Soil-borne disease suppression to *Rhizoctonia solani* AG8 in agricultural soils from a semi-arid region in South Australia

This thesis submitted in fulfilment of the degree of Doctor of Philosophy, Soil and Land Systems, School of Agriculture, Food and Wine, The University of Adelaide.

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Table of Contents

List of Figures

List of Tables

List of Appendices

Abstract

Soil-borne suppression to *Rhizoctonia solani* AG8 in agricultural soils from a semi-arid region in southern Australia

Rhizoctonia solani AG8 is a significant soil-borne pathogen of cereal roots in semi-arid Mediterranean regions of Australia and the Pacific North West region in the United States of America, causing severe productivity and economic losses to farmers. During the past twenty years the conversion of many farming systems to conservation tillage has meant that the mycelial network of the pathogen is no longer seasonally disturbed by cultivation which has subsequently increased the potential for greater incidence of Rhizoctonia root rot. There has been some success in reducing incidence by using modifications to direct drill seeding equipment enabling some disturbance to Rhizoctonia at sowing. However, a long term sustainable solution with both economic and environmental benefit, as concluded from a review of the literature (Chapter 1) is to harness the potential for biological control of the disease via natural or induced suppression in soils.

Biological suppression to specific disease organisms in soil has been reported worldwide from a range of environments. Further, the development of biological soil-borne suppression to Rhizoctonia root rot has been described for one specific agricultural location (Avon) in South Australia following a decade of stubble retention together with higher than average nutrient inputs (Chapter 1). The studies in this thesis investigate soil-borne suppression to Rhizoctonia in agricultural soils from a semi-arid region of South Australia called Eyre Peninsula (EP) that produces 40% of the State's grain. The context is that historically in Eyre Peninsula farming systems crop residue inputs to soil are inherently low, as are fertiliser N and P inputs. However, recent intensification of these systems with the implementation of continuous cereals and minimum or zero tillage has resulted in greater inputs of stubbles and fertilisers. Rhizoctonia root disease is prevalent in the mainly coarse textured alkaline soils of the region, and the reduction in cultural control associated with adoption of reduced till systems has highlighted a need for alternative control measures.

In a broad context, the key question addressed in this thesis is whether the soil ecology to suppress Rhizoctonia is present or can develop in these soils from a region considered an

extremely harsh environment climatically as well as edaphically. Specific key questions will be addressed in the discussion section of each chapter. The thesis, through a series of controlled environment studies, examines abiotic-biotic interactions between the soil, the *Rhizoctonia solani* pathogen and wheat seedlings. The work assesses how the soil organisms involved in disease suppression (both the pathogen *Rhizoctonia solani* AG8 organism and other antagonists or competitors) are influenced by cereal stubbles and fertilizer inputs to the system.

Through a series of preliminary experiments (Chapter 3) the important variables of soil moisture and amount of pathogen inoculum (e.g. number of pathogen infected agar plugs) suitable for a bioassay method were standardised, and used throughout the rest of the work described in this thesis.

Two controlled environment bioassay experiments (Chapter 4) were undertaken surveying soils from six sites across the region differing in physico-chemical and biological properties to elucidate the influence of abiotic and biotic factors on plant-soil-pathogen interactions and the potential for suppression of Rhizoctonia. A comparison was made with soil from the long term study site in SA (Avon) reported to be suppressive to Rhizoctonia. Studies growing wheat seedlings in sterilised soils demonstrated that the soils assessed were intrinsically different in terms of the growth supported by the abiotic matrix. Greatest shoot and root dry weight was observed in the soil from a region outside the EP (i.e. Avon) and the least was in an EP soil with extremely high calcium carbonate content (e.g. Streaky Bay) – a clear example of plant-soil abiotic interaction. Avon soil was confirmed as suppressive to Rhizoctonia root rot since the Avon soil inoculated with its own biotic component reduced root infection to 50% from more than 70% in the sterilised abiotic control. Whereas, for plants in the two EP soils with low calcium carbonate root infection was similar in the sterilised abiotic matrix to that in the soils inoculated with their biotic component, suggesting they were not biologically suppressive. Further evidence of the suppressive capability of the biotic component of Avon soil was obtained where it was inoculated into the two EP soils with higher carbonate and reduced root infection in plants grown in these two soils, although not in the lower carbonate content abiotic matrix of Minnipa, another EP soil. Surprisingly, considering the hostile edaphic conditions, root infection was reduced in the high calcium carbonate soil inoculated with its own biotic component, suggesting it was suppressive

but not to the same extent as Avon. It was hypothesised this was possibly related to the organic C content in that soil being similar to Avon and higher than the other two EP soils. Shifts in soil organism community structure were observed when plants were grown in sterilised soils inoculated with the biotic component from another soil (i.e. rhizosphere soil from plants grown in another non-sterile matrix). Overall this work suggested there was some biotic potential for suppression in EP soils but low organic C was likely to be a constraint. EP soils were not as suppressive as Avon and abiotic constraints were highly likely, for example, the high carbonate reducing availability of P due to chemical fixation.

