Effect of winter cover crops on nutrition and weeds of maize

A thesis submitted in partial fulfilment

of the requirements for the degree of

Master of Science (Research) in Biological Sciences

at

The University of Waikato

by

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THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2019

Abstract

Cover crops are being used in New Zealand to manage soil nutrients and weeds and can have positive or negative effects on the growth of the following main crop.

This study investigated the causes of lower yield of maize following a ryegrass cover crop compared to fallow or other cover crops at the Foundation for Arable Research Northern Crop Research site. Cover crops were sown in mixtures faba (*Vicia faba* L.), gland clover (*Trifolium glanduliferum* Boiss.) and rye (*Lolium perenne* L.) with fallow as a control and followed by maize. The effects on soil carbon to nitrogen ratios, and maize growth, yield and nutrition were monitored. Additional experiments in the laboratory and glasshouse investigated the effect of cover crops on weed seed germination.

Winter cover crops affected nutrient dynamics during growth of the maize crop. The total soil carbon to nitrogen (CN) ratio decreased as the proportion of clover in the cover crop mix increased. In the fallow and clover treatments nutrients were available earlier for maize to grow rapidly, resulting in higher yields compared to the rye and rye faba treatments. In the rye treatment, the CN ratio had fallen and nutrients had become available to the crop by harvest time, resulting in lower maize tissue CN ratios and higher tissue nutrient contents. Rye had a clear effect on the CN ratio in the cover crop mix whether sown at full rate or half rate. Rye also appeared to suppress clover and faba in the mixed treatments, possibly reducing the potential for supplementation of nutrients through the nitrogen fixation process. A clover-based cover crop was better than a rye-based cover crop, probably because it resulted in a lower soil CN ratio and higher extractable soil nitrogen at the time of establishment of the main crop, and may therefore release nutrients faster for the benefit of the main crop.

A laboratory experiment tested the inhibition effect of cover crop extracts on the germination of weed and crop species. Five gram aliquots of cover crop powder were soaked for 24 hours in 100 ml of water and the filtered extracts diluted before application to seeds of a range of test species. Cover crop extracts almost always inhibited seed germination, by up to approximately 40%, but the inhibitory effects

on seed germination varied with the species of cover crop and the test species. Serial dilution of extracts indicated that there was a dose response effect in the level of germination suppression by the cover crop species. Clover had the largest potential for reducing the weed and crop species germination and therefore was the most promising cover crop for exhibiting a true allelopathic effect on weed seed germination.

To further test the inhibition effect of clover and rye cover crops on germination of weed and crop species in the soil, root and shoot residues were tested on seeds of weed and crop species planted in pots. Root residues inhibited germination of volunteer and planted test species more than shoot residues, possibly because of a higher availability of inhibitory compounds, or because of differences in the time of setting up the root and shoot pots. The inhibitory effect on germination exerted by cover crop varied with the test species. This was shown by inhibition affecting one species but not others, which might suggest that some species were more sensitive to a particular cover crop treatment. Cover crop roots were more likely to affect germination, but cover crop shoots were more likely to reduce the growth of planted test species. The lack of a root effect on growth might be explained by loss or dilution of any allelopathic compounds that affected germination, or because their mode of action was specific to germination.

Overall, clover root and shoot treatments have the potential to inhibit germination and growth of weed and crop species. The results support the hypothesis that clover either as a crop residue or as an extract has a higher potential to suppress germination of other species compared to rye and is the most promising cover crop for exhibiting a true allelopathic effect on germination of weed seeds. It was also shown that a clover-based winter cover crop might be better than a rye-based cover crop in terms of its effects on nutrient availability to the main crop. The results have implications for the decision to keep crop residues in the field to reduce the germination and growth of weeds. There may be an economic benefit from using cover crop shoots for other activities (e.g. silage or cut-and-carry forage) while still benefiting from the weed controlling effects of roots residues that are left in the field. Further experiments on the effects of winter cover crops for maize production in New Zealand are recommended.

Acknowledgements

I would like to extend my sincere gratitude to Mike Clearwater, Trevor James and Michael Trolove for their supervision, inspiration and encouragement from the beginning of this study. Thank you to Mike for assistance with project planning, data analysis and writing. Trevor and Michael generously provided the project placement and supervision at Agresearch, and the opportunity and material support to work on their cover crop research project.

Many thanks to Michael Trolove for supporting me through all of the field work. Thank you to all of the staff of the Plant Protection section at AgResearch for accommodating me in their team throughout the period of my study.

I also, thank the Foundation for Arable Research for giving me the opportunity to work on the cover crop research programme.

I am grateful to Harold Henderson, Biometrician at AgResearch for assisting me with experimental design and data analysis using Genstat.

I thank Cheryl Ward, the Science librarian for being always available to support me, for providing lessons on how to arrange the data and for helping me to put together the pieces of thesis writing.

My appreciation also goes to all members of the Plant Physiology Laboratory and the University of Waikato, not forgetting to mention Stevie Noe who was very supportive and comforting throughout my research period, and Stephen Gardyne for his technical support when I was measuring the pH of the extract solutions.

Finally, I thank my family for being there for me, my wife who was always giving me support, my daughter Mwanjiwa who developed interest in the study I was carrying out, and my son Wangu who was always very understanding.

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Chapter 1

General introduction

1.1 Weeds in New Zealand

A weed in basic terms is defined as a plant growing in a place where it is not desired (Dadashi *et al.*, 2015). Later, this definition was modified to include any plant that interferes with people's objectives or requirements. In addition, any plant forming populations able to enter cultivated habitats to displace the resident plant populations that are deliberately planted for other purposes is considered a weed. A plant is a weed when it impacts negatively on the cultivated crop and causes economic damage to the crop (Welch *et al.*, 2016). Economic damage caused by weeds affects multiple sectors of a country's economy, such as production of agricultural crops, fruits and forest products (Büchi *et al.*, 2018).

The definition of a weed is generalized because any plant is potentially a weed when it grows in a place where it is not required. For example, the crop maize is regarded as of high value when it is cultivated. However, the same plant can be considered a weed when it grows in a place where another crop is being cultivated (Rajcan & Swanton, 2001).

A major importance of weeds to New Zealand is the reduction in crop quality and quantity that they cause, as a result of competition for resources such as nutrients, water and light. Their competition with cultivated plants results in the desired plants not acquiring enough resources for optimal growth (Allen & Lee, 2006). The most significant damage occurs at the initial growth stage of a plant when nutrients are required to build new tissues, and at intermediate levels of weed presence (Fig. 1.1). The problem of weeds is usually attributed to introduced exotic plants (Proce *et al.*, 2006; Fowler *et al.*, 2010). Despite the first weed control act being passed in New Zealand in 1954, the negative effects of weeds have continued. Control of weeds costs the New Zealand economy more than one billion dollars each year (Williams & Timmins, 2002; Sullivan *et al.*, 2009).

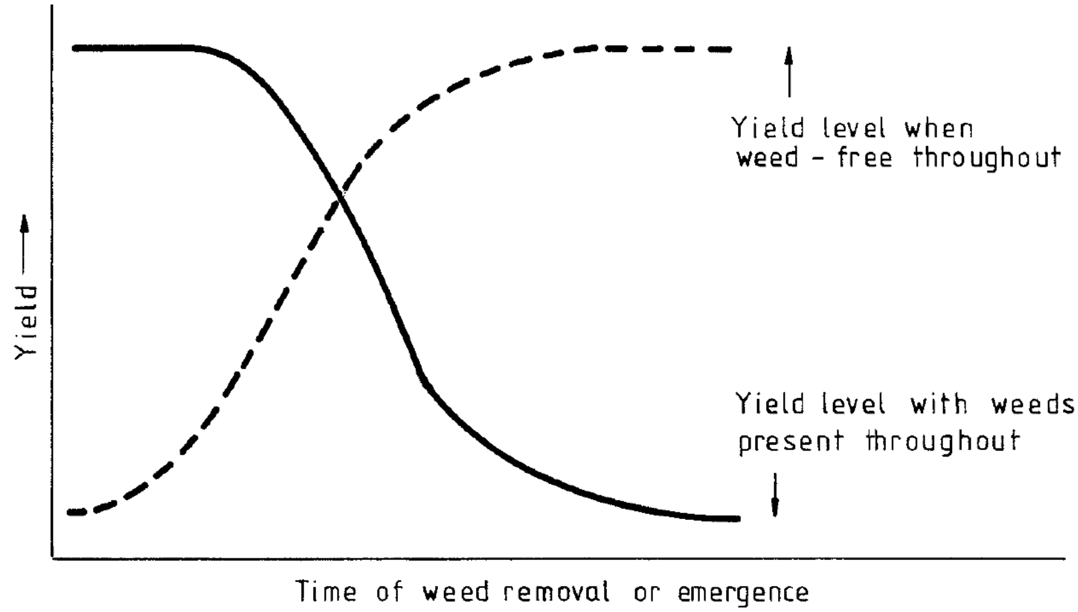

Figure 1.1. Relationship between yield and the amount of time that weeds are present in the field (solid line), or excluded from the field (broken line). The solid line represents the effect of allowing weeds in the field and broken line represents the effect of controlling weeds in the field. Image from (Naylor, 2008).

Throughout the process of crop domestication, weeds have been growing along with crops, necessitating the development of control measures. A range of methods of weed control have been developed, such as hand pulling and cutting using ancient and modern tools (Ch *et al.*, 2016). More recently, mechanical weed control and herbicide methods have been used to control weeds. These control measures have contributed to improved crop production. However, there are challenges associated with them (Rueda-Ayala *et al.*, 2015). For example, mechanical control of weeds is very expensive to manage, and herbicides result in herbicide resistant weeds and damage to the environment.

The problems caused by use of synthetic herbicides in the environment has resulted in increased efforts to discover sustainable and effective weed management tools. The exploitation of allelopathic interactions for weed control is one of the possible sustainable solutions (Campiglia *et al.*, 2011; Martinez-Feria *et al.*, 2016). In cropping systems the natural occurrence of toxins in certain cover crops can exert phytotoxic effects against weeds (Poffenbarger *et al.*, 2015), and therefore offers an alternative to reliance on herbicide control. Cover crops may also be of benefit for management of soil fertility and losses of nutrients to the surrounding environment.

1.1 Cover crops and soil nutrients

Cover crops are defined in simple terms as crops that are grown to cover the soil (Dabney *et al.*, 2010). Another definition includes any crop cultivated to provide soil protection and soil improvement between periods of normal crop production (Kaspar & Singer, 2011). The first use of cover crops was to reduce soil erosion during the period a field was uncultivated in annual cropping systems (Lawson *et al.*, 2013). Cover crop residues that remain in the field before planting the succeeding crop can also improve the soil organic matter content (Snapp *et al.*, 2005). Cover crops can add organic matter to the soil by residue decomposition. Soil organic matter is important for improving soil productivity by stabilizing soil aggregates and making the soil easier to cultivate (Ding *et al.*, 2006).

Soil structure and soil stability are primary physical characteristics influenced by organic matter in the soil (Nguyen *et al.*, 2016). Organic compounds physically and chemically bind soil particles in the aggregate, thereby increasing the stability of aggregates and reducing their breakdown during wetting (Kumar *et al.*, 2018). The stability of aggregates has an influence on the infiltration of water in the soil and improves water holding capacity (Brye *et al.*, 2003). However, despite the benefits of increased soil organic matter, it is not certain that cover crops will always improve the nutrition and growth of the succeeding main crop (Brennan & Boyd, 2012).

A cover crop should enhance the availability of nitrogen in the soil by recovery of mineral nitrogen from the soil nutrient pool and later passing it on to the succeeding crop, thereby preventing nitrogen leaching into the groundwater during the fallow period (Fan *et al.*, 2006). For example, the inclusion of cover crops in the production system regulates the amount of nitrogen escaping into groundwater and surface water bodies. Winter cover crops are among the crops that are used to take up mineral nitrogen from the soil sink to pass on the benefit to the succeeding crop (Gil & Fick, 2001). Inorganic soil nitrogen accumulates in the cover crop in an organic form and the following crop benefits as the cover crop residue decomposes (Laird *et al.*, 2010). Some leguminous cover crops can further improve soil nitrogen fertility through symbiotic nitrogen fixation, increasing the potential benefit to the succeeding crop (Tonitto *et al.*, 2006).

The mixing of carbon and nitrogen in the soil has an important role in the availability of nutrients to plants. A biomass carbon to nitrogen ratio of 24:1 is ideal for decomposition of crop residues (Baggs *et al.*, 2000). A carbon to nitrogen (CN) ratio away from the optimum takes longer to decompose because more nitrogen must be sourced from the soil by micro-organisms in order to reach the optimum ratio, reducing the availability of soil inorganic nitrogen. Immobilization or mineralization of nitrogen in the organic matter of cover crops depends on the CN ratio (Hoorman, 2009; Finn *et al.*, 2015).

1.2 Allelopathy

Allelopathy is a subject that deals with the effects of chemicals produced by plants, on the growth, development and distribution of other plants in natural communities or agricultural systems (Aslam *et al.*, 2017). The word allelopathy is derived from a Greek word and refers to mutual suffering (Belz & Hurle, 2004). It was earlier defined as a chemical interaction that exists among plants and other organisms (Álvarez-Iglesias *et al.*, 2014a). Later on the definition of allelopathy was refined to include the release of chemical compounds by a plant into the environment that influences germination, growth and development of surrounding plants (Ehsan *et al.*, 2011).

The release of allelopathic phytotoxins by plants may be essential to enhance the competitive ability of a plant in a new environment, by inhibiting the growth and life processes of other plant species (Adler & Chase, 2007). This is understood as a complex process occurring in nature to suppress growth of susceptible associated species from the common habitat (Jabran & Farooq, 2013).

In addition, allelopathy is a biological phenomenon where organisms produce one or more biochemicals that affect growth, survival, and reproduction of other organisms (Li *et al.*, 2016). The biochemicals released are called allelochemicals and are a part of products released by plants to reduce competition for resources (Gniazdowska & Bogatek, 2005). These allelochemicals are found in various types of plant species and can be released into the environment through a variety of pathways, including from the roots as exudate, by leaching from the leaves or leaf litter, by decomposition, or as a volatile gas (Fig. 1.2) (Farooq *et al.*, 2011). The phenomenon of allelopathy is very important in the functioning of natural communities because allelopathy also impacts on microorganisms and other soil organisms (Chon & Kim, 2002).

Plant allelochemicals can have strong effects, such that even small doses have the potential to inhibit germination and growth of other plants (Lawson *et al.*, 2013). The impact of these compounds on surrounding plants is greater at early growth stages, resulting in the inhibition of seed germination and growth of seedlings (Khan *et al.*, 2011). Allelochemicals operate by disrupting physiological and metabolic functions of plants such as photosynthesis, uptake of nutrients and water, DNA synthesis and respiration (Shah *et al.*, 2016b).

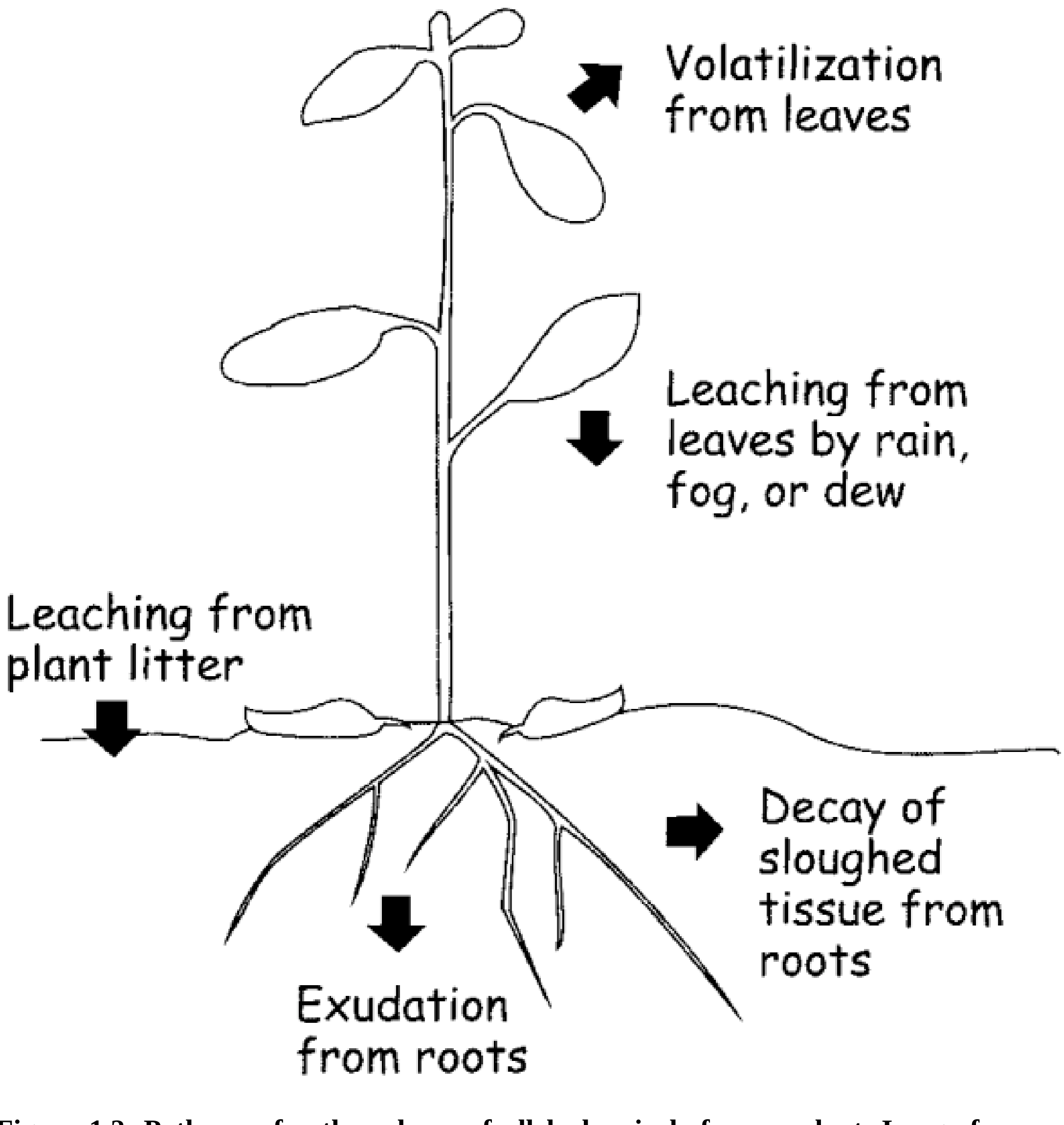

Figure 1.2. Pathways for the release of allelochemicals from a plant. Image from (Birkett *et al.*, 2001)

1.3 Application of allelopathy in crop production

The agricultural sector is facing the challenge of balancing increased productivity and maintaining a sustainable and clean environment (Mischler *et al.*, 2010; Mirsky *et al.*, 2011). In order to meet the challenge, practices of crop production need to be modified to support increased yield and reduced pollution of the environment (Haig, 2008). Use of herbicides in the control of weeds results in traces escaping and finding their way into surface- and ground-waters (Campiglia *et al.*, 2011; Martinez-Feria *et al.*, 2016). High accumulation levels result in adverse effects on the environment and human health. To reduce the impact on the environment, methods that are environmentally friendly have been researched that do not release any detrimental chemicals to the environment (Burgess *et al.*, 2002). One solution is based on observing natural interactions between plants. This includes plant released toxins that suppress germination and growth of weeds in agricultural systems (Hiltbrunner *et al.*, 2007; Mirsky *et al.*, 2011). Cultivation of clover and ryegrass as cover crops is an example of the application of allelopathy in crop production, and the chemicals associated with the inhibition effect include flavonoids and quinones (Dabney *et al.*, 2001). Secondary products from plants such as phenolic compounds, including simple phenols, phenolic acids, and tannins, are all potential allelopathic compounds that affect the germination and growth of other plants (Sisodia *et al.*, 2010).

Allelopathic control of agricultural weeds can involve the use of whole plants or plant extracts to suppress germination and growth of weeds (Bais *et al.*, 2004). In some examples, plant allelochemicals may be formed during the growth of the crop and released while the plant is still alive to affect the germination and growth of surrounding weeds (Soltys *et al.*, 2013), or the allelochemicals may be a product of crop decomposition that affects the germination and growth of succeeding weeds (Jamil *et al.*, 2009). Another method is the use of extracts from allelopathic cover crops that may be applied to the field or main crop to reduce germination and growth of weeds (Cheng & Cheng, 2015).

