab	1.33
Oat	1.34 ab	1.32 ab	1.30
Mean of sampling time	1.35 X	1.29 Y	

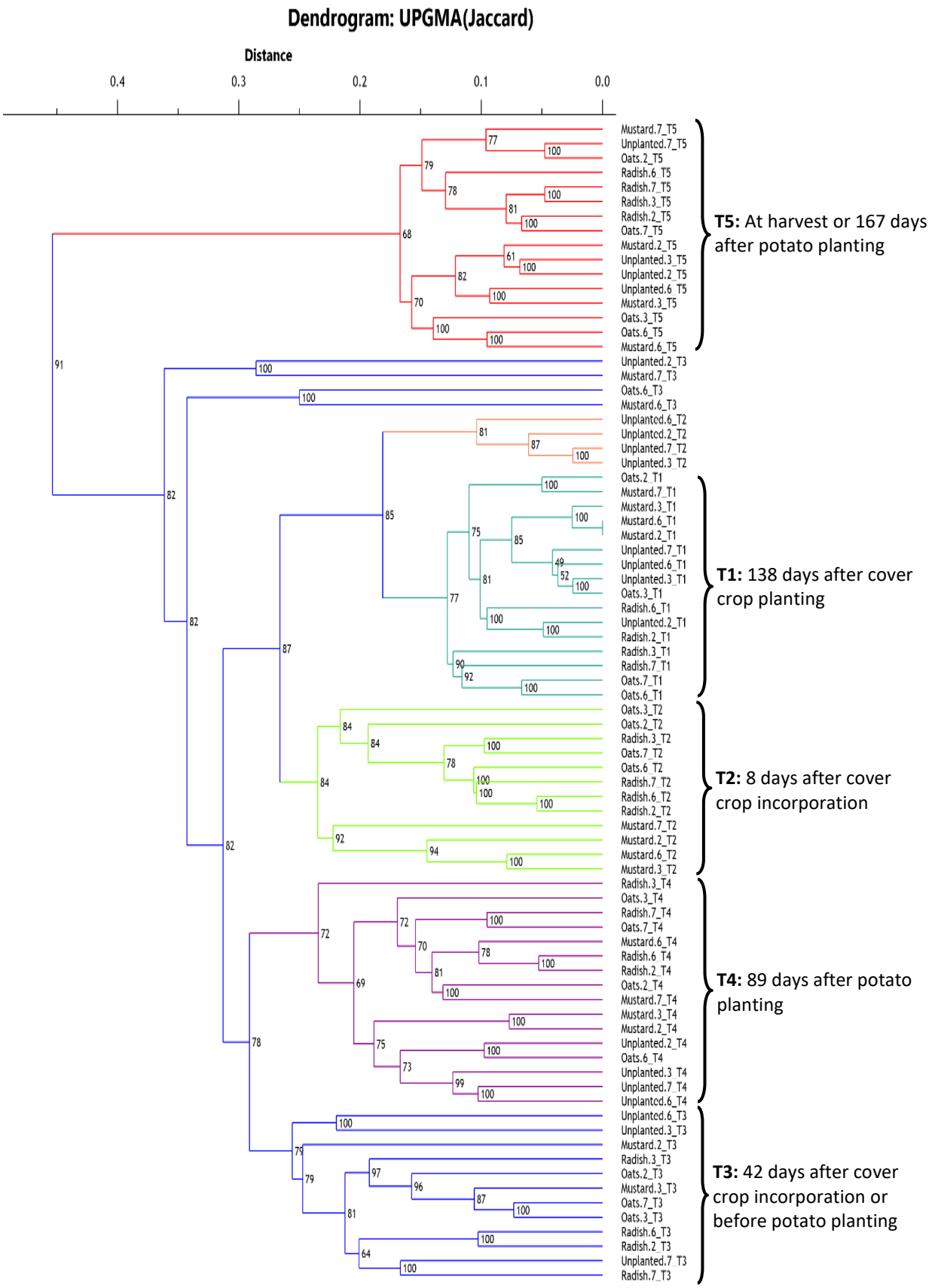
Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.