A long term glasshouse study (Chapter 5) was undertaken to measure the effect of carbon addition to two EP soils, as stubble or young root residues, on the potential to suppress Rhizoctonia. Other measurements in this experiment were microbial biomass carbon and quantitative PCR for DNA of pathogen and other specific micro-organisms implicated as contributing to disease suppression. C input to EP soils suppressed Rhizoctonia infection in wheat seedlings (despite abiotic constraints). C input as young roots increased DNA of *Rhizoctonia solani* and beneficial soil organisms *Microbacterium* spp. and *Pantoea agglomerans*. C input as stubble increased the populations of the beneficial soil organism, *Trichoderma* spp.

A further bioassay experiment (Chapter 6) investigated the effect of N and P fertiliser inputs on plant growth and Rhizoctonia suppression in two EP soils. The bioassay further investigated the interaction of these fertiliser nutrients with added available C in these two EP soils, one of which was highly calcareous. There was a positive plant growth response to added ammonium–N in both soils but no effect on Rhizoctonia infection. Addition of fertilizer P to the highly calcareous soil increased shoot and root growth and also Rhizoctonia infection without compromising effects on plant growth. Addition of available C (sucrose) with P fertiliser in the highly calcareous soil markedly suppressed Rhizoctonia infection.

Two final experiments focussed on measuring the changes in pathogen and other microbial communities in response to inputs of fertiliser and C in a highly calcareous EP soil, since Rhizoctonia root rot impacts are considered a particularly big issue in this soil type. In the first experiment (Chapter 7) it was hypothesised that fertiliser P may affect suppression of Rhizoctonia root rot not only via increasing plant growth but also by

altering microbial community composition. Results showed that virulence of *Rhizoctonia solani* was unaltered by P addition although pathogen DNA in soil and plant root infection increased. The effect of P fertiliser on plant growth compensated for the effect of P on increased pathogen population and root infection. Whilst fertiliser P increased microbial activity no shifts were detected in communities so the effects of P on soil organisms involved in suppression of Rhizoctonia root rot were not conclusive. However, in the last experiment (Chapter 8) there were measured shifts in populations of organisms resulting from addition of fertiliser P in conjunction with stubble. The known suppressive soil organisms *Pantoea agglomerans* and *Microbacterium* spp. increased whereas *Rhizoctonia solani* (DNA) remained constant and hence Rhizoctonia infection decreased.

In summary, some soils from the EP region of South Australia expressed a degree of suppression to Rhizoctonia root rot via their biotic component in pot culture experiments. Furthermore, some of the soils, although not necessarily the same ones, contained soil micro-organisms implicated by other studies in suppression of Rhizoctonia root rot. The biotic component from some of the EP soils, whilst not suppressive in the soil matrix it was extracted from did demonstrate the potential to suppress Rhizoctonia root rot when transferred into another soil matrix, indicating an abiotic constraint to suppression. It is postulated that important abiotic properties in these EP soils were calcium carbonate content, with organic carbon and to a lesser extent mineral N and P also important since these latter properties bridge the abiotic to biotic divide. Important biotic properties are likely to be microbial activity, microbial community structure and the population of the pathogen, *Rhizoctonia solani* AG8.

Results from this thesis work suggest that suppression to Rhizoctonia root rot can occur in EP soils despite abiotic and biotic constraints of limited C and P. Improvement and maintenance of a high suppressive capacity in soils in this semi-arid environment will require integrated agronomy aimed at maintaining a healthy crop using fertilisers, particularly P. Available carbon appears to be the most limiting constraint to microbe based biological disease suppression of Rhizoctonia root rot in these soils. Therefore it is essential that adequate available C is supplied via stubble input to develop and maintain a highly functioning soil biota.

Although these results highlight that disease suppression to Rhizoctonia root rot is indeed possible in the constrained soils of the EP, the time required to develop this suppressive capacity in a field situation remains to be investigated.

Thesis Overview Chart

Chapter1 Introduction and Literature Review

Soil-borne disease suppression has been reported as an important component for controlling Rhizoctonia root rot in sustainable semi-arid farming systems, there is a limited understanding of factors affecting its development and expression.

General hypothesis: Eyre Peninsula South Australia is a hostile climatic and edaphic environment so may pose biotic and abiotic constraints to the development of soil-borne suppression to Rhizoctonia root rot

Chapter 2 Materials & Methods

Chapter 3 Preliminary Experiments to standardise bioassay

Standardisation of soil moisture content and number of pathogen plugs for bioassay.