1.4 Use of Cover crops

Agricultural systems have benefited from the introduction of cover crops. Benefits include soil improvement and weed management (Belz & Hurle, 2004; Tabaglio *et* *al.*, 2013). The use of cover crops in weed management requires a balance to achieve profitable crop production and environmental protection (Lu *et al.*, 2000). The inhibition effect on reducing weed impact could be in the form of living plants or as plant residue (Wakjira *et al.*, 2005). This form affects the mechanism in which cover crops could be used in weed management. As a crop residue, cover crops are left in the field and through decomposition the inhibition effect is released to prevent germination of weed species. When used as living plants, they are planted along with weed species to suppress germination and growth (Adler & Chase, 2007; Farooq *et al.*, 2013; Worthington & Reberg-Horton, 2013; Lou *et al.*, 2016).

Cover crop inhibition effects on seed germination and growth are associated with compounds that are toxic to other plants (Worthington & Reberg-Horton, 2013). According to studies on the subject of allelopathy, some of the compounds associated with potential allelopathy include vanillic, ferulic, phenylacetic, benzoic and salicylic acids (Bhadoria, 2011a; Samanta *et al.*, 2011; Itani *et al.*, 2013). Other compounds associated with allelopathy include alkaloids, glucosinolates and isothiocyanates (Yasumoto *et al.*, 2011; Gfeller *et al.*, 2018) or phenolic and hydroxamic acids (Sisodia *et al.*, 2010). These compounds are believed to be allelopathic and have been isolated and identified. In nature, the subject of allelopathy is difficult to prove because of interactions with other factors that contribute to inhibition of weed germination and growth (Conklin *et al.*, 2002). The inhibition effect on weed species germination and growth can vary from one plant part to another (Fageria *et al.*, 2005). For example, the root and shoots parts of a cover crop might differ in the amount of allelopathic compounds that are produced.

In New Zealand cover crops have been used to control some weed species, with common cover crops including faba beans, oats, clover and ryegrass (Farooq *et al.*, 2013). The most important cover crops that are used are clover and ryegrass. These two cover crops are used as crop residues left in the field before planting of the main crop, with the goal of suppressing the germination and growth of weeds during growth of the main crop (Gibson & Liebman, 2003). The use of extracts from clover and ryegrass to control weed species in the field is not common and is mostly restricted to field and laboratory experiments (Khan *et al.*, 2011).

Gland clover (*Trifolium glanduliferum* Boiss.) is an annual self-regenerating leguminous plant that is native to a northern hemisphere Mediterranean type of climate. In nature, this species commonly grows in open fields and under a forest canopy together with other annual leguminous plants (Hayes *et al.*, 2008). The cultivar of gland clover known as Prima was developed from the germplasm collected in 1976 by J.S Katznelson from the Yehudiyya forest in Golan, Israel (Masters *et al.*, 2006).

In some countries, gland clover production and cultivation is relatively new in agricultural systems. However, there is a great potential for the use of this legume in pasture and cropping production due to its suitability for climatic zones ranging from low to high moisture conditions (Nichols *et al.*, 2007). In addition, gland clover adapts to a wide range of soil types, with the pH in the range of 4.8 to 7.5 and waterlogged to well-drained soil types. Prima gland clover has been selected as a cover crop to fix nitrogen to improve soil fertility and assist in the management of weeds (Moot, 2013).

Ryegrass (*Lolium perenne* L.) is a perennial grass that was introduced to New Zealand by early European pastoralist (Zheng *et al.*, 2017). It has been grown as a cultivated species for livestock grazing, for fodder, as a cover crop, for grass as lawn and pasture improvement (Griffith *et al.*, 2017). Like clover, ryegrass is also known for its potential to produce allelopathic chemicals. It is effective in weed suppression in the form of living mulch, as rye residues, and as root leachates. Residues from rye have been found to inhibit the germination and growth of various weed species. Certain allelochemicals have been discovered in residues from rye, such as benzoxazolinone. In addition, decomposed rye residues contain phenolic compounds that are inhibitory to weeds. Young rye tissues contain higher concentrations of allelochemicals compared to the decomposing residues. In organic agriculture, a rye cover crop is used as an inter-crop to control weeds (Gale *et al.*, 2016).

1.5 Knowledge gaps and objectives of research

The 2015/2016 growing season was the first year of the project that the Foundation for Arable Research (FAR) is conducting in conjunction with AgResearch at its northern crop research site in Tamahere to evaluate the effectiveness of cover crops for reducing weed pressure in maize production. The trial demonstrated that cover crops grown as part of maize production could suppress weed growth and still maintain high silage and grain yields (Trolove *et al.*, 2017).

Additional field trials were conducted in the following growing season of 2016/2017 to evaluate the effects of winter cover crop residues on maize production compared to the practice of fallow. In that year the study included the effects of cover crops on the growth of weeds and grain yield of the succeeding maize crop. The findings indicated that clover, rye and oats as cover crops all suppressed the growth of weeds. However, the results showed slower development and lower yield of maize in treatment plots with oat and rye cover crop residues. Clover residue plots had higher maize yield than the fallow treatment. It was believed that oats and rye took longer to decompose compared to prima gland clover and therefore reduced the availability of soil nitrogen during early growth of the main crop. Reduced maize growth with oats and rye could have been a result of the low availability of soil nitrogen and there was therefore a need to carry out research to study how the cover crops affected soil nutrients over the period of growing season.

Clover and rye were also selected as cover crops with more persistent residual effects on weeds in the field. There was therefore a need to investigate how rye and clover are having their weed suppression effects. In particular, whether a form of allelopathy is involved. For quick analysis, the extracts of cover crops can be applied to seeds of selected crop and weed species to test for inhibition effects (Kumar & Sahoo, 2011; Satish *et al.*, 2015). Crop extracts have been used to detect the effect of allelopathy on germination. However, few experiments have compared rye and clover extracts. Also, many studies do not include a variety of weed and crop species in their tests.

Most of the studies on allelopathy have been conducted under laboratory conditions. The test of inhibition effects on seed germination does not assess the effect of a cover crop on growth. This contributed to the need to conduct a study in the glass house environment to test the inhibition effect of the two main cover crops on germination and growth of weed and crop species. Also, it has not been established whether it is the crop residue roots (belowground parts) or shoots (aboveground parts) that provide the highest potential allelopathic effect on weeds.

The aim of this thesis was therefore to examine how the most important cover crops used in the FAR trials are having their effects on weed and main crop growth. The study was divided into three objectives.

Objective One was to investigate the causes of the lower yield of the maize crop following a ryegrass crop compared to fallow or clover. The effects of winter fallow, clover and rye cover crops on nutrient availability to the main crop were monitored during the 2017/2018 maize growing season. Soil samples were taken throughout the maize growing season, and maize growth, yield and nutrient contents were measured. The hypothesis was that planting clover as a winter cover crop would increase the nutrient levels in the soil at the time of maize sowing compared to rye. The results of this study are described in *Chapter 2*.

Objective Two was to test rye and clover for allelopathic effects on seed germination by other species under laboratory conditions. Rye and clover shoots were harvested from the field and used to prepare extracts that were applied to seeds of test species. Dilutions of extracts were compared to determine the dilution rate at which the inhibition of germination was reduced. The hypothesis was that extracts of clover inhibit seed germination of test species more than extracts of rye. The results of this study are described in *Chapter 3*.

Objective Three was to test rye and clover crop residues for allelopathic effects on seed germination in soil. Rye and clover plants were grown in soil in pots under glasshouse conditions until flowering. The shoots were then cut from the roots and placed on soil in a second set of pots, before seeds of test species were sown into the root and shoot pots. The effects of the rye and clover residues on volunteer weed and test species germination and growth were then recorded. The hypothesis was that clover root and shoot residues would inhibit seed germination more than rye root and shoot residues. The results of this study are described in *Chapter 4*.

Chapter 5 provides a summary of the key findings, general conclusions, and recommendations for future research.

Chapter 2

Effect of winter cover crops on soil nutrients and maize growth and yield.

2.1 Introduction

Residues of cover crops left in the field before planting the maize crop improve the soil organic matter (Hiltbrunner *et al.,* 2007). Proper selection of the type of cover crop can increase farm annual production and the total annual dry matter yield. Cover crops provide organic matter to the soil by residue decomposition. Soil organic matter is important in improving soil productivity by stabilizing soil aggregates and making the soil easier to cultivate (Kaspar & Singer, 2011). Soil structure and soil stability are primary physical characteristics influenced by organic matter in the soil (Nguyen *et al.*, 2016). Organic compounds physically and chemically bind soil particles in the aggregate, thereby increasing, the stability of aggregates and reducing their breakdown during wetting (Kumar *et al.,* 2018). The stability of aggregates has an influence on the infiltration of water in the soil and improves water holding capacity (Brye *et al.*, 2003). However, despite the benefits of increased soil organic matter, it is not certain that cover crops will always improve the nutrition and growth of the succeeding main crop. An important question is whether the cover crops had an effect on the soil nutrients and the yield of maize.

A cover crop should enhance the availability of nitrogen in the soil by recovery of mineral nitrogen from the soil nutrient pool and later passing it on to the succeeding crop, thereby preventing nitrogen leaching into the groundwater (Fan *et al.*, 2006). For example, the inclusion of cover crops in the production system regulates the amount of nitrogen escaping into groundwater and surface water bodies. Winter cover crops are among the crops that are used to take up mineral nitrogen from the soil sink to pass on the benefit to the succeeding crop (Gil & Fick, 2001). Inorganic soil nitrogen accumulates in the cover crop in an organic form and the following crop benefits as the cover crop residue decomposes (Laird *et al.,* 2010). Furthermore, some leguminous cover crops can further improve soil nitrogen fertility through symbiotic nitrogen fixation, increasing the potential benefit to the succeeding crop (Lawson *et al.*, 2013).

The mixing of carbon and nitrogen in the soil has an important role in the availability of nutrients to plants. A biomass carbon to nitrogen ratio of 24:1 is ideal for decomposition of crop residues (Burgers 2002). A carbon to nitrogen (CN) ratio away from the optimum takes longer to decompose because more nitrogen must be sourced from the soil by micro-organisms in order to reach the optimum ratio, reducing the availability of soil inorganic nitrogen. Immobilization or mineralization of nitrogen in the organic matter of cover crops depends on the ratio of carbon to nitrogen (Finn *et al.*, 2015).

The 2015/2016 growing season was the first year of the project that the Foundation for Arable Research is conducting in conjunction with AgResearch at its northern crop research site in Tamahere to evaluate the effectiveness of cover crops for reducing weed pressure on maize production. The trial demonstrated that cover crops grown as part of maize production could suppress weed growth and still maintain the high silage and grain yields (Trolove *et al.*, 2017).

In the following growing season of 2016/2017, another field trial was conducted to evaluate the effects of winter cover crop residues on maize production compared to the practice of fallow. In that year the study included the effects of cover crops on the growth of weeds and grain yield of the succeeding maize crop. The findings indicated that prima gland clover, ryegrass and oats as cover crops all suppressed the growth of weeds. However, the results showed slower development and lower yield of maize in treatment plots with oat and ryegrass cover crop residues. Prima gland clover residue plots had higher maize yield than the fallow treatment. It was hypothesized that oats and ryegrass took longer to decompose compared to prima gland clover and reduced the availability of soil nitrogen during early growth of the main crop. Therefore, there was a need to study the changes in soil nutrients over the period of the maize growing season.

Therefore, in the present growing season (2017/2018) a trial was conducted to investigate the causes of the lower yield of the maize crop following a ryegrass cover crop compared to fallow or other cover crops. This was done by analysing the carbon to nitrogen ratio of the soil from the time of maize planting until the end of the growing period, and the ratio in the maize plant material itself. Other parameters that were measured included crop growth and yield. The final crop nutrient content was observed at the end of growing period.

2.2 Methods

2.2.1 Site selection

The field trial was established at the Northern crop research site (37.5022° S, 175.2224° E) of the Foundation for Arable Research at Tamahere, Waikato, New Zealand. The experiment site was established in the growing season of 2015/2016 for the first season of cover crop assessment on silt loam soil (pH 6.8, TC 4.4%). The site had been used to grow maize in past seasons. The preceding maize crop was harvested, and the field was left fallow for 3 weeks in the winter of 2017 before planting of the cover crops.

2.2.2 Cover crops treatments

Plots were planted by east-west column orientation and replicated four times in a randomised block design along with fallow plots as a control. Five cover crop mixtures were tested. Clover (*Trifolium glanduliferum* cv. 'Prima') was sown along with ryegrass (*Lolium multiflorum* cv. 'Tama') at the rate 6.6 kg/ha clover and 2.5 kg/ha rye (clover rye 0.1), clover sown with ryegrass at 6.6 kg/ha clover and 6.25 kg/ha rye (clover rye 0.25), faba beans (*Vicia faba* cv. 'Ben') with ryegrass at 150 kg/ha faba and 25 kg/ha rye (rye 0.5 faba), faba sown with ryegrass at 150 kg/ha faba and 12.5 kg/ha rye (0.5 rye 0.5 faba) and ryegrass alone at the standard rate of 25 kg/ha (rye). Cover crops were sown on 11 June 2017, onto the stalks that remained on the soil surface from the previous crop. The cover crops were left in the plots without destruction after reaching the heights in the range of 30 – 50 cm and sprayed with herbicide (glyphosate) at a rate of 2.7 l/ha using a quadbike mounted sprayer with water rate of 200 l/ha.

In spring (6 November 2017) of the planting season, maize was planted using no-till cultivation practice in two strips of four rows each, leaving an equal sized gap between each strip without maize being planted. The total number of plots was 48 with an area of 18 m^2 each (3 m wide x 6 m long). Only half of the plots were planted with maize. A standard pre-emergence and post-emergence herbicide was applied to control weeds. No fertilizer was applied to the cover crops and the maize crop. However, the previous experiment conducted in the same field had maize grown and a starter fertilizer was applied at the rate of 150 kg/ha and a stabilised urea fertilizer (SustaiN) was broadcasted at 92 kg nitrogen per hectare.

2.2.3 Soil sampling

Soil samples were taken six different times at a depth of 0-10 cm from each of the 48 plots. Six subsamples were taken across each plot, mixed and then frozen on the day of collection. The interval of soil sampling was two weeks for the first, second and third sampling dates and four weeks for the other sampling dates thereafter. The soils were subsequently dried in an oven for 48 hours at 35°C, then screened to 4 mm particles. The dried samples were then divided and repacked for two separate analyses.

2.2.4 Total soil carbon and nitrogen

Total soil carbon and nitrogen contents were measured at the University of Waikato Stable Isotope Laboratory. Five grams of soil was ground in a ball mill (MM400, Retsch, Germany) for 2 minutes. Sub-samples of 0.2 mg were weighed into foil capsules with samples weighed using a 6 place high precision microbalance (DeltaRange PM4800, Mettler, Ohio) to provide approximately 100 µg carbon and 20 µg of nitrogen. Total carbon and nitrogen content of the samples was measured using an analyser (Elementar Vario EL cube, Germany). Samples were referenced to the standard set.

2.2.5 Extractable soil carbon and nitrogen

Extractable soil carbon and nitrogen content were measured at the AgResearch laboratory. The samples were analysed for moisture content and pools of soluble carbon and nitrogen. The soils were sieved to 4 mm and extraction was done for 2 hours with 0.5 M potassium sulphate, then filtered through Whitman's #42 filter paper. Extracts were analysed for inorganic nitrogen in the segmented flow analyser (Skalar SAN++ KELADA 1050 sampler) and for the soluble organic carbon and total soluble nitrogen in the analyser (Shimadzu TOC-VCSH/TNM-1) (White & Nairn, 2007).

2.2.6 Maize plant growth

On 16 January 2018 (nine weeks after maize emergence), four maize plants were randomly selected per plot. The 7th emerged maize leaf at maturity was cut at the ligule from each plant and placed in sample bags. The bagged samples were stored in the chiller prior to leaf area measurements. A bench top leaf area meter (LI3000, Licor, Nebraska) was used to measure the area of sampled leaves. The average leaf area (in centimetres) of the four sampled leaves per plot was recorded.

Shortly after flowering on 27 February 2018, the heights of four randomly selected maize plants were measured using a 4 m long measuring pole and recorded. The measuring pole was placed alongside the maize plant and the height of the highest tip of the flower was recorded as plant height.

2.2.7 Maize plant yield

Maize plants were harvested for silage yield on the 15 March 2018 (17 weeks after emergence), from a representative strip 1.5 m wide in the second central line of each plot. All plants within the strip were cut at 10 cm above ground and their fresh weight recorded. Afterwards, two plants were randomly selected, mulched, and a 200 g sub-sample was taken for drying in an oven at 80°C for 48 hours to determine the moisture content.

Maize grains were hand harvested on 8 May 2018, after black layer formation in the kernels. All cobs within a 2 m strip from the third row of each plot were collected, counted and bagged. The cobs were air dried before shelling. Shelling was done using a Sheller (Vidhata B3 model) and all the grains from each plot were weighed together to provide an estimate of the total grain yield for each plot. In addition, a sub-sample from each plot was dried in an oven at 100°C for 48 hours to determine grain moisture content.

2.2.8 Plant nutrient analysis

A sub-sample of the dried silage sample was retained for determination of total nutrient content by the Eurofins New Zealand laboratory services. Nutrients analysis was only carried out on fallow and rye treatments. These had already been shown to be on the low and high extremes of results from the other measurements. Samples were analysed using the combustion elemental analyser (Mitsubishi NSX-

2100) by combustion at high temperature (1020 - 1800°C), followed by oxidation with pure oxygen and finally, samples were chromatographically separated.

2.3 Data analysis

One way and two-way analysis of variance (ANOVA) tests were performed to assess the effect of cover crop treatments and date, soil and plant nutrient contents and yield parameters. Duncan test were used for post-hoc pairwise comparisons between treatments. Data were checked for normality and homogeneity of variance and transformed where necessary. All analyses were completed in Statistica version 13.3 (Tibco 2017 Software Inc)

2.4 Results

2.4.1 Total soil nitrogen

Total soil nitrogen content at the time of sowing of maize crop did not differ between cover crop treatments (ANOVA, $p>0.05$; Fig. 2.1). Extractable nitrogen content was highest with a fallow treatment (no cover crop) and was usually higher after cover crops that included clover or faba, compared to rye, however, there was no significant effect of cover crops and fallow treatments (ANOVA, $p>0.05$; Fig. 2.1).

2.4.2 Soil carbon to nitrogen ratios

The total soil carbon to nitrogen ratio varied little during growth of the maize crop, and only differed significantly between the cover crop treatments on the first and last date of sampling (ANOVA, $p<0.05$; Fig. 2.2). Across all dates, the ratio was usually lowest for cover crops containing clover, highest for cover crops with high proportions of rye and intermediate for the fallow treatment (no cover crop). In all treatments, the ratio declined over the season, except for a small increase on the final date, but the range of variation across all dates and treatments was small (<5%; Fig. 2.2).

The extractable soil carbon to nitrogen ratio varied significantly between cover crop treatments, and over time during growth of the maize crop (Fig. 2.3). The differences between crops was largest during early growth of the maize crop (ANOVA, $p<0.001$). The ratio was lowest for the fallow treatment, followed by cover crops containing clover, and highest for cover crops containing rye. The extractable carbon to nitrogen ratio tended to decline during the season and became less different between cover crops but increased again in February following heavy rain (Fig. 2.3). Compared to the total soil carbon to nitrogen ratio (Fig. 2.2), the range of variation in extractable soil carbon to nitrogen ratio between treatments and dates was much larger (50-100% differences between lowest and highest values; Fig. 2.3).