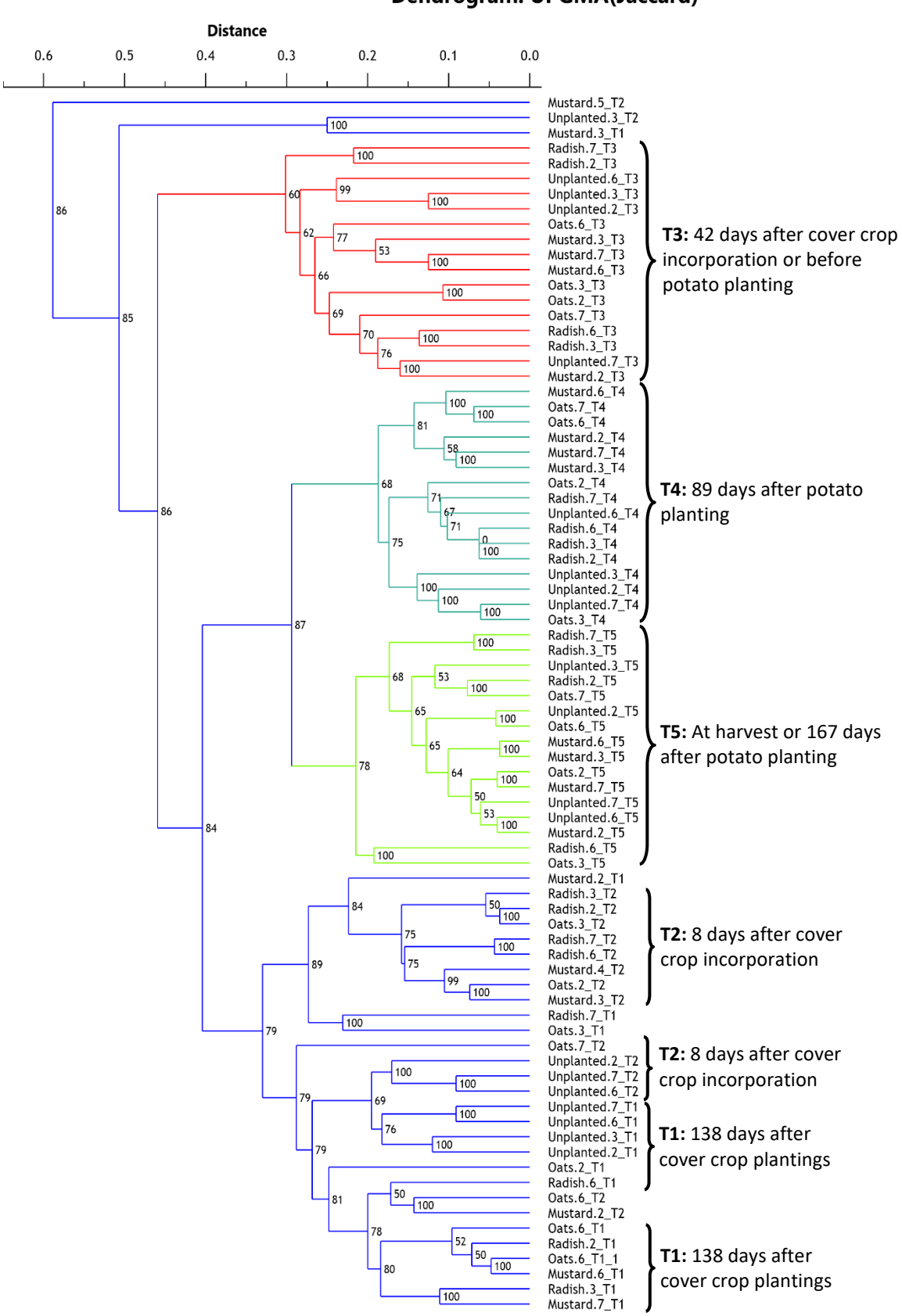
A.5.38 UPGMA (Jaccard) showing total fungi community structures influenced by different cover crop treatments ('Caliente' mustard, oat, 'Graza' radish and unplanted). Data assessed after 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17). Each colour represented for each sampling time.