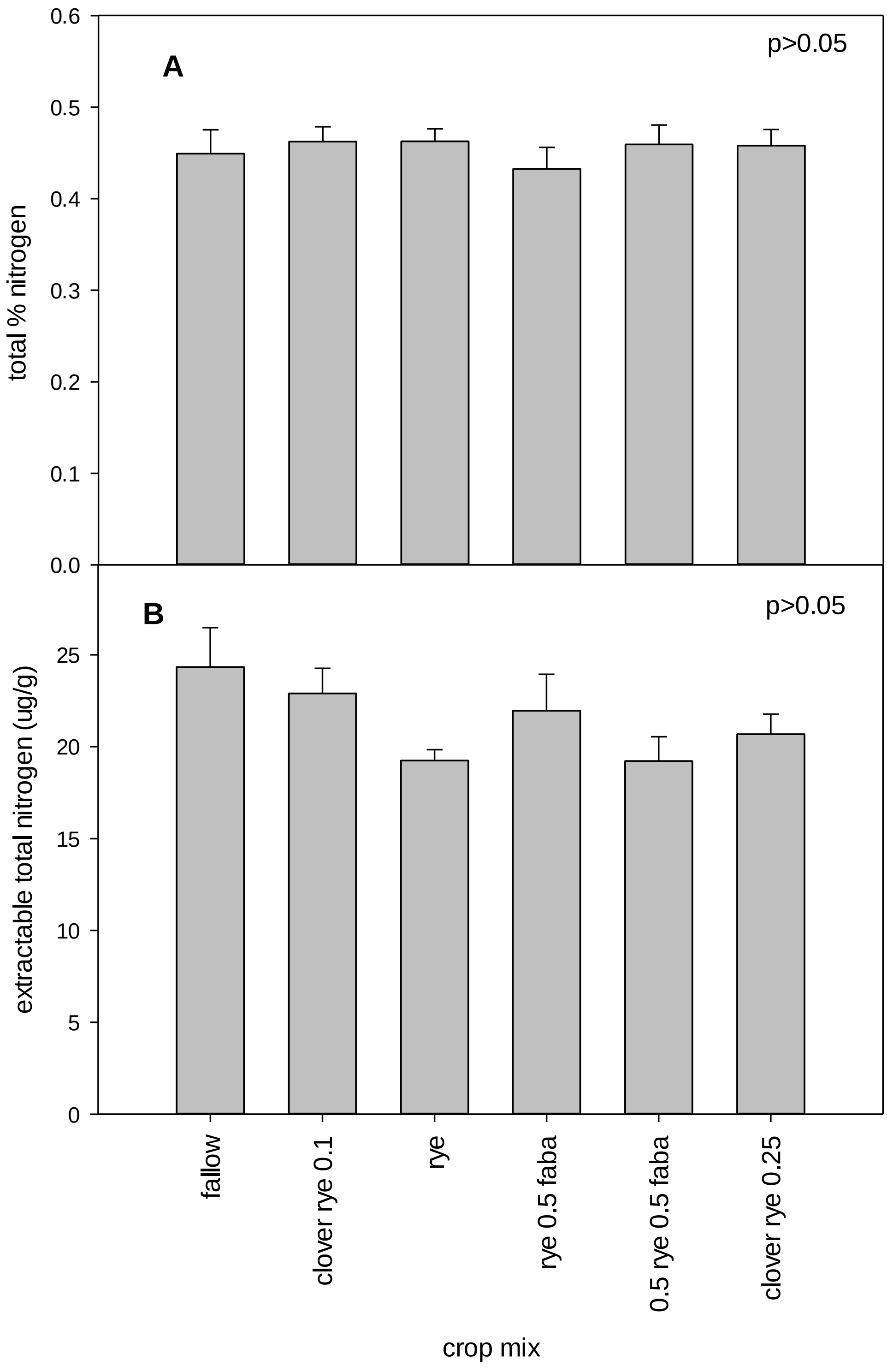

Figure 2.1. Soil nitrogen content for the cover crop and fallow treatments at Northern crop research site on 09 November 2017, at the time of sowing of the maize crop. (A) Total % nitrogen (B) Extractable nitrogen. Bars show sample means ± SE, n = 6

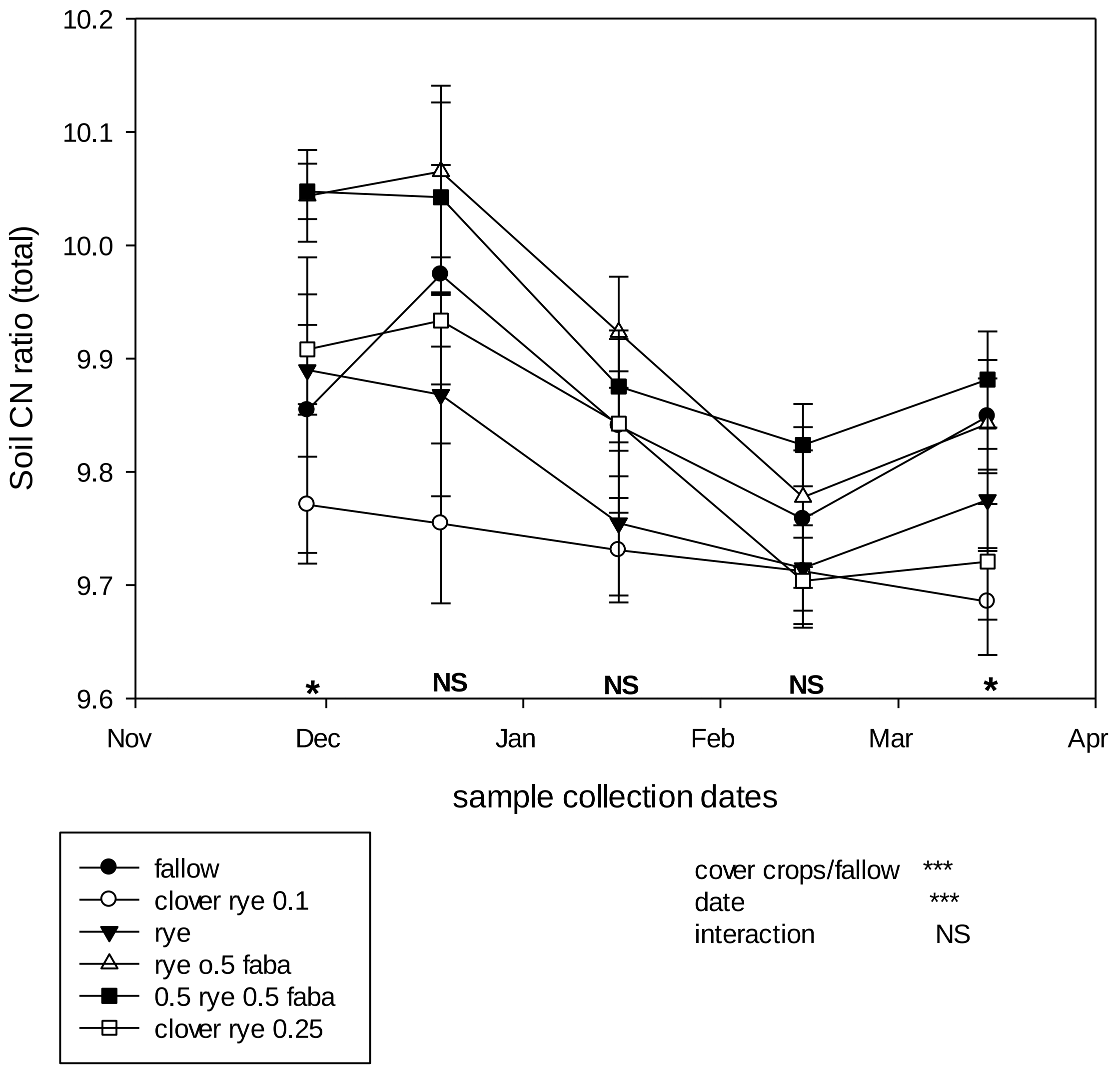

Figure 2.2. Total carbon to nitrogen ratio of soil samples collected at Northern crop research site Tamahere for 5 cover crop treatments and fallow treatment. Samples were collected 5 times during the maize growing period. Points show means ± SE, n = 6. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, NS not significant.

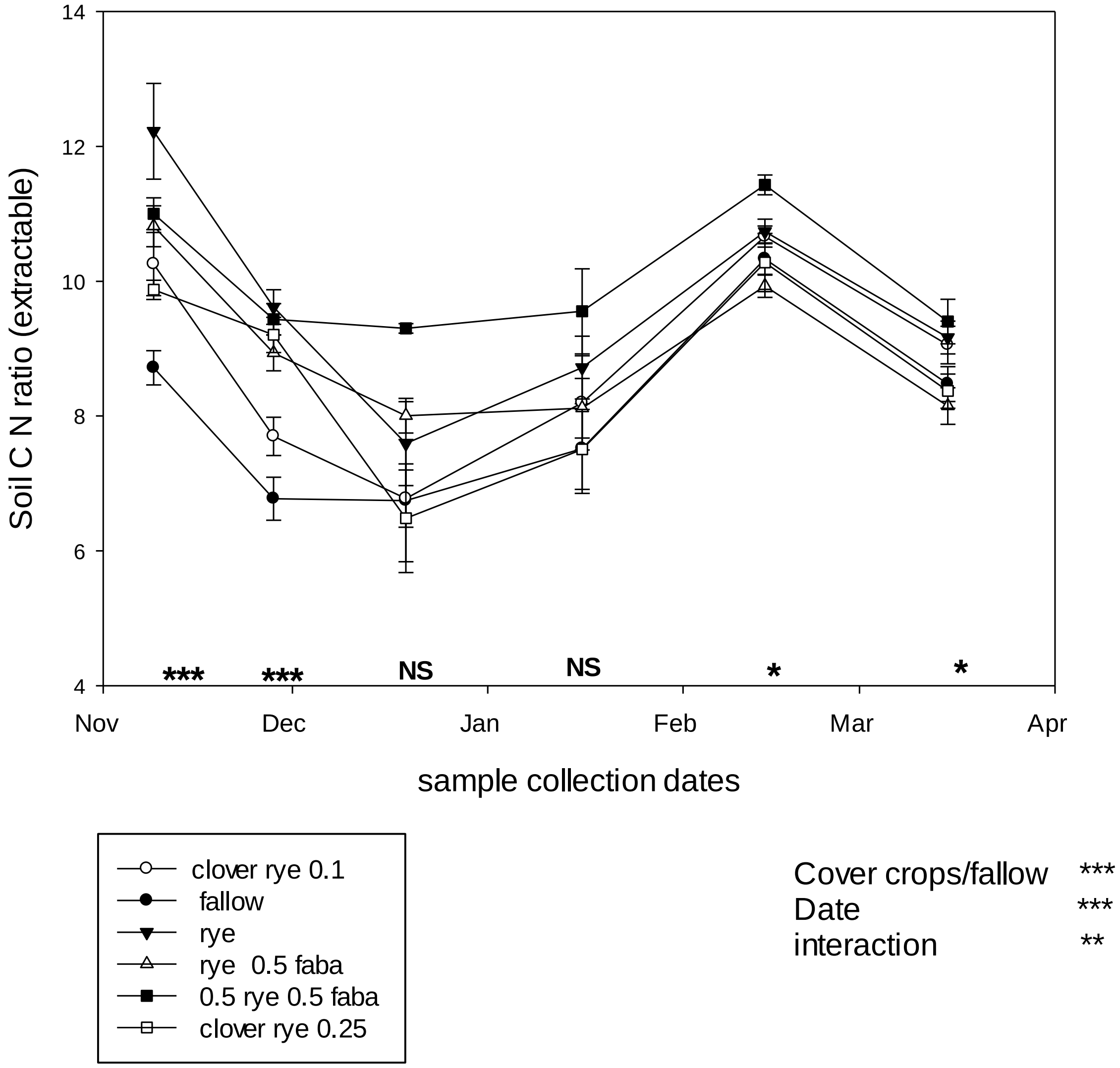

Figure 2.3. Extractable carbon and nitrogen ratio of soil samples collected at Northern crop research site Tamahere for 5 cover crop treatments and the fallow treatment. Samples were collected 6 times before and during the maize growing period. Points show means ± SE, n = 6. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and NS not significant.

2.4.3 Maize growth

The cover crop treatments had clear effects on the growth of the following maize crop, with faster growth in height and leaf area following the fallow and clover treatments, compared to rye and rye/faba (Fig. 2.4–2.7). Two weeks after planting, the maize plants appeared healthy, with no visible difference in the growth between treatments (Fig. 2.4). However, by the flowering stage, there were clear reductions in height and differences in leaf colour in plots treated with cover crops compared to winter fallow (Fig. 2.5).

Figure 2.4. The growth of maize crop at two weeks after planting. The plots indicate cover crop treatments of (A) rye treatment (B) fallow treatment and (C) clover treatment.

Figure 2.5. The growth of maize plants at flowering stage under the cover crop treatments plots. (A) rye treatment (B) clover mix treatment and (C) fallow treatment.

Leaf area prior to flowering was highest in the fallow treatment and lowest in the rye and rye/faba treatments (ANOVA, $p<0.01$; Fig. 2.6). Clover containing cover crop mixes resulted in a slight but non-significant reduction in leaf area compared to the fallow treatment (Duncan's Multiple Range Test, $p>0.05$; Fig.2.6).

Similarly, plant height was highest in the fallow treatment and approximately 20% lower in the rye treatment (ANOVA, $p<0.001$; Fig. 2.7). Compared to fallow, the clover containing cover crop mixes also caused a significant reduction in maize plant height (Duncan's Multiple Range Test, $p<0.05$; Fig. 2.7).

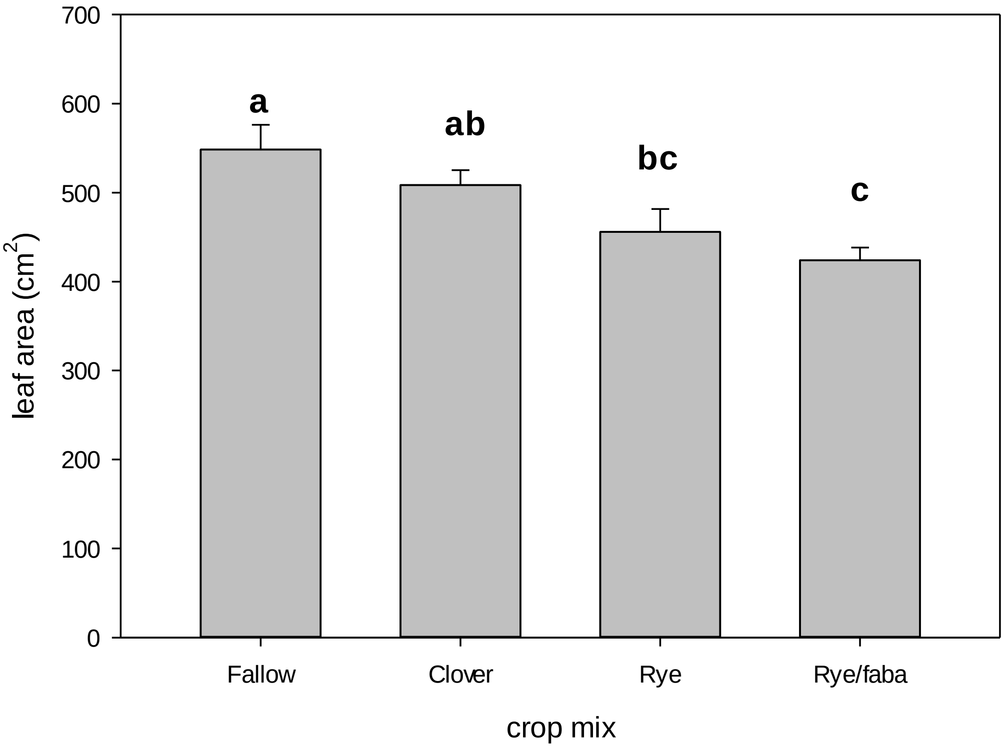

Figure 2.6. Average leaf area of four leaves per plot for maize plants measured before flowering stage for cover crop treatments and fallow. Clover mix treatments were grouped together as clover, rye and faba mixes were also grouped together as rye and faba. Bars show sample means ± SE, n= 4, ($p \leq 0.01$). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, $p<0.05$).

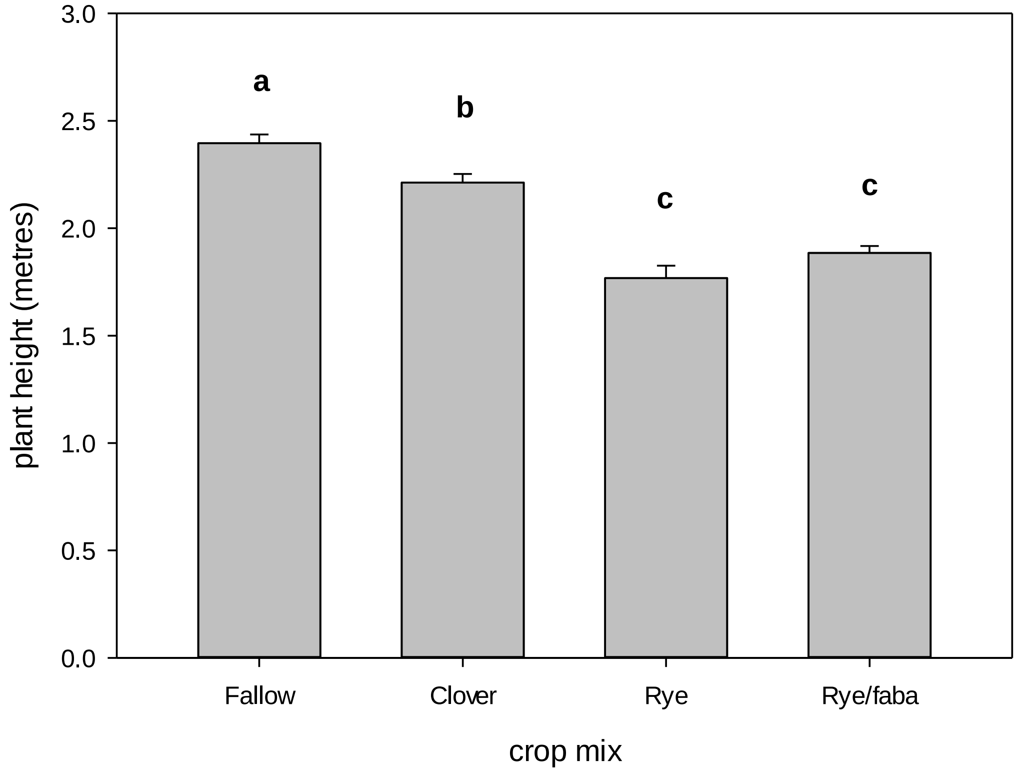

Figure 2.7. Plant height of maize plants measured at flowering stage for the cover crop treatments and fallow. All clover mix treatments were grouped together as clover, and rye and faba mixes were grouped together as rye and faba. Bars show sample means ± SE, n= 4, ($p \leq 0.01$). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, $p < 0.05$).

2.4.4 Maize yield

Cover crop treatments caused a two-fold difference in silage yield, with the fallow treatment producing the highest silage yield and the rye cover crop the lowest (ANOVA, $p < 0.01$; Fig. 2.8). Clover and faba cover crop mixes produced intermediate silage yields.

Similarly, the rye cover crop treatment produced the lowest grain yield and the fallow treatment the highest grain yield (ANOVA, $p < 0.01$; Fig. 2.9). Clover and faba cover crop mixes produced intermediate grain yields.

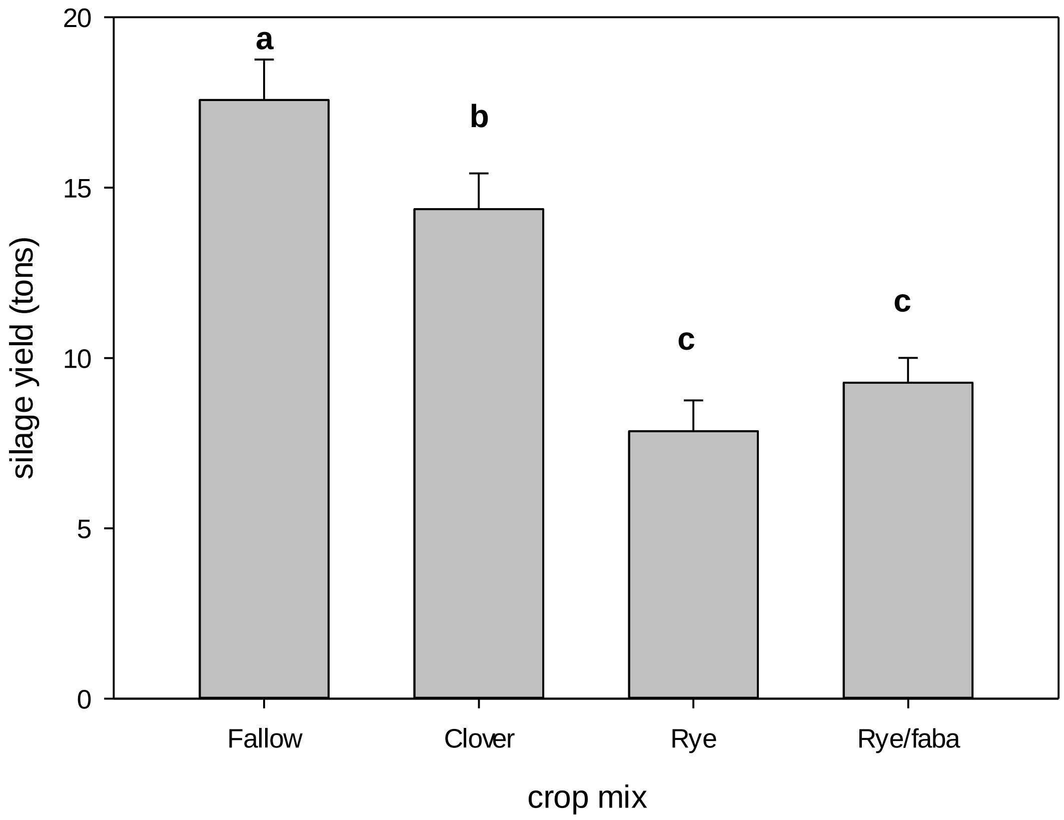

Figure 2.8. Silage yield of the maize plants grown after winter cover crops or fallow treatments, harvested before grain maturity. Clover mix treatments were grouped together as clover, rye and faba cover crop mix treatments were grouped together as rye and faba. Bars show sample means ± SE, n= 8 - 14, ($p \leq 0.01$). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, $p<0.05$).

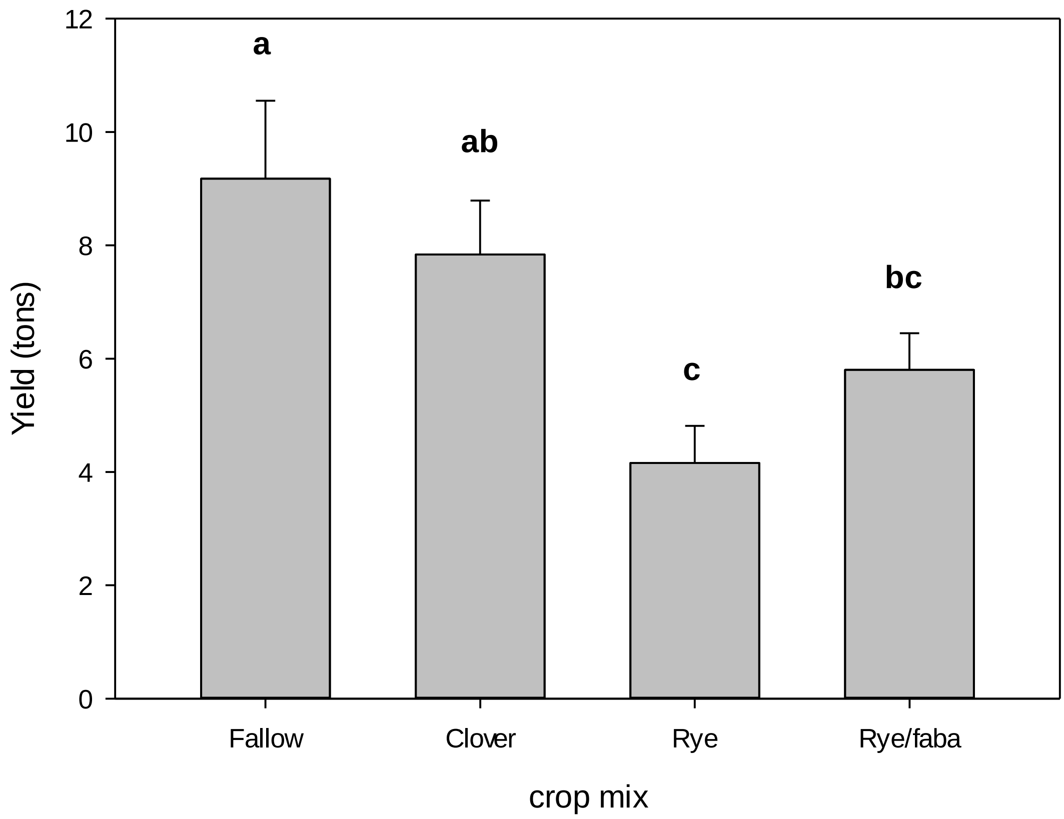

Figure 2.9. Grain yield in tonnes of the maize crop grown under the cover crop treatments and fallow treatment. All clover cover crop mix treatments were combined as clover, rye and faba cover crop mix treatments were combined as rye and faba mix. Bars show sample means ± SE, n= 8 - 14, (p = 0.02). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

2.4.5 Plant nutrients

The CN ratio of mature maize plant biomass was highest following winter cover crop mixes that included clover and lowest following rye containing cover crops (ANOVA, p=0.04; Fig. 2.10). Cover crop treatments with faba or fallow had an intermediate ratio (Fig. 2.10).

A full nutrients analysis was completed on the maize plant biomass for the fallow and rye treatments, the two extremes of cover crop treatment effects on maize yield. In five of the six elemental nutrients considered (N, P, K, Mg, S), percent nutrient levels were higher following a rye cover crop compared to the fallow treatment (t-test, not significant for N and K, significant for P, Mg, S; Fig. 2.11). Sodium was the only element that was not higher in concentration in the rye treatment compared to the fallow (t-test, p=0.35; Fig. 2.11).

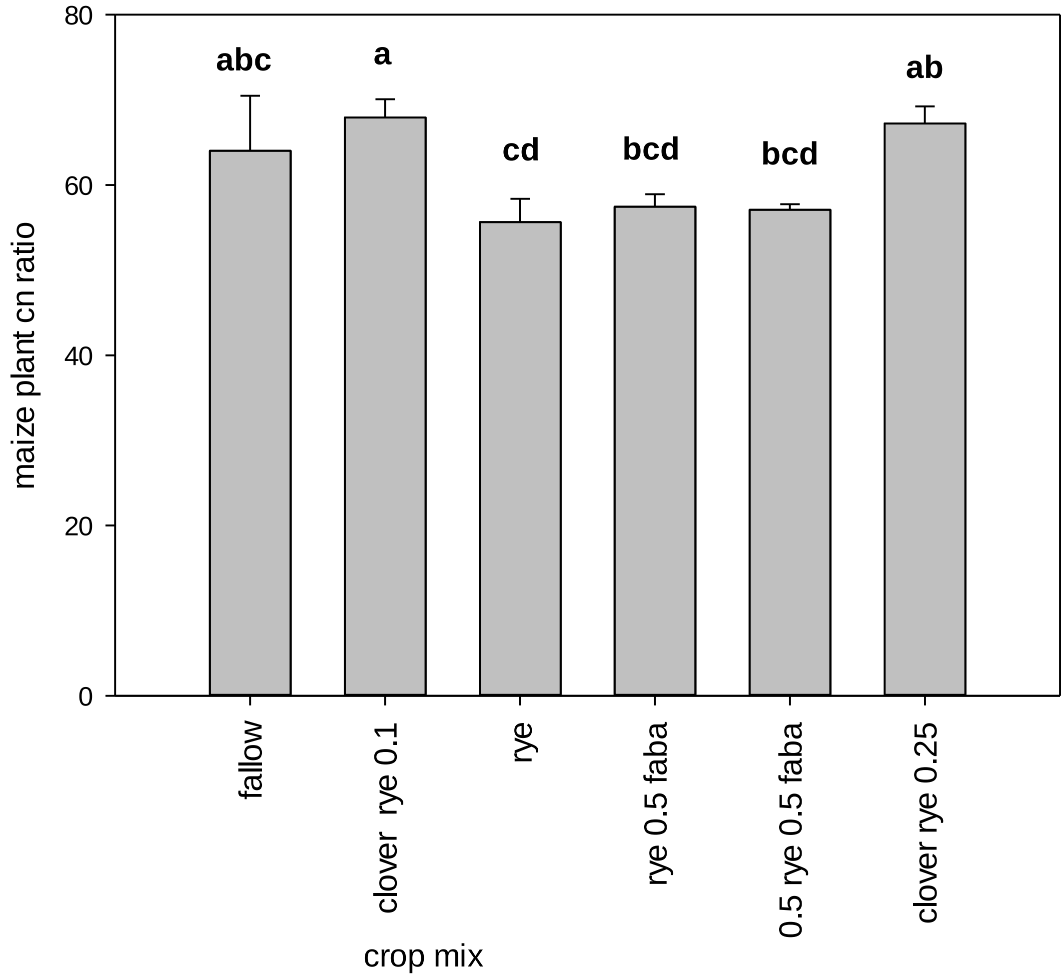

Figure 2.10. Carbon to nitrogen ratio of whole maize plant samples collected at plant maturity stage from the cover crop and fallow treatments. Bars show sample means ± SE, n= 4 - 6, (p=0.04). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

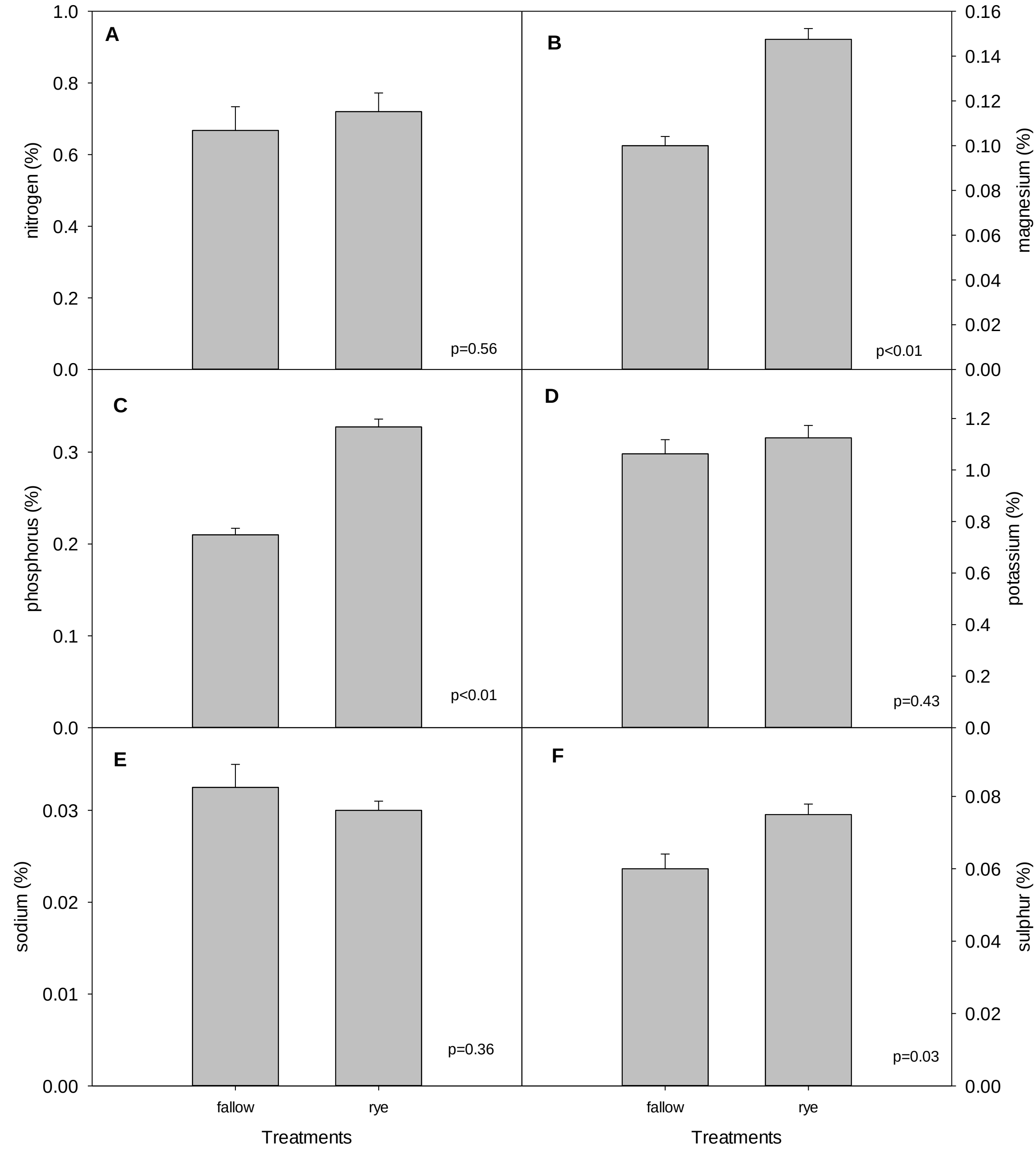

Figure 2.11. Nutrient content of maize plant biomass for rye and fallow treatments, collected at grain maturity and expressed as a percentage of dry weight. (A) % nitrogen (B) % magnesium (C) % phosphorus (D) % potassium and (E) % sodium (F) % sulphur. Bars show sample means ± SE, n= 8 - 14.

2.5 Discussion

The results demonstrate that winter cover crop treatments can have large effects on the yield of a summer maize crop and that these effects are not always positive. Examination of the soil and maize crop nutrient levels suggests that the cover crop's influence on maize yield were driven by the timing of availability of nutrients to the main crop. A winter fallow without cover a crop produced the highest yield because nutrients were not made temporarily unavailable by the cover crop. In this experiment, no nutrients were added between the fallow and main crops. Therefore, if the cover crop is to be used to control weeds, then nutrient availability to both the cover and main crops should be carefully considered. The results also show that a clover-based cover crop is better than rye-based cover crop, probably because it results in a lower soil CN ratio and higher extractable soil N at the time of establishment of the main crop and may therefore release nutrients faster for the benefit of the main crop.

2.5.1 Total soil nitrogen

Winter cover crop treatments did not affect the size of the total nitrogen pool at the beginning of the main growing season. This suggests that the amount of nitrogen fixed by the leguminous cover crops (clover and faba) was not significant compared to the total pool present in normal fallow soil. The cover crops may have served to reduce mobile nitrogen loses over the winter months, indicated by the slightly lower total soil nitrogen in the fallow plots, but overall there were no significant effects of cover crop treatment on total soil nitrogen. The total amount is probably more a function of the history of the field in terms of the amount of fertilizer applied to the field (Fan *et al.*, 2006; Kumar *et al.*, 2018).

The lack of difference in total percent nitrogen at the beginning of the trial also indicates that the cover crop treatment did not affect leaching of nitrogen over this period (Askegaard *et al.*, 2005; Fageria, 2016). This finding may depend on the conditions during the trial, in particular the amount of rainfall during the fallow or cover crop period.

2.5.2 Soil CN ratios

The main effect of cover crops was on the size of the organic fractions in the soil at the time of planting of the maize, and how readily this fraction decomposed to make the organically bound nutrients available to the main crop. The soil CN ratio decreased as the proportion of clover in the cover crop mix increased. The total CN ratio suggests that there was more nitrogen relative to carbon in the soil with clover treatment. Fallow treatment was intermediate, then rye and rye faba treatments had more carbon relative to nitrogen. For the clover mix treatments, the reduction in CN ratio was possibly due to a faster decomposition of the plant materials that contributed to additional nutrient becoming available in the soil nutrient pool (Majumder *et al.*, 2008). This result supports earlier findings of Kramberger *et al.*, (2009) that leguminous cover crops had a reduced CN ratio in the soil compared to non-leguminous cover crops.

At the end of the sampling period, rye and faba mix treatments had higher soil CN ratios than the fallow treatment. Even if the cover mix contained a high rye rate, faba as a leguminous crop was expected to contribute nitrogen to the soil that could have resulted in lower CN ratio in rye faba mix than the fallow treatment. However, the findings of this study suggest that the rye rate reached the point where the faba leguminous crop was suppressed, affecting the process of nitrogen-fixing. The nutrients from the nitrogen-fixing process could have caused a lower CN ratio in the rye faba mix compared to the fallow treatment (Ruffo & Bollero, 2003). Inhibition of the nitrogen fixation process in leguminous plants by non-leguminous plants was observed in rice attributed to phenolics produced by the rice that inhibited growth of nitrogen-fixing bacteria in the soil (Rice, 2012).

The range of differences in CN ratio (with date and cover crop treatment) is very small for total CN ratio measurement. This reinforces the conclusion that the cover crop did not have much effect on the total nutrient pools. The legume cover crop mixes did not add a lot of nitrogen into the soil nutrient pool. Therefore, the cover crops were having more effects on the timing of nitrogen release than the total amount. The high CN ratio recorded on the fifth sampling date for all the cover crops and the fallow treatment was probably due to the heavy downpour of rain received prior to the soil sampling. This was expected because rainwater leaches out the mobile nitrogen from the soil surface, causing a reduction in nitrogen content that leads to a higher CN ratio (Bauer & Roof, 2004). This further illustrates that it was the mobile (extractable) pool that was affected by rainfall and the cover crop treatments, not the total amount.

2.5.3 Maize growth and yield

The cover crop treatments had effects on the growth of the maize crop as shown from higher growth in the clover treatments and fallow. At the beginning of the trial, extractable nitrogen was higher in fallow and clover treatments and lower in rye treatment. The availability of nitrogen at the initial stage of growth contributed to earlier and faster growth of fallow and clover treatments (Snapp *et al.*, 2005). Clover treatments provided nutrients to the main crop through decomposition and fast release, but also delayed nutrient release and crop growth compared to fallow.

It was not surprising to find that the rye cover crop treatment had lower maize growth than the fallow treatment due to slower and lower release of nutrients by decomposition of residue (Gil & Fick, 2001). In addition, other studies showed that a rye cover crop in some cases caused significantly lower growth of the main crop than other treatments due to the removal of nitrogen from the available soil nutrient pool (Jackson, 2000).

Rye faba mix treatments showed a significant difference in growth compared to fallow treatment. Since the treatment contained the leguminous cover crop it was expected to promote growth in a subsequent crop of maize (Thorup-Kristensen *et al.*, 2003). According to other results, where faba was used as a monoculture cover crop, it caused significantly higher main-crop growth than the control treatment (Jensen *et al.*, 2010; Constantin *et al.*, 2011). This could be due to the high rates of rye used in our mixes affecting the nitrogen-fixing ability and suppressing the growth of faba, reducing the potential supply of nutrients from nitrogen fixation and decomposition of the faba and resulting in lower growth of the maize crop (Gartner & Cardon, 2004).

Yield measurement of both silage and grain showed similar results and confirmed the findings from the growth measurements. These findings show that there is not an automatic benefit of using a legume as a cover crop, the benefit probably depends on site fertility before sowing, soil type, and timing of cover crop residue breakdown (Trolove *et al.,* 2017). However, other studies have shown that yield can be higher in treatments involving leguminous cover crops compared to control and non-leguminous cover crops (Ladan & Jacinthe, 2017).

2.5.4 Maize plants nutrients

It was expected that rye and rye faba mix treatments would have a higher CN ratio in the maize plant biomass than in the maize from the clover mix and fallow treatments, because growth rates were lower in these treatments. Surprisingly, the maize plant CN ratio was higher in both the clover mix treatments and fallow treatment. The reason for high CN ratio is probably due to the timing of nutrient availability and growth rate (Yang *et al.*, 2009). In the clover and fallow treatments, the plants' earlier growth was faster and available nutrients were diluted by the large biomass of the plant. For the rye treatment, nutrients perhaps become available more gradually and accumulated later when there was less opportunity for increased photosynthesis and dilution by carbon (Gartner & Cardon, 2004; Majumder *et al.*, 2008). This carbon-dilution effect would explain the higher percent nutrient content of the rye treatment compared to fallow, for all but one of the elements analysed.

Another explanation could be that the larger, faster-growing plants in the clover and fallow treatments approached a CN ratio that was normal for mature maize plants. In smaller plants, maturity may have been delayed and grain yields lower, along with lower harvest indices, reducing the whole plant CN ratios (Burgess *et al.*, 2002). On the other hand, the finding that the CN ratio of high growth maize plants was higher than low growth maize plants is not supported by some other studies (Peoples *et al.,* 2009; Partey *et al.*, 2014; Poffenbarger *et al.,* 2015) that found that CN ratio was higher in slower growing plants. Again, these contrasting findings could be a result of timing effects, and differences between studies in the causes of differences in maize growth and final yield (Fageria, 2016). For example, fertilizing the crop at the flowering stage may cause it to take up nutrients and lower the CN ratio but there would not be much effect on yield.

2.6 Conclusion

This study has shown that winter cover crops affect nutrient dynamics during growth of a maize crop. The total soil CN ratio decreased as the proportion of clover in the cover crop mix increased. The narrow range in the total CN ratio for cover crops and fallow treatments during the trial period is due to total nitrogen not available in the mobile soil sink for plant use. The total CN ratio suggests that there was more nitrogen relative to carbon in the soil with a clover treatment compared to rye and rye faba treatments.