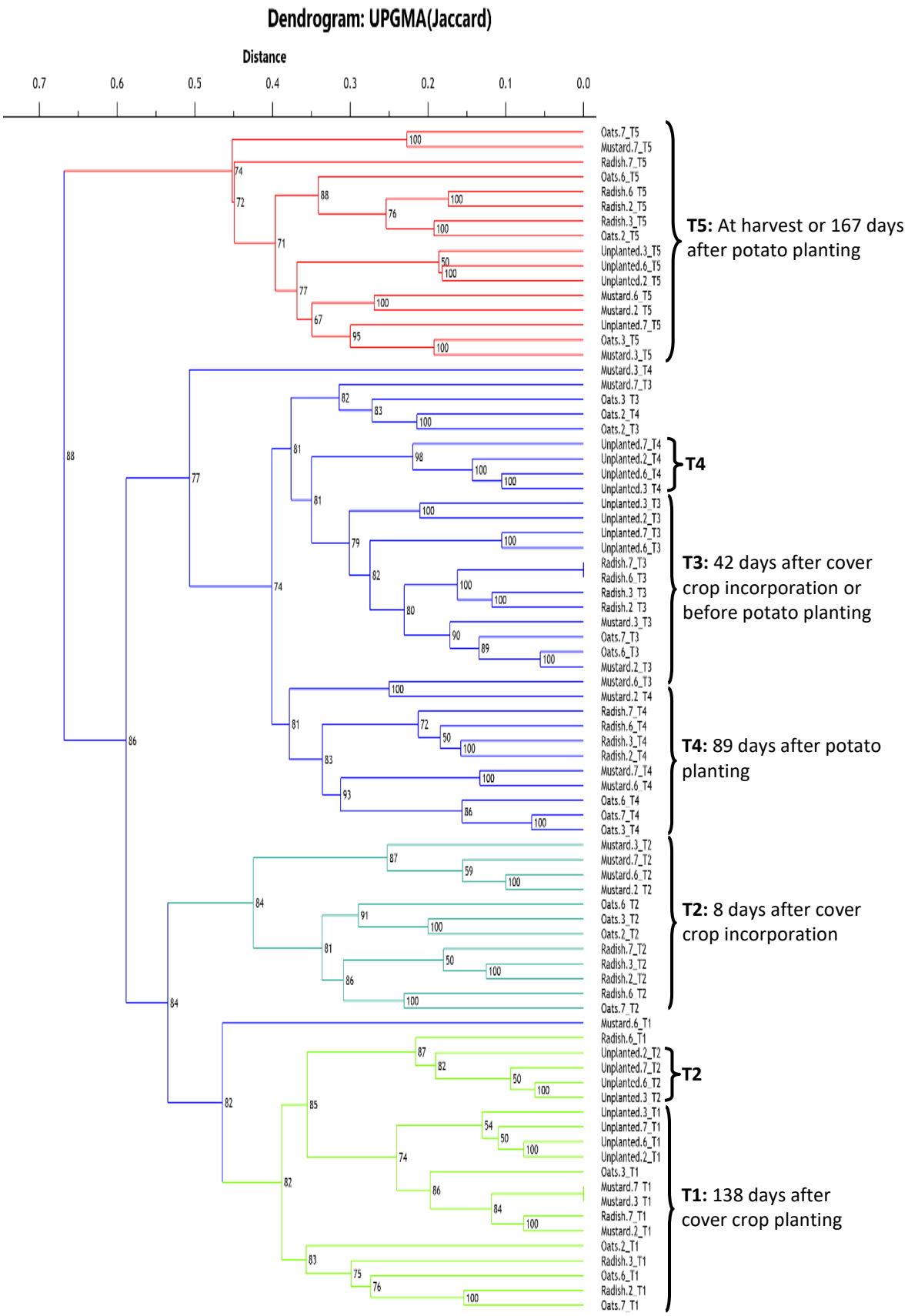
A.5.39 UPGMA (Jaccard) showing arbuscular mycorrhizal fungi community structures influenced by different cover crop treatments ('Caliente' mustard, oat, 'Graza' radish and unplanted). Data assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 days after potato planting) using PCR-DGGE in the Timaru field trial (2016/17). Each colour represented for each sampling time.



A.5.40 UPGMA (Jaccard) showing Alphaproteobacteria community structures influenced by different cover crop treatments ('Caliente' mustard, oat, 'Graza' radish and unplanted). Data assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 days after potato planting) using PCR-DGGE in the Timaru field trial (2016/17). Each colour represented for each sampling time.



A.5.41 UPGMA (Jaccard) showing Betaproteobacteria community structures influenced by different cover crop treatments ('Caliente' mustard, oat, 'Graza' radish and unplanted). Data assessed at 5 sampling times (T1: 138 days after cover crop plantings; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 days after potato planting) using PCR-DGGE in the Timaru field trial (2016/17). Each colour represented for each sampling time.



A.5.42 UPGMA (Jaccard) showing Gammaproteobacteria community structures influenced by different cover crop treatments ('Caliente' mustard, oat, 'Graza' radish and unplanted). Data assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/Before potato planting; T4: 89 days after potato planting; T5: At harvest/167 days after potato planting) using PCR-DGGE in the Timaru field trial (2016/17). Each colour represented for each sampling time.

A.5.43 The richness of the total fungi community after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	41.2 bcde	37.9 de	30.1 fg	38.7 cde	48.2 ab	38.8 a
'Caliente' mustard	39.9 cde	36.0 e	26.5 g	38.2 cde	45.4 abc	36.6 b
Oat	39.4 cde	34.6 ef	27.2 g	38.7 cde	45.4 abc	36.6 b
'Graza' radish	38.5 cde	38.7 cde	25.7 g	44.3 abcd	49.7 a	38.5 ab
Mean of sampling time	39.7 b	36.8 c	27.3 d	39.9 b	47.1 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.44 The richness of the arbuscular mycorrhizal fungi community after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	22.3	22.7	26.9	24.4	27.5	25.2
'Caliente' mustard	23.0	22.6	24.6	24.9	29.4	25.3
Oat	24.9	25.0	19.3	26.4	27.5	25.0
'Graza' radish	25.7	26.2	22.2	25.1	28.6	25.9
Mean of sampling time	23.9 b	24.0 b	23.1 b	25.2 ab	28.2 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.45 The richness of the Alphaproteobacteria community after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	39.5	39.7	39.4	38	42	39.7
'Caliente' mustard	39.3	36.5	38.2	36.7	39.4	38.0
Oat	41.7	35.7	35.6	37.2	41.0	38.1
'Graza' radish	42.0	37.0	38.8	38.2	41.5	39.4
Mean of sampling time	40.6 ab	37.2 c	37.9 abc	37.5 bc	40.9 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.46 The richness of the Betaproteobacteria community after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	22.2	19.2	21.4	32.7	23.7	23.4
'Caliente' mustard	19.5	20.5	22	30.2	25.5	23.2
Oat	18.9	22.4	22.9	30.8	23.5	23.4
'Graza' radish	19.5	24.7	20.4	30.5	26.4	24
Mean of sampling time	20.0 c	21.6 bc	21.6 bc	31.0 a	24.8 b	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.47 The richness of the Gammaproteobacteria community after incorporation of different cover crops ('Caliente' mustard, oat, 'Graza' radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	12.2 gh	14.7 defgh	17.5 bcde	18.2 abcd	19.5 abc	16.2 A
'Caliente' mustard	11.7 h	16.9 bcdef	16.1 cdefg	13.7 efgh	21.6 ab	15.7 AB
Oat	12.2 gh	12.4 gh	15.7 cdefg	13.9 defgh	21.4 ab	14.8 B
'Graza' radish	12.9 fgh	14.5 defgh	16.9 bcdef	17.7 bcde	23.6 a	16.7 A
Mean of sampling time	12.2 Z	14.5 Y	16.6 X	15.8 XY	21.5 W	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.48 The diversity index of the total fungi community after incorporation of different cover crops ('Caliente' mustard, oat, radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	1.52 a	1.50 a	1.34 bc	1.47 a	1.51 a	1.47 A
'Caliente' mustard	1.52 a	1.43 ab	1.26 c	1.47 a	1.47 a	1.43 BC
Oat	1.51 a	1.45 a	1.26 c	1.43 ab	1.47 a	1.42 C
'Graza' radish	1.50 a	1.49 a	1.34 c	1.43 ab	1.51 a	1.45 AB
Mean of sampling time	1.51 W	1.47 XY	1.30 Z	1.45 Y	1.49 WX	