The extractable CN ratio results indicated a higher nitrogen availability relative to carbon in fallow and clover mix treatments at the beginning of the trial. This suggests that early during maize growth nitrogen release and mobility were higher with the clover mix and fallow treatments compared to the rye treatments.

The nutrients were available early for maize to grow rapidly and resulted in higher yields for fallow and clover mixes treatments. In the rye treatment, by harvest time the CN ratio had fallen and nutrients had become available to the crop resulting in lower maize tissue CN ratios and higher tissue nutrient contents. However, it was too late to contribute to growth and yield.

It is indicated from this study that ryegrass has a great effect on the CN ratio in the cover crop mix whether sown at full rate or half rate. It was able to suppress the faba in the mixed cover crop treatment, possibly reducing the potential for supplementation of nutrients through the nitrogen fixation process.

It can be concluded that the type of cover crop and the influence of cover crop on the timing of availability of nutrients to the main crop have important effects on the final yield. Fallow treatments produced higher yields in this experiment because nutrients were not temporarily held by cover crop residues. A clover-based cover crop is better than rye-based because clover biomass has a lower CN ratio and releases nutrients faster.

Chapter 3

Effect of ryegrass and clover extracts on seed germination of grass and leguminous species

3.1 Introduction

Observations of natural interactions between plants in the wild have led to natural measures to control weeds (Jamil *et al.*, 2009). These involve the use of whole plants or plant extracts to suppress germination and growth of weeds in agricultural production systems (Bais *et al.*, 2004). In some examples, plant allelochemicals may be formed during the growth of cover crop and released to affect the germination and growth of surrounding weeds, or the allelochemicals may be a product of residue crop decomposition that affects the germination and growth of weeds during the growth of the following main crop. Secondary products from plants such as phenolic compounds that include simple phenols, phenolic acids, flavonoids, quinones, and tannins have been identified as potential allelopathic compounds that affect the germination and growth of other plants (Sisodia *et al.*, 2010).

The use of plants in nature that contain these allelochemicals is a sustainable and effective weed management tool (Soltys *et al.*, 2013). The control of weeds in agricultural cropping systems is possible by using cover crops with the potential to release these allelochemicals to suppress germination and growth of weed species. Another method is the use of extracts from allelopathic cover crops that are applied to the field to reduce germination and growth of weeds (Cheng & Cheng, 2015).

In New Zealand cover crops have been used to control some weed species, with the most common choices being faba beans, oats, clover or ryegrass. The most important cover crops used are clover and rye, with cover crop residues left in the field before planting the main crop to suppress the germination and growth of weeds (Gibson & Liebman, 2003). The use of extracts from clover and ryegrass to control weed species in the field is not common in New Zealand but is the subject of field and laboratory experiments (Khan *et al.*, 2011).

Gland clover (*Trifolium glanduliferum*) is an annual self-regenerating leguminous plant that is native to northern hemisphere Mediterranean-type climates. In nature, this species commonly grows in open fields and under a forest canopy together with other annual leguminous plants (Hayes *et al.*, 2008). The cultivar of gland clover known as Prima was developed from germplasm collected in 1976 by J.S Katznelson from the Yehudiyya forest in Golan, Israel (Masters *et al.*, 2006).

In some countries, gland clover cultivation is relatively new in agricultural systems. However, there is a great potential for the use of this legume in pasture and cropping production due to its suitability for a wide range of climatic zones from low to high moisture conditions (Nichols *et al.*, 2007; Zheng *et al.*, 2017). In addition, gland clover adapts to a wide range of soil types, with the pH in a range between 4.8 and 7.5 and from waterlogged to well-drained soils. Prima gland clover has been selected in New Zealand as a cover crop to fix nitrogen to improve soil fertility and assists in the management of weeds (Moot, 2013). Previous trials suggested that prima gland clover had an allelopathic effect on the weeds in the field (Trolove *et al.*, 2017). This is one of the reasons for including this crop in the experiments conducted in this chapter to check the effect of cover crop extracts on seed germination by weeds and other species.

Ryegrass (*L. perenne*) is a perennial grass that was introduced to New Zealand by early European pastoralists (Zheng *et al.*, 2017). It is grown as a cultivated species for livestock grazing, for cut fodder, as a cover crop, as a lawn grass, and for pasture improvement (Griffith *et al.*, 2017). Like gland clover, ryegrass has been selected as a cover crop to assist in management of weeds and nutrients. Ryegrass may have a more persistent residue effect (Martinez-Feria *et al.*, 2016) in the field compared to clover (Chapter 2). Since these two cover crops have been used to suppress the growth of weeds in the fields, there was a need to investigate how rye and clover have their weed suppression effects, and in particular, whether it is a form of allelopathy or not.

The aim of this study was to test the effect of cover crop extracts on seed germination of the test species. The term ‘test species’ refers to the species from which seeds were used to test the effect of cover crop extracts on seed germination. These test species included well-known weeds such as fathen and summer grass, as well as readily available crop species like amaranth and lettuce. The first objective was to compare the effect of extracts from a range of cover crops on amaranth and summer grass. The range of cover crops was then reduced to just rye and gland clover, and the second objective was to test the effect of extract dilution and pH on germination of an expanded range of test species (amaranth, summer grass, fathen and lettuce). It was hypothesised that clover extracts would inhibit test species germination more strongly than the other cover crop extracts. If the inhibition of germination by cover crop extract was truly an allelopathic effect, then it was expected that inhibition of germination would decrease in a dose dependent manner as extract dilution was increased.

3.2 Methods

3.2.1 Drying of plant materials

Shoots of cover crops were collected from the trials at the Northern crop research site (37.5022° S, 175.2224° E) in Tamahere. The cover crops were planted on silt loam soil (pH 6.8, TC 4.4%) on a site that had been used to grow maize in previous seasons. The cover crops included faba (*Vicia faba* cv. 'Ben'), clover (*Trifolium glanduliferum cv.* 'Prima'), oats (*Avena sativa*) and rye (*Lolium multiflorum* cv. 'Tama'). The materials were harvested when the flowers of the cover crops had dried, and consisted of leaves and stems without the roots. The samples were dried in an oven (Universal CONVAIRE CAREL air 33, Schwabach, Germany) at 60°C for 48 hours and stored at room temperature in readiness for grinding process.

The dried plant materials were cut using the secateurs into smaller pieces of between 2 and 4 cm in length then ground to a powder with a mortar and pestle.

3.2.2 Petri dish experiment

Five gram aliquots of cover crop powder were measured on a scale (Mettler Toledo SB 3200) and soaked for 24 hours in 100 ml of deionized (MilliQ) water that was considered free from organic or inorganic impurities. The stock extracts were filtered using filter paper (Whatman discs), brought back to 100 ml and stored at room temperature for 24 hours in readiness for application to the seeds of the test species.

Two hundred healthy amaranth (*Amaranthus tricolor* cv. 'Mekong red') and summer grass (*Digitaria sanguinalis*) seeds were selected at random from well-mixed pure seed. One hundred seeds were uniformly spaced on an eight cm diameter filter paper disk to minimise the effect of adjacent nearby seeds on seedling development. The filter paper was placed in eight cm diameter Petri dishes and 10 ml of cover crop extract from clover, ryegrass, faba beans or oats added. Each cover crop treatment and test species combination made up five replicate Petri dishes with their lids, with each dish containing one hundred seeds of the test species. The control treatment consisted of seeds with distilled water. The Petri dishes were sealed with tape and positioned randomly in the laboratory incubator set at 25°C temperature with diffused light during the day. Three days after plating, the number of germinated seeds was recorded.

3.2.3 Paper towel experiment

Harvested shoots materials used for rye and clover extracts were taken from the field (Figure 3.3). Five grams of clover and rye powder were measured on a scale (Mettler Toledo SB 3200) and soaked for 24 hours in 100 ml of deionized (MilliQ) water that was considered free from organic or inorganic impurities. The stock extracts were filtered using filter paper, brought back to 100 ml and stored at room temperature for 24 hours in readiness for application to the seeds of the test species. Extracts were further diluted using distilled water to half, quarter, one eighth and one sixteenth dilutions. The pH of the stock and diluted solutions was measured using a pH meter (EDT instruments, series 3, BA 350, Kent, UK).

A between paper method was used in which seeds were held in between two layers of paper towel, loose enough to allow for air circulation around the seeds. Fifty healthy seeds of each test species were selected at random from the well-mixed pure seed. The seeds were uniformly spaced on the paper towel to minimise the effect of adjacent nearby seeds on seedling development. The paper towel was used due to its ability to hold sufficient water and provide continuous movement of water to the seeds. The test species were fathen (*Chenopodium album*), summer grass, amaranth and lettuce (*Lactuca sativa* cv. 'webbs wonderfull iceberg'). Ten mls of extract solution of either rye or clover was applied to each paper towel. The control treatment was ten mls of distilled water applied to the test species seeds. The experimental design was fully randomised with four replicate towels per cover crop and test species combination, and 50 seeds per towel. Thereafter, paper towels were sealed in plastic bags and randomly arranged in a temperature-controlled room at a temperature of 25°C. Every 24 hours the paper towels were observed for germination and after 10 days a final germination count was conducted on all paper towels. All seeds that reached a stage where essential structures (roots and shoots) were clearly formed were counted as germinated. Hard seeds or seeds without any sign of emerging structures were counted as not germinated.

3.3 Data analysis

Data were checked for normality and homogeneity of variance and transformed where necessary.

For the Petri dish experiment, a one-way analysis of variance (ANOVA) was performed for each test species to assess the effect of cover crop extract on seed germination. Duncan's Multiple Range Tests were used for post-hoc pairwise comparisons between treatments. Analyses were completed in Statistica version 13.3 (Tibco 1984-2017 software Inc)

For the paper towel experiment, a factorial plus added control ANOVA was performed for each test species, with extract type (rye, clover or water) and dilution nested within extract (Cochran & Cox, 1957). Analyses were completed in Genstat 9th edition (2018 VSN International Ltd).

3.4 Results

3.4.1 Petri dish experiment

For summer grass seeds the control and rye extracts had the highest number of seeds germinated and were not significantly different (ANOVA, $p<0.01$; Fig. 3.1). Faba extract had the lowest number of seeds germinated. Clover and oat extracts caused intermediate levels of germination. Compared to rye extracts, the clover extracts caused a significant reduction in the number of germinated summer grass seeds (Duncan's Multiple Range Test, $p<0.05$; Fig. 3.1).

For amaranth seeds the control treatment had the highest number of seeds germinated and extracts of all of the cover crops caused a reduction in germination (ANOVA, $p<0.01$; Fig. 3.2). Oat extract had the lowest number of seeds germinated. Clover, rye and faba extracts had an intermediate number of seeds germinated. Clover extract resulted in a slight but non-significant reduction in amaranth seed germination compared to the rye extract (Duncan's Multiple Range Test, p>0.05; Fig. 3.2).

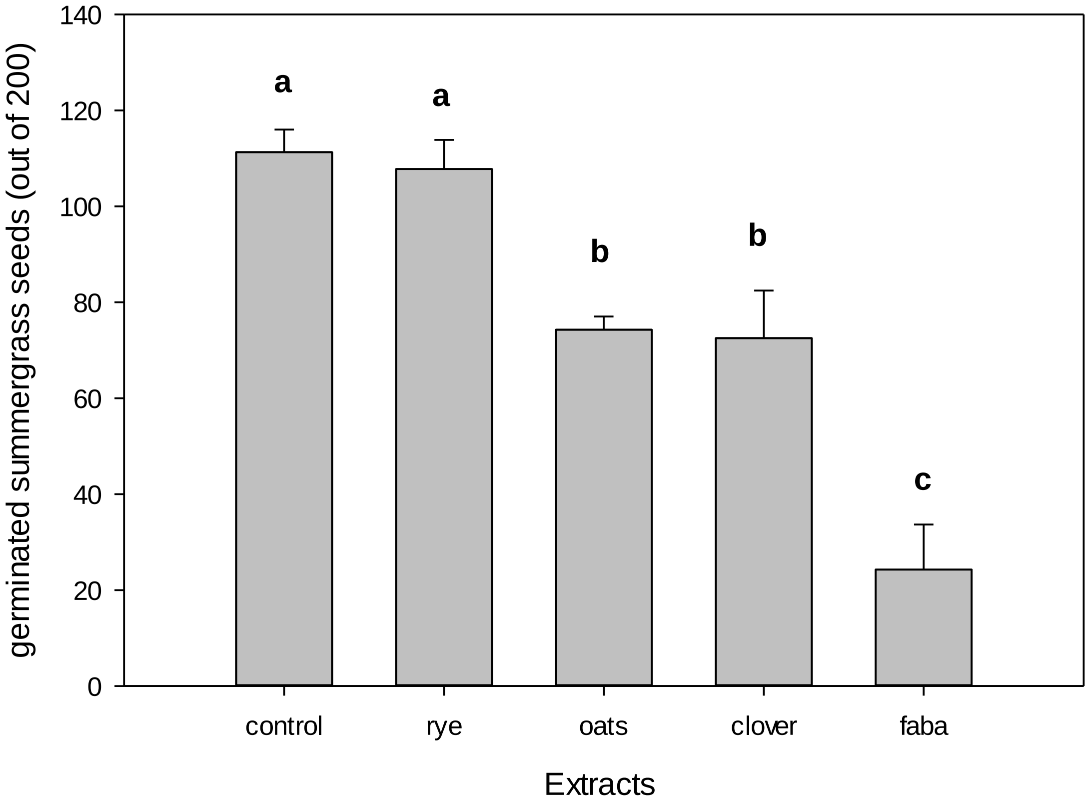

Figure 3.1. Cover crops extract without dilution and a pure water control tested on 200 seeds of summer grass in petri dishes. Bars show sample means ± SE, n= 5, (p<0.01). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

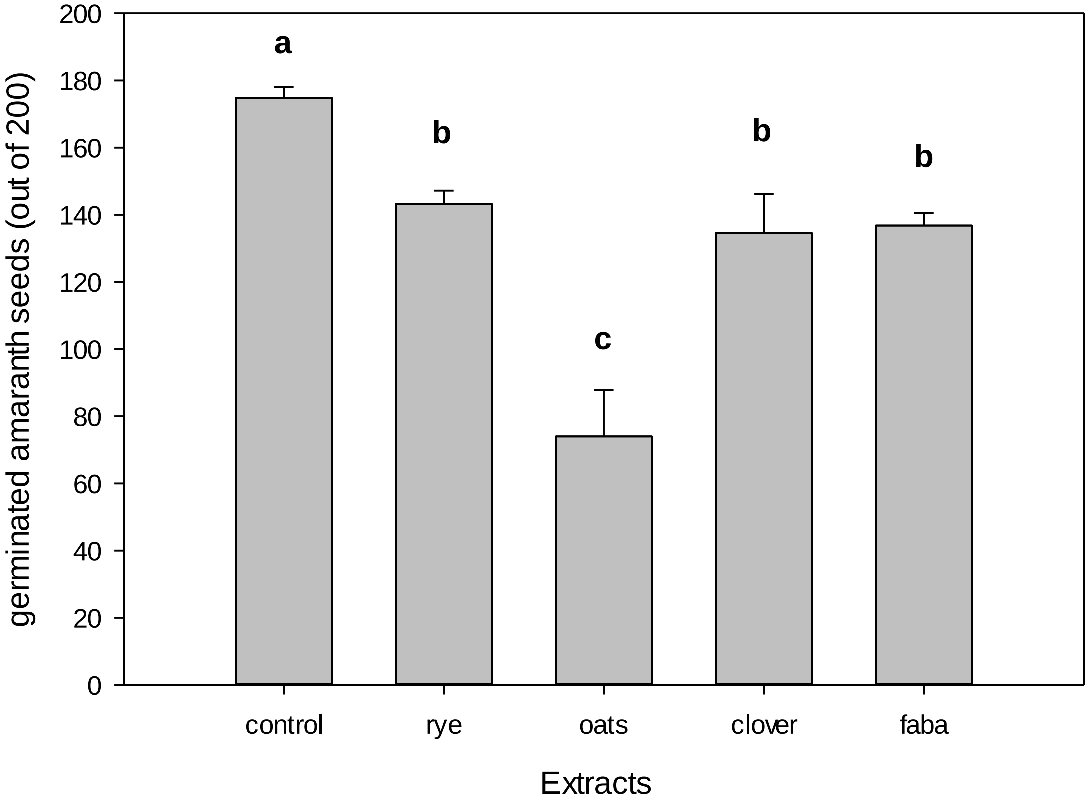

Figure 3.2. Cover crops extract without dilution and a pure water control tested on 200 seeds of amaranth in petri dishes. Bars show sample means ± SE, n= 5, ($p<0.01$). Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, $p<0.05$).

3.4.2 Paper towel experiment

Rye extracts had a lower pH than clover extracts. The pH increased in both the rye and clover extracts from the undiluted solution to the sixteenth dilution solution (Table 3.1).

Figure 3.3. Mixed cultivation of clover and rye in the field at northern crop research site in Tamahere before the harvest. Plant materials were cut for extraction after the flowering stage to use for testing as cover crop species.

Table 3.1. pH of the extract solutions of clover and rye before and after dilution.

	Clover	Rye
Undiluted solution	5.58	4.50
Half diluted solution	5.75	4.58
Quarter diluted solution	5.73	4.58
Eighth diluted solution	5.58	4.74
Sixteenth diluted solution	5.84	4.84

3.4.3 Summer grass

The number of summer grass seeds that germinated was reduced in less diluted extracts of both cover crops (Fig. 3.4). The germination rate with undiluted extracts of clover and rye was approximately 25-35% lower than the sixteenth dilution rate. The water control and the sixteenth dilution rates had the highest number of seeds germinated, and there was no difference between these two treatments. Clover extracts resulted in a lower number of seeds germinated at all the dilution rates compared to rye extracts (ANOVA, p<0.01; Fig. 3.4).

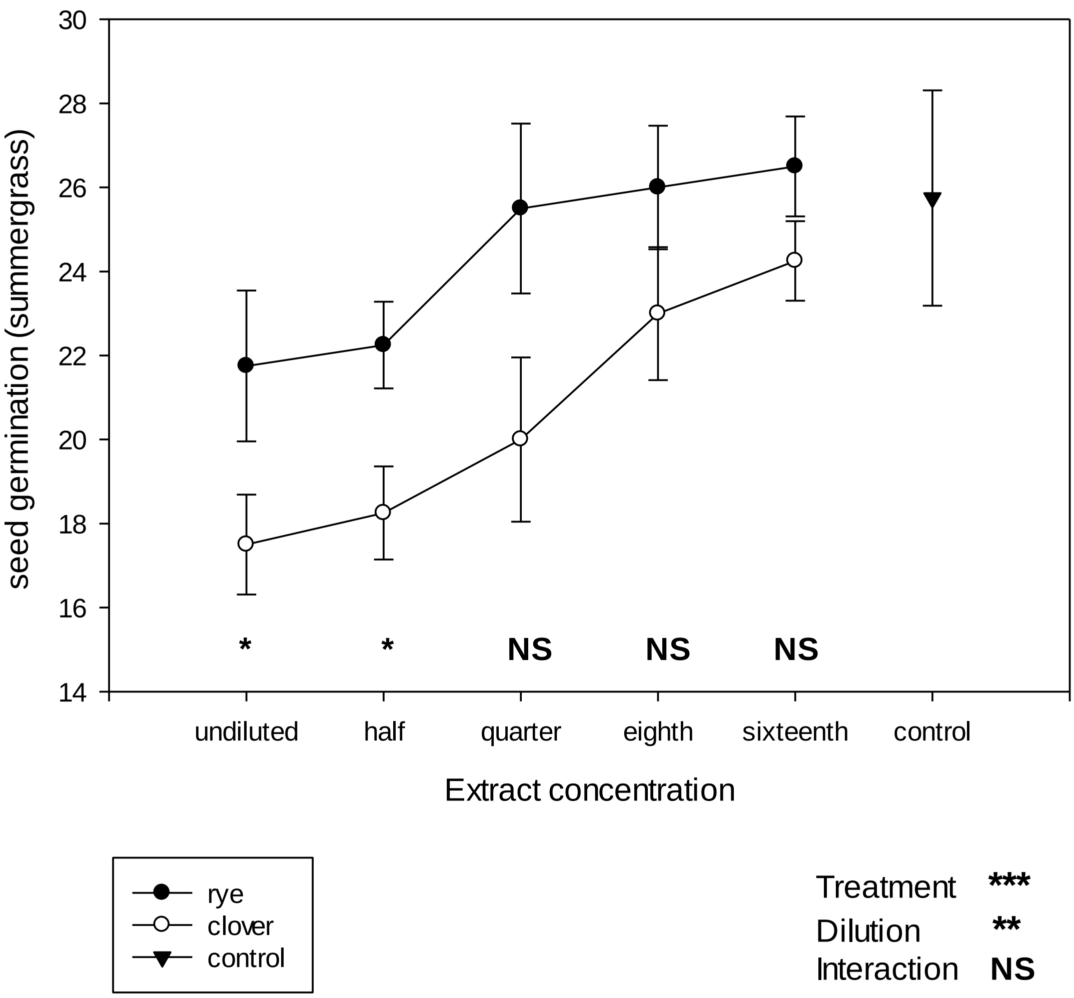

Figure 3.4. Shoots extracts of clover and rye harvested at maturity stage and applied to 50 seeds of summer grass at a range of dilution rates. The seeds were incubated on paper towels and sealed in plastic bags. Points show means ± SE, n = 4. * stands for P ≤ 0.05, ** stands for p ≤ 0.01, * stands for ≤ p 0.001 and NS stands for No significant difference.**

3.4.4 Amaranth

The number of amaranth seeds that germinated was reduced in all dilutions of extracts of both cover crops (Fig. 3.5). The germination rate with undiluted extracts of clover and rye was approximately 25-40% lower than the sixteenth dilution rate. The water control had the highest number of seeds germinated. Clover extracts resulted in a lower number of seeds germinated at quarter and eighth dilution rates compared to rye extracts (ANOVA, p<0.05; Fig. 3.5).

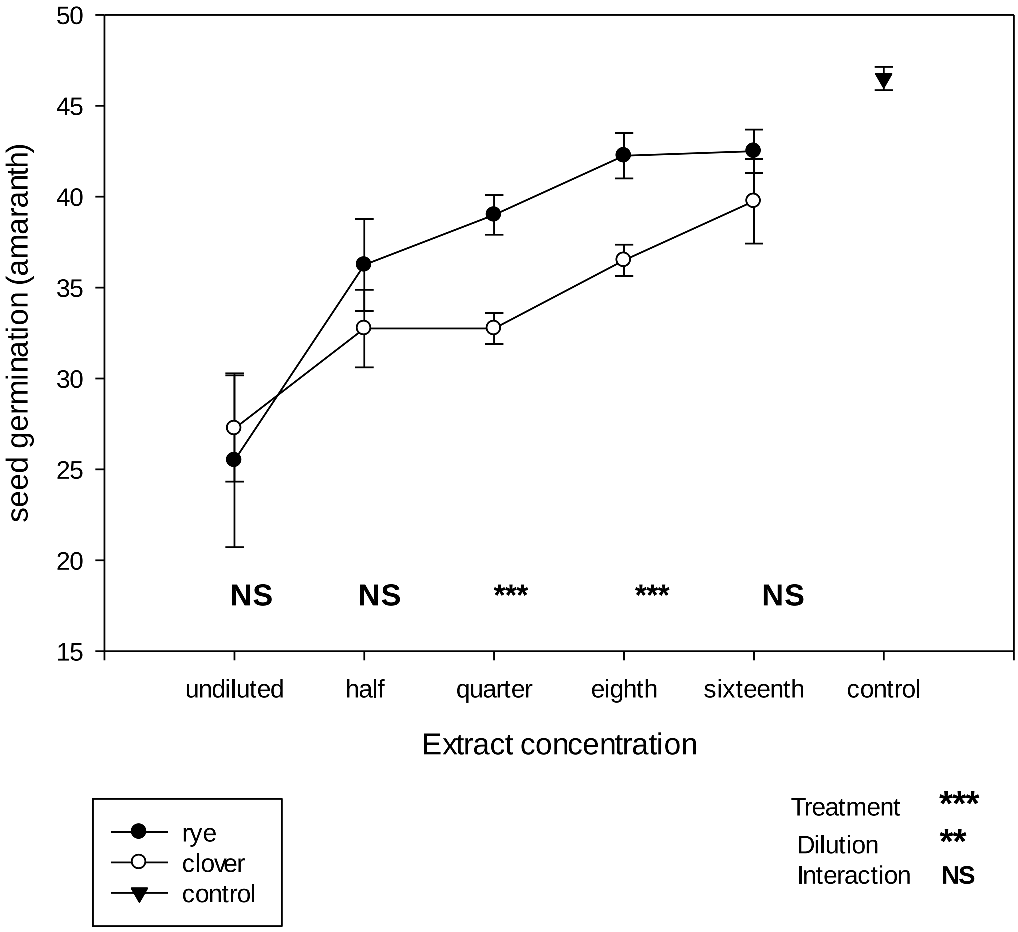

Figure 3.5. Shoots extracts of clover and rye harvested at maturity stage and applied to 50 seeds of amaranth at a range of dilution rates. The seeds were incubated on paper towels and sealed in plastic bags. Points show means ± SE, n = 4. * stands for P ≤ 0.05, ** stands for p ≤ 0.01, * stands for ≤ p 0.001 and NS stands for No significant difference.**

3.4.5 Fathen

The number of fathen seeds that germinated was reduced in less diluted extracts of both cover crops (Fig. 3.6). The germination rate with undiluted extracts of clover and rye was approximately 30-40% lower than the sixteenth dilution rate. The water control and the sixteenth dilution rates had the highest number of seeds germinated and there was no difference between these two treatments. Clover extracts resulted in a lower number of seeds germinated at half and eighth dilution rates compared to rye extracts (ANOVA, p<0.01; Fig. 3.6).

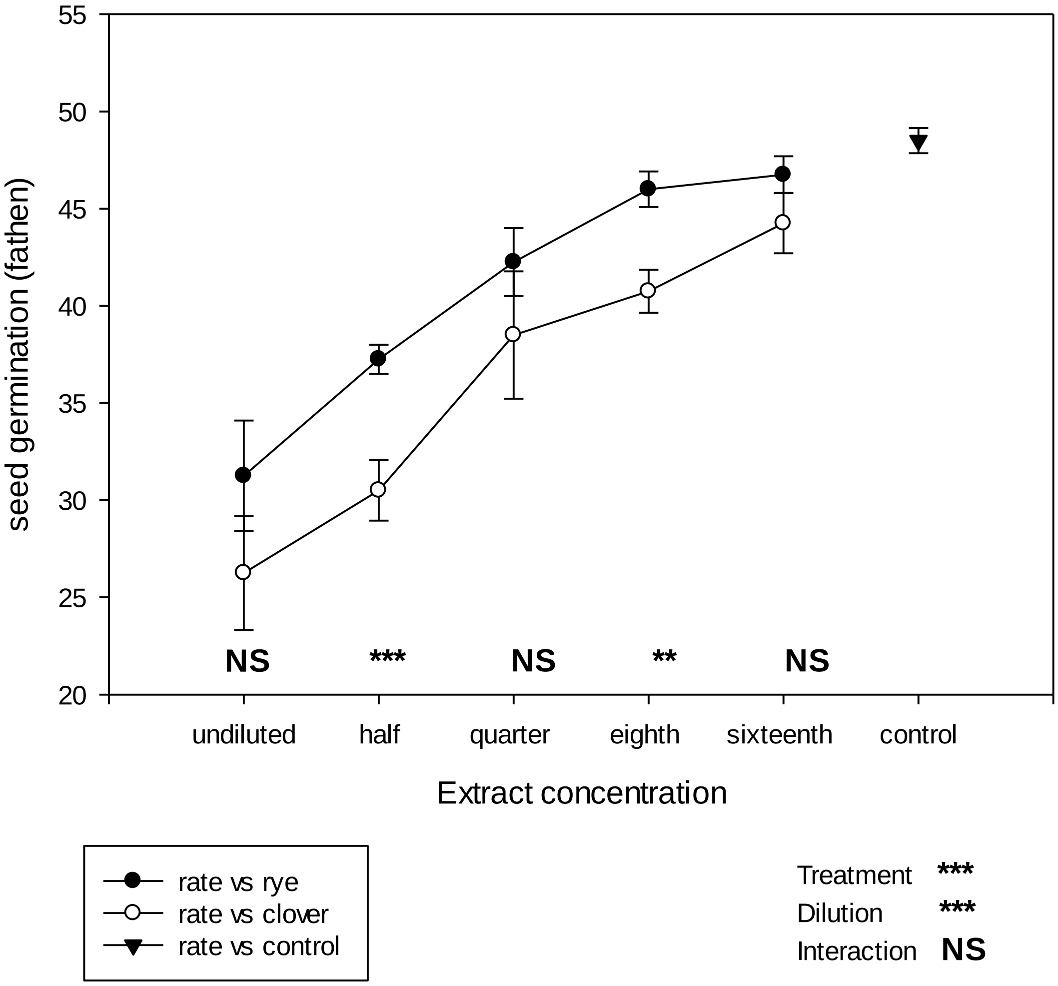

Figure 3.6. Shoots extracts of clover and rye harvested at maturity stage and applied to 50 seeds of fathen at a range of dilution rates. The seeds were incubated on paper towels and sealed in plastic bags. Points show means ± SE, n = 4. * stands for P ≤ 0.05, ** stands for p ≤ 0.01, * stands for ≤ p 0.001 and NS stands for No significant difference.**

3.4.6 Lettuce

The number of lettuce seeds that germinated was reduced by all dilutions of extracts of both cover crops (Fig. 3.7). The germination rate with undiluted extracts of clover and rye was approximately half that of the sixteenth dilution rate. Clover extracts resulted in a consistently lower number of seeds germinated compared to rye at all dilutions, with a significant pairwise difference at the eighth dilution rate (ANOVA, $p<0.05$; Fig. 3.7).

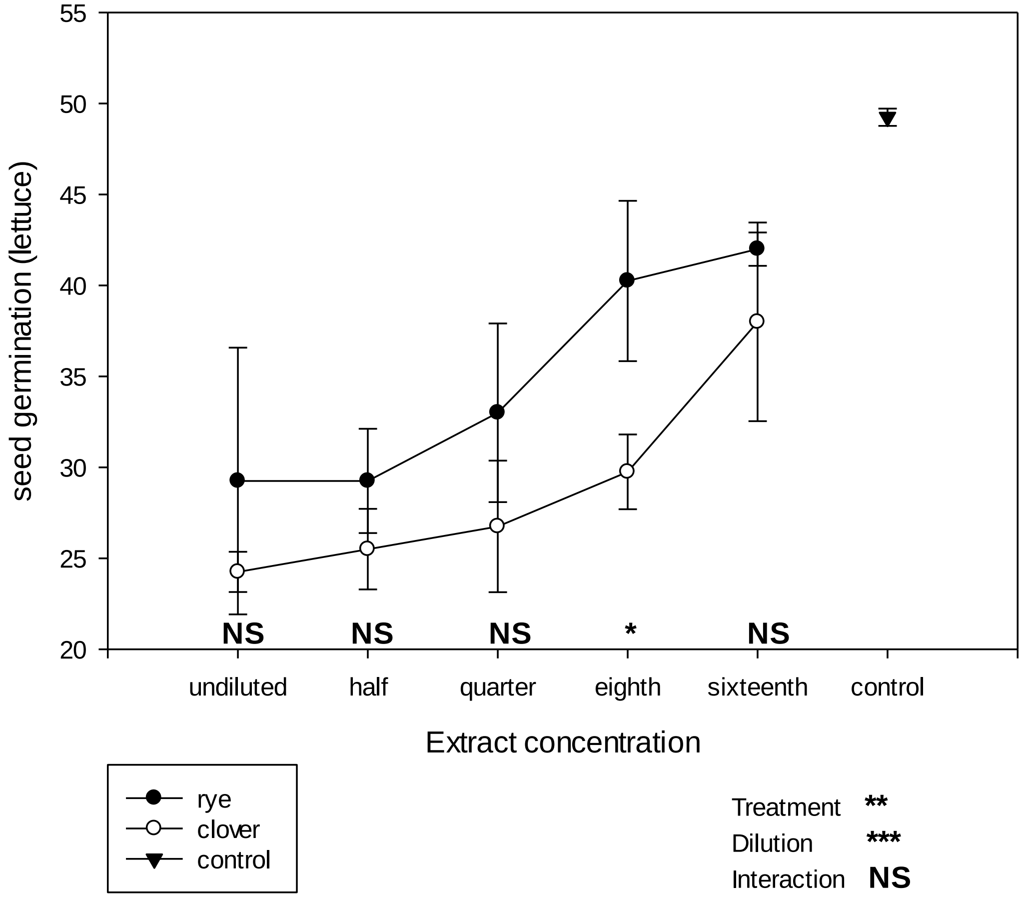

Figure 3.7. Shoots extracts of clover and rye harvested at maturity stage and applied to 50 seeds of lettuce at a range of dilution rates. The seeds were incubated on paper towels and sealed in plastic bags. Points show means ± SE, n = 4. * stands for P ≤ 0.05, ** stands for p ≤ 0.01, * stands for ≤ p 0.001 and NS stands for No significant difference.**

3.5 Discussion

The overall results show that residues from cover crops may well have inhibitory effects on seed germination, and that these effects vary with the species of cover crop. The first experiment showed that all cover crops have some effect, but the level of response depended on the test species. The second experiment used serial dilution to show that there was a dose response effect, and that there were consistent differences in level of suppression between the two main cover crop species. Clover is the most promising cover crop for exhibiting a true allelopathic effect on weed seed germination.

The results of this experiment support the hypothesis that extracts of clover inhibit seed germination of test species more than extracts of rye. Clover extract consistently reduced germination of test species more than rye extract, across the full range of dilutions. The only exception was for amaranth, where the undiluted extracts of both cover crop species resulted in similar reductions in germination. The dilution of extracts caused an increase in pH, which might have contributed to reducing the inhibition effect. The dilution rate of the extracts had an effect on the number of seeds that germinated. Lower concentration extracts resulted in more seeds germinated than highly concentrated extracts, supporting the conclusion that germination is being inhibited by a compound or compounds present in the extracts.

3.5.1 Petri dish experiment

The effect of cover crop extracts on seed germination was dependent on the species being tested (Álvarez-Iglesias *et al.*, 2014b). This was seen from the difference in seed germination of summer grass and amaranth using the same extracts, but the number of seeds that germinated was different. For example, faba extracts resulted in lower summer grass germination and higher amaranth germination compared to controls. In contrast, oat extract resulted in higher summer grass and lower amaranth germination. This is supported by the results obtained in other studies that showed that the effect of cover crop on germination depended on the test species (Jama *et al.*, 2000; Turk & Tawaha, 2003; Maharjan *et al.*, 2007; Lou *et al.*, 2016).

3.5.2 Paper towel experiment

The shoots used to extract the solution applied on test species were harvested at maturity. The plant at maturity stage is associated with reaching the full potential accumulation of compounds that contribute to allelopathy in the extracted solution (Sisodia *et al.*, 2010; Jabran & Farooq, 2013).

The dilution of extracts could have caused an increase in pH, which might contribute to reducing the inhibition effect. A study of lettuce seed germination in response to plant extracts found that dilution of the extracts resulted in the change in pH (Rice, 2012).

The inhibition trend from this experiment shows that the concentration of the extract solution affected the number of seeds germination. Undiluted extracts with a high concentration of the extracted materials prevented a higher number of seeds from germinating than the diluted extracts with low concentrations of extracted materials. Both clover and rye extracts showed a similar trend signifying that undiluted extract solutions have more potential to inhibit germination of seeds than the diluted extract solutions. This dilution effect is expected for allelopathic compounds because undiluted extracts should have higher concentrations of allelopathic compounds in the solution than diluted solutions (Gfeller *et al.*, 2018).

3.5.3 Chemical effects on seed germination

Higher inhibition of germination by clover extracts may be associated with higher concentrations of allelopathic compounds or different compound(s) compared to the other cover crop species. The allelopathic potential of clover is associated with the presence of isoflavonoids and phenolic acids (Kruidhof *et al.*, 2008). These compounds in clover were believed to have contributed to the inhibition of germination of weed seeds (Conklin *et al.*, 2002).

According to some studies, the allelopathic potential of rye is associated with the presence of compounds like benzoxazinones, cyclic hydroxamic acid and phenolic acid (Schulz *et al.*, 2013). These compounds in rye extract contributed to the inhibition of germination of weed species compared to the controls (without extract application) (Burgos *et al.*, 2004).

The compounds associated with the potential of allelopathy as shown by other studies include vanillic, ferulic, phenylacetic, benzoic and salicylic acids (Putnam, 1988; Bhadoria, 2011a; Samanta *et al.*, 2011; Itani *et al.*, 2013). Alkaloids have also been associated with allelopathy to inhibit germination of weed and crop seeds (Sisodia *et al.*, 2010). Also, glucosinolates and isothiocyanates have been associated with a detrimental effect on seed germination of weeds (Yasumoto *et al.*, 2011). Other potential allelopathic compounds detected in extracts include phenolic acids and hydroxamic acids (Weih *et al.*, 2008; Schulz *et al.*, 2013).

Since rye extracts have been found to exhibit the potential of inhibiting germination of other seeds (Dahiya & Singh, 2004), it could be that rye extracts had lower concentrations of allelochemicals than extracts of clover. When applied to the test species it might be that the lower concentration of allelopathic compounds in rye extracts suppressed the germination of seeds less than the higher compound concentration in clover extracts.

The inhibition of germination of test species seeds as seen from this experiment reduced at the dilution rate of sixteenth for both rye and clover extracts. At this rate, the inhibition of seed germination was not different compared to the control treatment except for amaranth and lettuce. It might be that concentration of allelopathic compounds at this dilution rate reduced to levels that could not inhibit germination of seeds. The five grams of dry powdered cover crop prepared per 100 mls of water was diluted from this starting concentration. This dilution rate corresponds with other studies that used five grams of powder to 500 mls of water and had inhibition effects on the germination of seeds (Hussain *et al.*, 2007). Other studies also, showed that there was a dilution level at which the inhibition potential reduced for the extracted solution (Oyerinde *et al.*, 2009).

3.6 Conclusion

This study has shown that extracts from cover crops may well have inhibitory effects on seed germination that vary with the species of cover crop. The first experiment showed that all cover crops had some effect, but the level of effect varied with test species. The second used serial dilution to show that there was a dose response effect and consistent differences in level of suppression between the two main cover crop species. Clover was the most promising cover crop for exhibiting a true allelopathic effect on weed seed germination.

According to the results obtained from this experiment, clover had the largest potential for reducing the test species germination. This suggests that it might be good for suppressing weeds in the field. Further experiments should be conducted in which extracts of clover are applied in the field or glasshouse to suppress germination of weeds. Alternatively, clover might be grown in the field and the crop residues could be reserved for suppressing germination of weeds. It is recommended that an experiment be conducted with soil in pots to further test the effect of rye and clover cover crop residues on the germination and growth of weed species under more natural conditions (Chapter 4).

Laboratory experiments should also, be conducted to identify the compounds responsible for the inhibitory effect of clover on seed germination. This could be done by fractionating the crude extract through separation methods such as liquid chromatography, and testing each fraction individually for inhibitory effects. Further fractionation and chemical characterisation of the most active fraction should lead to the identification of the compound(s) responsible. Alternative sources of inhibitory compounds could be identified and used to suppress weeds, or cover crops could be deliberately bred and selected to produce higher concentrations that provide better weed suppression.

Chapter 4

Effects of clover and rye roots and shoots on the germination of selected weed and crop species.

4.1 Introduction

Agricultural systems have benefited from the introduction of cover crops. Some of the benefits include soil improvement and weed management (Belz & Hurle, 2004; Tabaglio *et al.*, 2013). The use of cover crops in weed management requires a balance to achieve profitable crop production and environmental protection (Lu *et al.*, 2000). The inhibition of weeds by the cover crop may be achieved by the living plants, as plant residue, or both (Wakjira *et al.*, 2005). How the effect is achieved will influence the way in which cover crops can be used in weed management. As crop residue, cover crops are left in the field and through decomposition, inhibitory compounds may be released that prevent germination of weed species. When used as living plants, they are planted at the time that weed suppression is required, and suppress weed germination and growth while still living (Adler & Chase, 2007; Farooq *et al.*, 2013; Worthington & Reberg-Horton, 2013; Lou *et al.*, 2016).