Means within each column or row followed by the same letter are not significantly different according to Tukey's HSD test at *P*=0.05.

A.5.49 The diversity index of the arbuscular mycorrhizal fungi community after incorporation of different cover crops (‘Caliente’ mustard, oat, ‘Graza’ radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	0.84	0.9	1.06	1.01	1.13	0.99
‘Caliente' mustard	0.88	0.91	0.95	1	1.16	0.98
Oat	0.99	0.96	0.81	1.06	1.07	0.98
‘Graza’ radish	1.07	1.06	0.94	1.04	1.15	1.05
Mean of sampling time	0.95 b	0.96 b	0.94 b	1.03 ab	1.13 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey’s HSD test at *P*=0.05.

A.5.50 The diversity index of the Alphaproteobacteria community after incorporation of different cover crops (‘Caliente’ mustard, oat, ‘Graza’ radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	1.54	1.51	1.45	1.51	1.52	1.51
‘Caliente' mustard	1.53	1.47	1.35	1.48	1.54	1.47
Oat	1.48	1.49	1.47	1.50	1.54	1.5
‘Graza’ radish	1.49	1.49	1.45	1.50	1.54	1.49
Mean of sampling time	1.51 a	1.49 ab	1.43 b	1.50 a	1.54 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey’s HSD test at *P*=0.05.

A.5.51 The diversity index of the Betaproteobacteria community after incorporation of different cover crops (‘Caliente’ mustard, oat, ‘Graza’ radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	1.24	1.21	1.20	1.40	1.28	1.26
‘Caliente' mustard	1.20	1.21	1.22	1.35	1.30	1.26
Oat	1.18	1.24	1.25	1.35	1.28	1.26
‘Graza’ radish	1.20	1.28	1.21	1.34	1.32	1.27
Mean of sampling time	1.20 b	1.23 b	1.22 b	1.36 a	1.30 a	

Means within each column or row followed by the same letter are not significantly different according to Tukey’s HSD test at *P*=0.05.

A.5.52 The diversity index of the Gammaaproteobacteria community after incorporation of different cover crops (‘Caliente’ mustard, oat, ‘Graza’ radish and unplanted) assessed at 5 sampling times (T1: 138 days after cover crop planting; T2: 8 days after cover crop incorporation; T3: 42 days after cover crop incorporation/before potato planting; T4: 89 days after potato planting; T5: At harvest/167 day after potato planting) using PCR-DGGE in the Timaru field trial (2016/17).

Treatment	Sampling time					Mean of treatment
	T1	T2	T3	T4	T5	
Unplanted	0.92 f	1.03 cdef	1.10 abcd	1.10 abcd	1.15 ab	1.06
‘Caliente' mustard	0.95 ef	1.15 abc	1.09 abcd	0.99 def	1.15 ab	1.06
Oat	0.96 ef	1.02 def	1.02 def	1.02 def	1.16 ab	1.03
‘Graza’ radish	0.95 ef	1.06 bcde	1.04 bcde	1.08 abcd	1.20 a	1.07
Mean of sampling time	0.95 Z	1.06 Y	1.06 Y	1.05 Y	1.17 X	

Means within each column or row followed by the same letter are not significantly different according to Tukey’s HSD test at P=0.05.