Apart from competitive and nutrient cycling effects, the inhibitory effect of cover crops is associated with compounds that are toxic to other plants (Worthington & Reberg-Horton, 2013). Compounds that are believed to be allelopathic have been isolated and identified. Under natural conditions, allelopathy can suppress and exclude species that are susceptible to harmful biological substances. These biological substances reduce the rate of germination and plant growth (Yasin *et al.*, 2012). Understanding the role of cover crops in the release of allelopathic compounds is essential for the control of weeds in agricultural systems. In nature, the subject of allelopathy is difficult to prove because of interactions with other factors that contribute to inhibition of weed germination and growth (Conklin *et al.*, 2002). The compounds associated with allelopathy may be produced and released from specific parts of the plant (Fageria *et al.*, 2005), and their inhibitory effect on weed germination and growth can therefore vary from one plant part to another. For example, the roots and shoots of a cover crop might differ in the amount of allelopathic compounds that are produced.

In New Zealand cover crops have been used to control some weed species, with the most common choices of cover crop being faba beans, oats, clover or ryegrass. The most important cover crops used are clover and rye, with cover crop residues usually left in the field before planting the main crop to suppress the germination and growth of weeds. Cover crops such as clover and rye are believed to contain allelopathic potential and are therefore used in rotations with a main crop, such as maize, to control weeds (Yasumoto *et al.*, 2011; Trolove *et al.*, 2017).

Cover crop residues that have potential allelopathic effects may be a remedy to control the germination and growth of weed species (Dabney *et al.*, 2001; Burgess *et al.*, 2002; Yasumoto *et al.*, 2011; Aslam *et al.*, 2017; Trolove *et al.*, 2017). There is therefore a need to identify which cover crops have the highest inhibitory effect towards weeds. Also, it is essential to assess whether it is the root or shoot residues that contribute most to weed inhibition. If the assessment shows that the root residues have more inhibition effect, the economic advantage could be passed to farmers to use the shoot residues for other activities. It is also important to determine how long the allelopathic compounds might remain in the soil and affect the growth of other species. Based on previous field experiments, clover appeared to have the best ability to suppress weeds, although it was not known which part of the plant might be having more of this effect (Trolove *et al.*, 2017).

This experiment was set up to test the allelopathic potential of aboveground and belowground parts of rye and clover on selected species planted in pots. Clover and rye were grown in soil in pots before the shoots were harvested and placed on the surface of separate pots filled with soil. The pots with root and shoot residues were then compared for their potential to reduce germination of volunteer weed seeds, and their effect on germination and growth of the planted seeds of the test species fathen, amaranth, maize, summer grass and lettuce. The hypothesis under test in this experiment was that clover roots or shoots would have the strongest allelopathic effects.

The term 'test species' in this experiment refers to the species of seeds that were used to test the effect of cover crop on seed germination.

4.2 Methods

On 16 May 2018, soil was collected from the AgResearch Ruakura farm in a two-tonne trailer and stored in large bags. Basal dressing fertilizer (at the rate of 0.2 g per pot) was mixed with the soil. The fertilizer nutrient content was Nitrogen 15%, Phosphorous 8.4%, Potassium 10% and Sulphur 9.2%. Black pots were filled with soil to a volume of 0.0015 m^3.

On 28 September 2018, the experiment was set up in a glass house at the AgResearch Ruakura facility. Five pots were filled with soil as a control without any planting of seeds. Another five pots were filled with soil and 20 seeds of rye (*Lolium multiflorum* cv. 'Tama') were planted in each pot. In another set of five pots were planted 20 seeds of clover (*Trifolium glanduliferum* cv. 'Prima'). The experiment was replicated six times to a total of 90 pots arranged in a complete randomised block design. Weeds were removed from all pots immediately after they appeared. After six and seven weeks of growth, a liquid fertilizer (Nitrates of Calcium, magnesium, ammonium and potassium, potassium sulphate and sodium chloride at the rate of one gram per 4.5 litres of water) was applied to the ryegrass and the clover, respectively, to improve their nutrition. Each pot received 200 ml of the liquid fertilizer twice a week. On 23 November 2018 at the flowering stages of both clover and rye the plants were harvested by cutting about 2 mm above the soil level.

On 23 December 2018, five test species were planted (amaranth (*Amaranthus tricolor* cv. 'Mekong red'), maize (*Zea mays*), fathen (*Chenopodium album*), summer grass (*Digitaria sanguinalis*) and lettuce (*Lactuca sativa* cv. 'webbs wonderfull iceberg')). Each test species was planted in a set of six pots containing control (control shoot and root), clover (clover shoot and root) and rye (rye shoot and root). The control root treatment was the pots that were set together with clover and rye planted pots at the beginning of the experiment, but without seeds sown in the pots. Rye root were the pots that had rye planted at the beginning of the experiment and the plants were cut at two cm from the base. Clover root were the pots that had clover planted at the beginning of the experiment and the plants were cut before planting the test species. The control shoot treatment was pots newly filled with soil later in the experiment (one week before planting of test species), but without cover crop shoots laid on the soil surface. Rye shoot were the pots that had rye shoots (harvested from the rye planted in the pots earlier and chopped without drying) laid on top of pots newly filled with soil. Clover shoot were the pots that had clover shoots (harvested from the clover planted in the pots earlier and chopped without drying) laid on top of pots newly filled with soil, unlike the control root and root pots, which were filled when the rye and clover were sown. The soil surface of each pot designated for shoots was covered with 10 grams of rye or 13 grams of clover after the planting of test species. The total of 180 pots were arranged in a complete randomized block design with six replicates. Three replicates were placed on the western side of the glasshouse, and three on the eastern side. Ten maize seeds were planted in each pot for all the set of pots designed for maize test species. For the other test species, twenty seeds were planted in each pot.

Three weeks after the seeds were planted all the volunteer weeds were counted. The volunteer weeds were removed immediately after completing the counting process. Also, the number of test species that had germinated in each pot were counted.

Four plants in each pot were randomly measured for plant heights on 24 January 2019. Plant heights were taken using a 1 m metallic rule to measure the highest point of each plant. The following day all the plants in each pot were harvested by cutting 2 mm from the base. The harvest was done by species from each replicate, and the material weighed fresh using a balance (SB3200, Mettler Toledo, Greifensee, Switzerland). The plant materials were placed in paper bags and dried (CONVAIRE CAREL air 33, Universal, Schwabach, Germany) at 80°C. After 72 hours of drying plant materials were kept in the storeroom in preparation for dry weight measurements. The weights of dried plant materials were measured using the three decimal place scale (PM6000, Mettler Toledo, Greifensee, Switzerland).

4.3 Data analysis

Data were checked for normality and homogeneity of variance and transformed where necessary. The analyses were performed for each test species to assess the inhibition effect of cover crop on seed germination and growth. A two-way analysis of variance (ANOVA) was performed for the species and root and shoot treatments.

Analyses were completed in Genstat 9th edition (2018 VSN International Ltd). Duncan's Multiple Range Tests were used for post-hoc pairwise comparisons between treatments completed in Statistica version 13.3 (Tico 1984-2017 software Inc).

4.4 Results

Volunteer weeds

The highest volunteer weed counts were in the clover shoot, rye shoot and control shoot treatments. Clover root, rye root and control root had lower numbers of volunteer weeds across all the test species (Fig. 4.1). The clover root treatment usually had the lowest number of volunteer weeds compared to the other treatments. However, this was only significantly lower than rye root with maize as the test species (ANOVA, p=0.024; Fig. 4.1 B), and it was not significantly different from the control (no roots) with any test species.

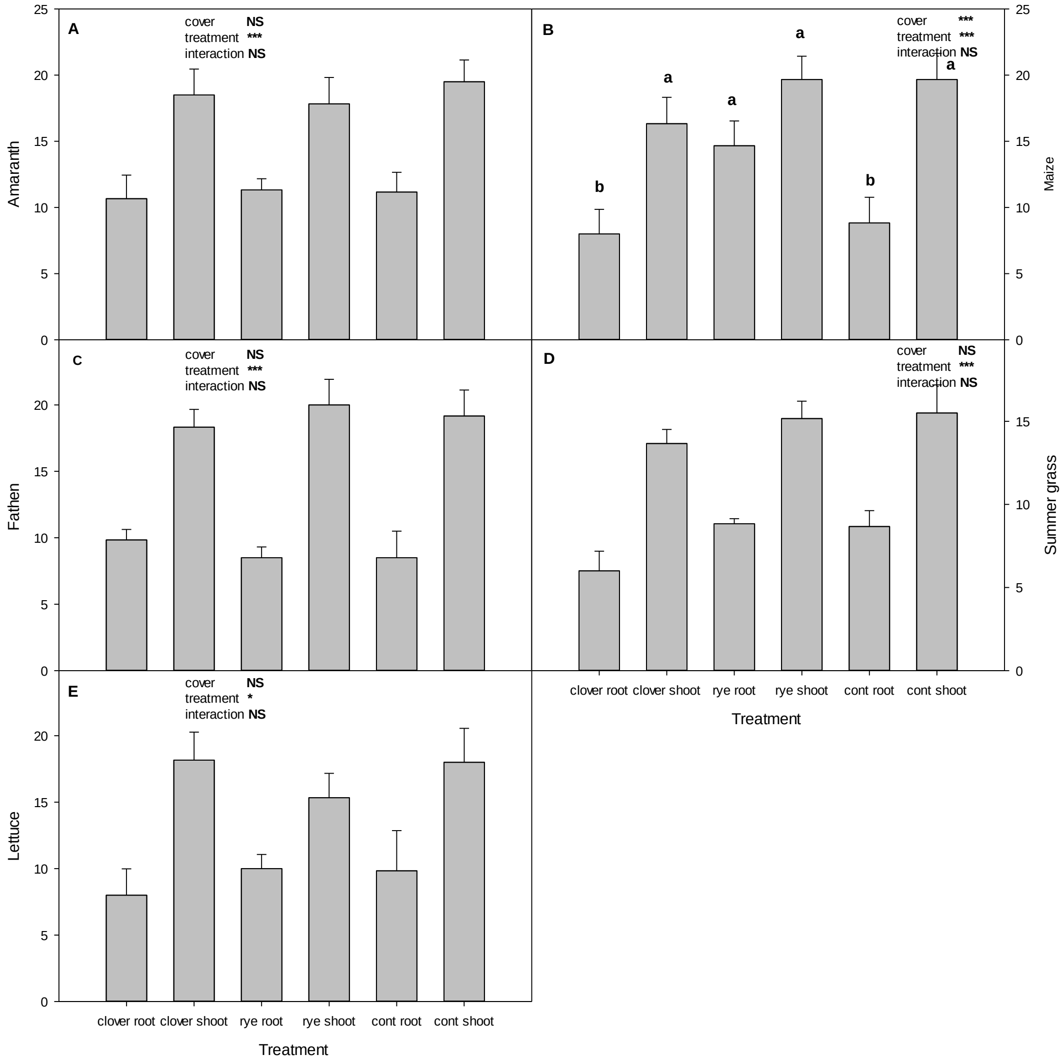

Figure 4.1. The number of volunteer weeds that had germinated in the pots at three weeks after planting of the test species. (A) amaranth (p<0.001). (B) maize (p<0.001). (C) fathen (p<0.001). (D) summer grass (p<0.001). (E) lettuce (p<0.001). Bars show sample means ± SE, n= 6. Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

4.4.1 Test species germination

Of the five test species, cover crop treatments (clover and rye, roots or shoots) only affected the germination rate of summer grass and lettuce (Fig. 4.2). With summer grass as the test species, the root treatments caused lower germination than shoots (Fig. 4.2 D), and clover roots caused the lowest germination of all. Across all test species, there was a tendency for clover roots or shoots to cause the lowest rate of germination (Fig. 4.2). With lettuce, the root treatments also tended to cause lower germination than shoots, except that clover roots had a significantly higher germination count than clover shoots (ANOVA, p<0.001; Fig. 4.2E).

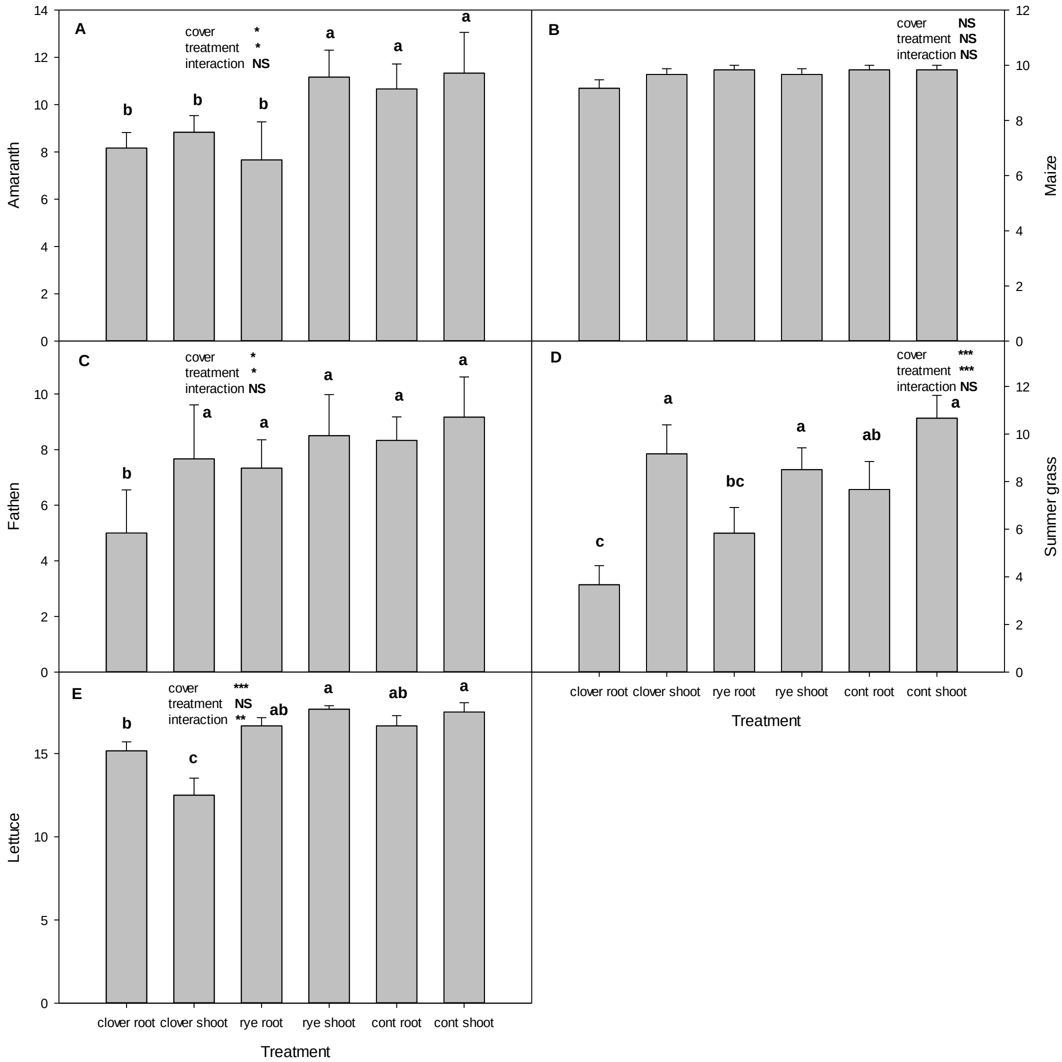

Figure 4.2. The test species population counted at three weeks after germination. (A) amaranth (p=0.15). (B) maize (p=0.22). (C) fathen (p=0.41). (D) summer grass (p<0.001). (E) lettuce (p<0.001). Bars show sample means ± SE, n= 6. Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

4.4.2 Plant height

Cover crop treatments only affected the height growth of maize (Fig. 4.3). Clover, rye, and control shoots tended to reduce the height growth of all test species, but the main effects of cover crop and treatment (roots or shoots) were only significant for maize, which had shorter shoots when grown with the clover and rye shoot treatments (ANOVA, p≤0.01; Fig. 4.3B).

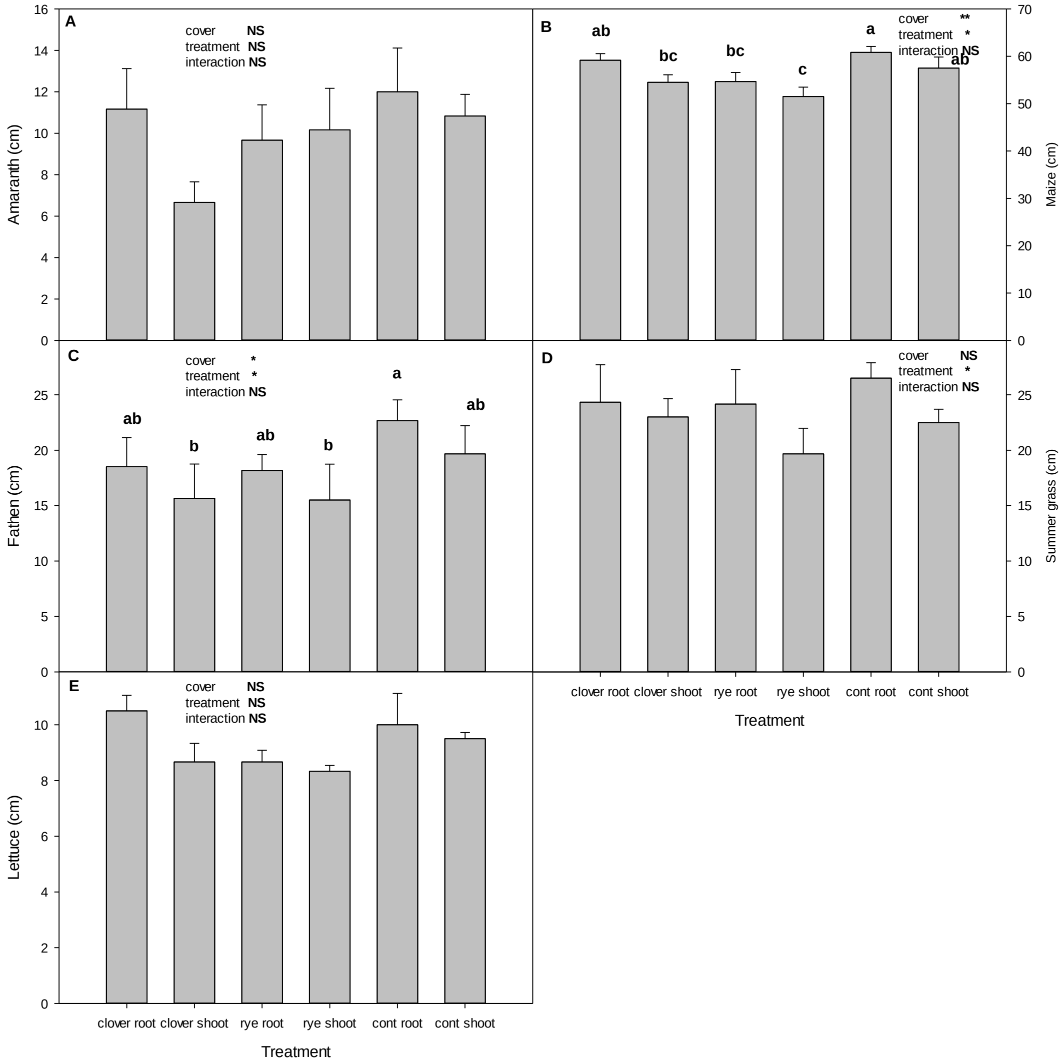

Figure 4.3. Plant heights of the test species taken at six weeks after germination. (A) amaranth (p=0.33). (B) maize (p=0.01). (C) fathen (p=0.38). (D) summer grass (p=0.46). (E) lettuce (p=0.12). Bars show sample means ± SE, n= 6. Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

4.4.3 Plant biomass

Shoot treatments tended to decrease test species dry biomass compared to root treatments (Fig. 4.4). This effect was significant for maize, fathen, lettuce and summer grass (ANOVA, p<0.01, p<0.001, p<0.001). The effect of cover crop was also significant for maize, fathen and lettuce, but there was no consistent effect of crop type across the test species, with rye, control and clover roots producing the largest maize, fathen and lettuce plants, respectively (Fig. 4.4).

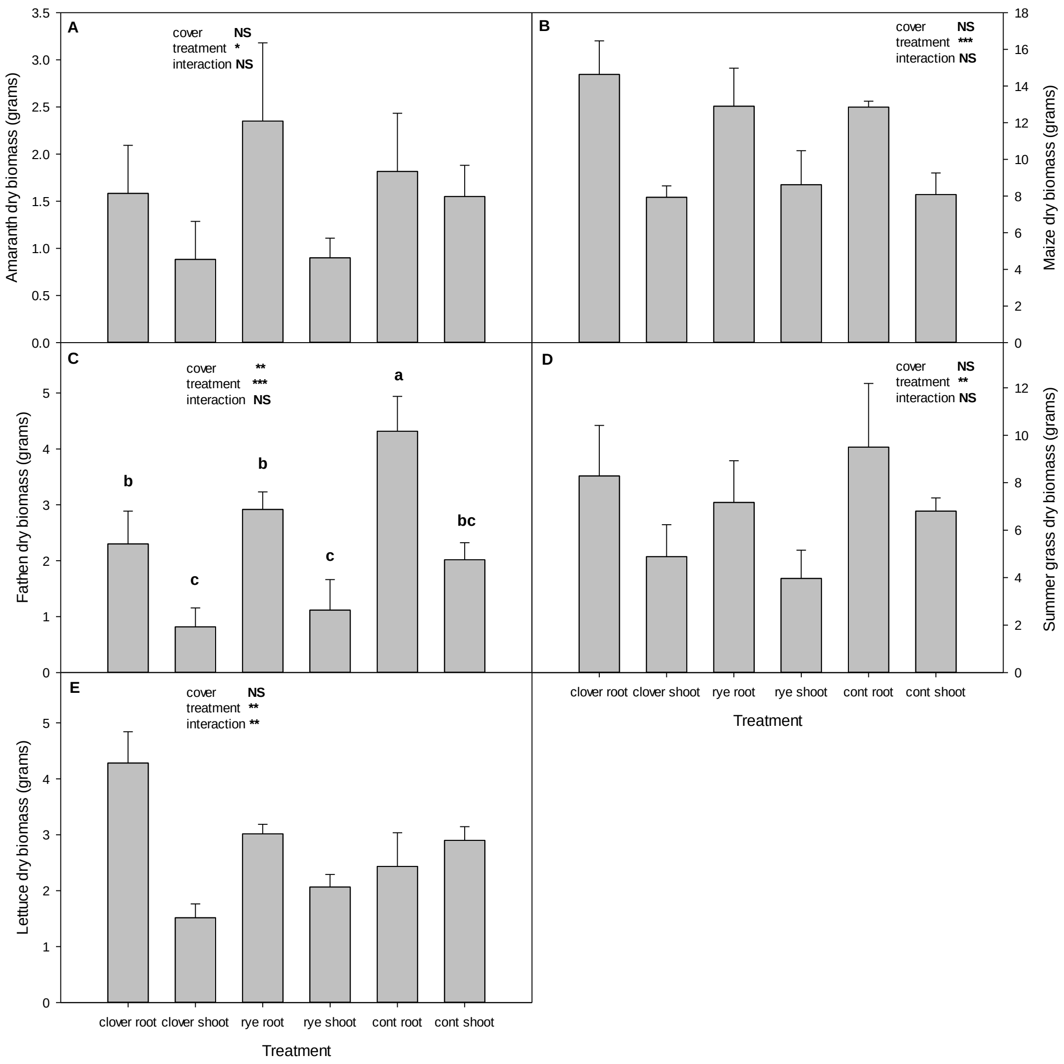

Figure 4.4. Test species dry biomass measured at the end of the experiment. (A) amaranth (p=0.36). (B) maize (p<0.01). (C) fathen (p<0.001). (D) summer grass (p=0.25). (E) lettuce (p<0.001). Bars show sample means ± SE, n= 6. Bars with the same letter are not significantly different (Duncan's Multiple Range Tests, p<0.05).

4.5 Discussion

This study is in agreement with the hypothesis that clover had a higher potential to suppress germination of other species than rye. It also showed that root treatments inhibited germination of volunteer and test species more than the shoot treatment. The root treatment suppressed more volunteer weed germination than the shoot treatment, possibly because of a higher availability of inhibitory compounds, or because of the differences in the time of setting up the root and shoot pots for the two cover crops. The cover crop treatments showed that clover inhibited germination more than rye, suggesting that phenolic acids, compounds that are known to be produced by clover (Bhowmik, 2003), may have contributed to the inhibition effect.

In addition, the results indicate that the inhibitory effect on germination exerted by cover crop varies with the test species. This was shown by inhibition affecting one species but not others, which might suggest that some species are more sensitive to a particular cover crop treatment. Cover crop roots were more likely to affect germination, but cover crop shoots were more likely to reduce the growth of planted test species. The lack of a root effect on growth might be explained by loss or dilution of any allelopathic compounds that affected germination, or because their mode of action was specific to germination. Lower growth by some test species with shoot treatments may have been the result of interactions between volunteer weed germination and test species growth, and illustrates the difficulty of separating true allelopathic effects from competitive interactions in these types of experiments (Duke, 2015).

4.5.1 Volunteer weeds

The number of weeds that germinated in the root treatments was lower than in the shoot treatments for all the test species, suggesting that the decaying roots of the cover crops contributed to the reduction in the number of germinating volunteer weeds. Compounds that are associated with allelopathy that are released in the soil include glucosinolates and isothiocyanates (Gardiner *et al.*, 1999). It might be that more of these allelochemicals were present and released from the roots (Chon & Kim, 2002), contributing to a lower rate of volunteer weed germination.

The lower number of volunteer weed seeds that germinated in the root treatments could also be a result of the difference in timing of treatment set up. The pots containing root treatments were the same pots used previously to grow the clover and rye cover crops. When these crops were growing, the weeds that germinated in the pots initially were being uprooted as they appeared, thereby reducing the seed bank available to germinate later. For the shoot treatments, a new set of pots was filled with the same soil as the root pots, on the day that the cover crop shoots were harvested from the root pots. Fresh disturbance of the weed seed bank at the beginning of the shoot treatments may have contributed to the higher germination rate of volunteers from the start of the cover crop treatments. This increased germination also occurred in the control shoot pots, compared to the control root pots. A better design may have been to prepare the shoot pots at the same time as the root pots, and to keep them in the glasshouse while the cover crops were being grown. With either approach, it is difficult to separate the effects of cover crop growth from the later effects of cover crop residues on volunteers.

The compounds associated with allelopathy in clover include isoflavonoids and phenolic acids (Kruidhof *et al.*, 2009). In rye, the compounds associated with allelopathy include cyclic hydroxamic acid and phenolic acids (Schulz *et al.*, 2013). The clover root treatment had a lower number of volunteer weeds that germinated, possibly because the allelopathic compounds were in higher concentrations or were released more quickly than from the other root treatments.

4.5.2 Test species germination

Overall, compared to volunteer germination, the cover crops had less consistent effects on the germination of deliberately planted seeds. Clover shoots reduced lettuce seed germination compared to the other treatments. It was not clear why this effect was only observed for lettuce and not the other test species. Clover and rye root treatments reduced summer grass germination compared to shoots and the controls. These results indicate that the germination response to cover crop treatments was species dependent. It may be that the allelochemicals present in the treatments had the potential to reduce germination of seeds for one species but failed to reduce the germination of the other species. One study suggested that weed species germination suppression depended on the type of cover crop and the weed species under control (Kruidhof *et al.*, 2009).

The lack of significant effects on test species germination could also be the result of the relatively low number of seeds that were planted in the pots. For example, only ten seeds were planted per pot for maize (60 seeds in total over six reps). For differences in germination rate to be detected, there was a need for a strong allelopathic effect and a large change in germination rate. The present experiment was already quite large, requiring 180 pots. To better detect possible allelopathic effects, further experiments could increase replication by using only the most promising cover crop (clover), testing only smaller-seeded test species, and planting more seeds in each pot.

4.5.3 Plant height

Cover crops had no effect on test species plant height, except for maize. This may be an indication that the cover crops have less effect on the inhibition of growth of other species. It may also indicate that the inhibition potential was not strong enough to impact growth because the allelochemical effect was becoming diluted or depleted. This is not supported by some studies that showed that the inhibition potential of cover crops can last the whole growing season (Rice, 2012). These findings may not apply directly to this study because the treatments were administered in pots where the allelopathic effect could be depleted through dilution by continuous watering and drainage of the pots.

The shoot and root treatments had a difference in plant height only with maize as the test species. The higher growth potential of maize could have contributed to faster detection of differences in height, which did not occur with the other test species. Within three weeks of germination, the maize plants had reached a height where differences in growth could be detected. Maize height tended to be reduced in shoot treatments compared to root treatments, an effect that might be explained by competition by volunteer weeds, as explained below.

4.5.4 Biomass

As for plant height, cover crop treatments had no consistent effects on test species biomass, suggesting that inhibition had less effect on the growth of test species than germination, or that its effect was species dependent. However, root and shoot treatments did cause clearer differences in dry biomass. The difference is not easy to explain because the root treatment had higher biomass than the top treatment. If inhibition was occurring (as suggested by germination), it was expected that the shoot treatment would have higher biomass than the root treatment. The results support the idea that the mechanism that inhibited germination did not affect the later growth of the test species. This conclusion is not supported by the study that showed that roots and shoots had a similar inhibition effect on the growth of lettuce (Bertin *et al.*, 2003). An alternative explanation for higher biomass growth in the root treatments is because of the lower germination rate of volunteer weeds, caused either by cover crop or experiment design. Higher numbers of volunteers in the shoot treatments may have reduced test species growth through competition for nutrients and light, even though the volunteers were counted and removed as they appeared.

4.6 Conclusion

This study has shown that a clover cover crop may have the potential to suppress germination of weeds better than a rye cover crop. It has also shown that clover roots may inhibit germination of other species more than shoot residues. The inhibition effect was exerted more on the germination of the test species than on the growth of the test species. The root treatment suppressed more volunteer weeds than the shoot treatment possibly due to the availability of inhibition compounds, or because of a difference in timing of setting up the pots for the experiment. The cover crop treatments showed clover inhibited germination more than rye, suggesting that allelopathic compounds might have contributed to the inhibition effect.

The number of germinated seeds was species dependent since the inhibition effect was felt on one species but not on another, suggesting that some species were more sensitive to potential allelopathic effects. The germination inhibition effect did not extend to the growth of the test species, suggesting that the allelopathic compounds were degraded or diluted. The interactions between experimental design, germination rate and later growth of the test species illustrate the difficulty of detecting true allelopathic effects, and separating them from competitive effects when plants are growing in close proximity (Duke, 2015; de Jesus Jatoba *et al.*, 2016; Uddin & Robinson, 2017).

The results obtained from this study are important for the farmers that are keeping the crop residues in the fields to reduce the germination and growth of weeds. There is an economic benefit for the farmers to use the shoots of crop residues for other activities while they may still benefit from the weed controlling effects of roots residues. It suggests the potential to cut and carry the cover crop shoots for use in silage or for direct feeding to stock that are held on feed pads.

It is recommended that another experiment to be conducted in the field for the growth inhibition to be explored where the allelochemicals are not restricted. The weed suppression effect should be compared between the root and shoot treatments, with the soil prepared at the same time for both treatments, to avoid the effect of pre-germination of volunteer weeds that occurred in the pot experiment reported above. The experiment should include an extended growth period to cater for measurements of the yield of the test species and to check the rate of release of allelochemicals from the cover crop residues. Like in the cover crop extract study (Chapter 3), the root and shoot crop residues could be tested by fractionating a crude extract to identify the compounds responsible for inhibition.

Chapter 5

General discussion

Cover crops are very important for maintaining soil fertility, through decomposition of residues that retain nutrients in the soil. Another benefit of using cover crops is the potential to inhibit germination and growth of weed species. The aim of this study was to investigate the mechanism for weed suppression and impacts on maize growth caused by cover crops that were observed in previous field trials. The first objective was to investigate the causes of the lower yield of maize crop following a ryegrass crop compared to fallow or clover. The second objective was to test rye and clover for allelopathic effects on seed germination by other species under laboratory conditions. The third objective was to test rye and clover crop residues for allelopathic effects on seed germination in soil.

This study has shown that clover root and shoot treatments have the potential to inhibit germination and growth of weed and crop species. The results support the hypothesis that clover has a higher potential to suppress germination of other species compared to rye. The study has also shown that clover either as a crop residue or as an extract is the most promising cover crop for exhibiting a true allelopathic effect on germination of weed seeds. It was also shown that a clover-based winter cover crop may be better than a rye-based cover crop in terms of its effects on nutrient availability to the main crop.

In Chapter Two it was found that winter cover crop treatments impact the growth and yield of the main crop. Examination of the soil CN ratio showed that clover treatments resulted in lower total and extractable soil CN ratios than the rye treatments. This suggested that there was a higher nitrogen availability relative to carbon in clover treatments at the beginning of the experiment. It also suggested that nitrogen was more mobile and nitrogen release was higher during early maize growth following a clover crop. The early nutrient availability contributed to faster early growth of the maize and resulted in higher yields with the clover treatment. In the rye treatment, lower initial availability of nutrients resulted in lower total growth and yields but higher crop nutrient concentrations at the end of the growing season. Compared to winter fallow (no cover crop), all cover crops, including clover and rye, decreased early nutrient availability to the main crop. During normal commercial use, a fertilizer application at the time of planting could mitigate this effect of cover crops.

In Chapter Three it was shown that the effect of cover crop extracts on seed germination was dependent on the species being tested. For example, there was a difference in seed germination of summer grass and amaranth using the same extracts. Faba extracts resulted in a lower number of summer grass seeds germinating, and higher amaranth seed germination. In contrast, oat extract resulted in higher summer grass and lower amaranth germination. This result agrees with other studies that showed that the effect of cover crop on germination depended on the test species (Sodaeizadeh *et al.*, 2009; Al-Sherif *et al.*, 2013).

In addition, the inhibition trends observed in Chapter Three showed that the concentration of the extract solution affected the number of seeds that germinated. Undiluted extracts with a higher concentration of the extracted materials prevented a higher number of seeds from germinating than the diluted extracts with lower concentrations of the extracted materials. This effect of dilution on germination is expected for a genuine allelopathic mechanism because undiluted extracts should have higher concentrations of allelopathic compounds in the solution than diluted solutions (Abdelhalim *et al.*, 2019).

It was also found in the dilution experiment that the inhibition of germination of test species seeds was reduced to close to that of the water control at the dilution rate of one-sixteenth for both rye and clover extracts. It might be that the concentration of allelopathic compounds at this dilution rate was reduced to levels that could not inhibit germination. This dilution rate corresponds with other studies that extracted five grams of plant material in 500 mls of water and observed inhibition effects on germination reduce at higher dilution rates (Bhadoria, 2011b; Farooq *et al.*, 2013; Álvarez-Iglesias *et al.*, 2014b).

Chapter Four showed that allelopathic effects on germination could also be detected in soil, and these effects varied with the type of cover crop, the plant parts tested, and the test species used. The number of volunteer weeds that germinated in the root-residue treatments was lower than in the shoot-residue treatments for all test species, and the germination of the seeds of some test species was also lower with root-residues, indicating that the decaying roots of the cover crops contributed to the reduction in the number of germinating volunteer weeds. According to other studies (Shah *et al.*, 2016a; Sturm *et al.*, 2018) the compounds associated with allelopathy, that include glucosinolates, phenolic acids and isothiocyanates, might have been present and released from the roots therefore contributing to lower rates of germination. However, this result was confounded by a lower rate of volunteer seed germination in control root compared to control shoot treatments, a potential artefact of later preparation of shoot pots compared to root pots.

In addition to the effect of plant part (roots or shoots), Chapter Four showed that the species of cover crop also affected seed germination. The clover root treatment had a lower number of volunteer weeds that germinated than the rye treatment, possibly because the allelopathic compounds were in higher concentrations or were released more quickly than from the other root treatments. This result supports previous observations from field trials that gland clover showed promise as a cover crop that suppressed weed germination during early growth of the maize crop (Trolove *et al.*, 2017). However, overall, compared to volunteer weed germination, the cover crop treatment inhibition effect on the germination of deliberately planted seeds was inconsistent. For example, clover shoots reduced lettuce seed germination compared to the other treatments. Also, clover and rye root treatments reduced summer grass germination compared to shoots and the controls. These results suggest that the germination response to cover crop treatments was species dependent. It may be that the allelopathic compounds present in the treatments had the potential to reduce germination of seeds for one species but failed to reduce the germination of the other species. This finding is in agreement with a study that suggested that weeds species germination suppression depended on the type of cover crop and the weed species under control (Rice, 2012).

The growth inhibition effect of cover crop treatments on test species was also not consistent. This may suggest that the cover crops have less effect on the growth of other species. It may also indicate that the inhibition mechanism was not strong enough to impact growth of the test species. The explanation for this could be that the allelopathic compounds available in cover crops were becoming diluted or depleted as the experiment progressed. However, this is not supported by a study (Khalid *et al.*, 2002; Grove *et al.*, 2012) that found that the inhibition potential of cover crops can last the whole growing season. Paradoxically, the clearest effect on biomass growth in the present experiment was higher growth with the root treatment, the opposite of the effect of roots on germination. This result may also be an artefact of the later preparation of shoot pots, and higher numbers of volunteer weeds in these pots that could have competed with test species growth. The experiment reported in Chapter 4 was the second attempt at a glasshouse test of allelopathic effects, after the first attempt failed because of inconsistent growth of the cover crops and test species. These results illustrate the well-known difficulty of separating genuine allelopathic effects from competitive interactions between species (Duke, 2015).

5.1 Final Conclusions and Recommendations

This thesis achieved the overall aim of learning more about how the cover crops included in the FAR trials are having their effects on weeds and growth of the main crop. Cover crops have significant effects on nutrient cycling that influence main crop growth, and they can have complex allelopathic effects on germination of weeds. Both of these findings have significant implications for the use of cover crops under New Zealand conditions.

The results mean that if cover crops are used, their effect on nutrient cycling must be accounted for. They may reduce nutrient loss by leaching during winter, but they can also reduce nutrient availability compared to fallow at the time of planting of the maize. Fertilizer application regimes should take account of this effect – for example there may need to be an altered timing or higher rate of fertilizer application in spring, and a reduced rate later on. The choice of cover crop is also important. Graminaceous cover crops like rye or oats may be more likely to slow down nutrient release to the main crop. Further studies should consider the net effect of cover crop on nutrient loss by leaching. If fertilizer rates must be increased to account for the immobilization in organic matter and delayed release to the main crop, then there may be no net benefit in terms of total application rate and loss to surface waters. Further research should also investigate whether winter planted clover crops do fix significant amounts of atmospheric nitrogen by the time they are cut and the main crop planted. There is also a need to investigate the reason clover used in the experiment had no effect on total nitrogen. Further research could be conducted to select alternative clovers or other leguminous cover crops that can be used to fix more nitrogen during winter.

The finding that root treatments are more effective for reducing germination than the shoot treatments are important for the farmers that are keeping the crop residues in the fields to reduce the germination and growth of weeds. There is an economic benefit for the farmers to use the shoots of cover crops for other activities while they may still benefit from the weed controlling effects of roots residues. It suggests the potential to cut and carry the cover crop shoots for use in silage or for direct feeding to stock that are held on feed pads. The major cover crops used in New Zealand such as faba, oats, rye and clover could be tested in an experiment to compare whether the inhibition effects of roots and cutting the shoots are the same. While cutting and carrying cover crop residues might inhibit germination of weed species, it could cause a problem for nutrient recycling that affects the main crop. This could negatively impact on soil fertility. Since clover should have shown the potential to add nutrients to the soil this negative impact could be minimised when clover is used as winter cover crop. To support soil fertility, the farmer might be required to increase application of nutrients to the soil and to increase nutrient availability at critical times.

Laboratory experiments should also be conducted to identify the compounds responsible for the inhibitory effect of clover on seed germination. This could be done by fractionating the crude extract through separation methods such as liquid chromatography, and testing each fraction individually for inhibitory effects. Further fractionation and chemical characterisation of the most active fraction should lead to the identification of the compound(s) responsible. Alternative sources of inhibitory compounds could be identified and used to suppress weeds, or cover crops could be deliberately bred and selected to produce higher concentrations that provide better weed suppression. It is recommended that another experiment be conducted in the field where the allelochemicals are not restricted in their movement. The weed suppression effect should be compared between the root and shoot treatments, with the soil prepared at the same time in both treatments, to avoid the effect of pre-germination of volunteer weeds that occurred in the pot experiment reported above. The experiment should include an extended growth period to cater for measurements of the yield of the test species and to check the rate of release of allelochemicals from the cover crop residues.

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