Chapter 4 Survey of six EP soils and comparison to suppressive soil Avon

Hypothesis: EP soils may not be suppressive due to abiotic and/or biotic constraints

- Some Biotic potential for suppression in EP soils but low organic C possibly a constraint and may impose C limitation
- EP soils not as suppressive as Avon - abiotic constraints highly likely, especially high carbonate causing less available P due to fixation

Chapter 5 Stubble or root C input to three EP soils, one highly calcareous

Hypothesis: Input of C to EP soils will suppress Rhizoctonia root rot

- C input to EP soils suppressed *Rhizoctonia* infection in wheat seedlings (despite abiotic constraints)
- C input as young roots increased DNA of *Rhizoctonia* and beneficial soil organisms *Microbacterium* spp. and *Pantoea agglomerans*.
- C input as stubble increased the beneficial soil organism (*Trichoderma* spp.)

Chapter 6 Fertiliser and available C input to 2 EP soils, one highly calcareous

Hypothesis: Removing nutrient limitations in EP soils will directly or indirectly suppress Rhizoctonia root rot, but it may require additional C

- Positive plant growth response to added ammonium –N in both soils
- Addition of P to highly calcareous soil increased shoot & root growth and also *Rhizoctonia* infection without compromising effects on plant growth.
- Addition of available C (sucrose) with P fertiliser in a highly calcareous soil markedly suppressed *Rhizoctonia* infection

Chapter 8 Stubble and P fertiliser input to a highly calcareous EP soil

Hypothesis: P fertiliser plus a complex C source will increase suppressive organisms

- *Pantoea agglomerans* and *Microbacterium* spp. increased and *Rhizoctonia solani* DNA did not
- Rhizoctonia infection decreased

Chapter 7 P fertiliser rates in a highly calcareous EP soil

Hypothesis: Fertiliser P affects suppression of Rhizoctonia by increasing plant growth and altering microbial community composition

- Virulence of *Rhizoctonia* unaltered by P addition although pathogen DNA in soil and plant root infection increased
- P effect on plant growth overrides P effect on increased pathogen and infection
- Fertiliser P increased microbial activity but no shifts detected in communities so effects of P on soil organisms involved in suppression of *Rhizoctonia* inconclusive

Chapter 9 General Discussion

Thesis studies suggest soil-borne disease suppression to *Rhizoctonia* can occur in EP soils despite abiotic and biotic constraints of limited C and P

Requires integrated agronomy which aims to maintain a healthy crop and soil biota using fertilisers, particularly P to ensure adequate available C via crop residue inputs

Declaration

I certify that this work contains no material which has been accepted for the award of another degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Standing on Chris' head, Glenelg, 2011. Photo taken by Matthew Wren.

Chapter 1

Introduction & Literature Review

1. Literature Review

1.1 Introduction

Social concerns about the environmental effects of intensive agriculture have deepened, resulting in a shift of agricultural systems thinking towards "sustainability" (Janvier et al. 2007). Adoption of conservation tillage practices, decreased use of agrochemicals such as herbicides and reduced or targeted application of fertilisers are some of the current strategies considered to be integral to a sustainable farming systems. In semi-arid dry land agricultural regions, adoption of these strategies especially conservation tillage, has been hindered by an increase in soil-borne diseases. Most notable of these is root rot of cereals caused by *Rhizoctonia solani* AG8 (Rovira 1986; Roget 1995; Schroeder et al. 2006).

One possible strategy to control soil-borne diseases under conservation tillage is to implement a farming system that supports biological suppression of soil-borne diseases. The term soil-borne disease suppression is defined by Weller et al. (2002) as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil."

Disease decline towards *Rhizoctonia solani* AG8 was reported in a long-term conservation tillage trial at Avon in South Australia (Roget 1995). At the start of the trial, incidence of disease increased significantly before decreasing to lower levels and after 10 years had disappeared completely. In an attempt to understand the mechanisms behind this disease decline, (Wiseman et al. . 1996) carried out a range of experiments and concluded that disease decline was linked to a biological factor. Although disease decline and subsequent disease suppression have been observed for many years, the mechanisms are not clear.

The following literature review will provide some background information on semi-arid dry land wheat growing regions in Australia and the specific impact of Rhizoctonia root rot on wheat crops in the southern region especially, for the Upper Eyre Peninsula in South Australia, the region this thesis study focuses on. The review will discuss the effects of past and current management practices in semi-arid farming systems on the incidence

of Rhizoctonia root rot in cereal crops. It will then examine the concept of disease suppression and the current state of knowledge regarding mechanisms involved in the complex biotic – abiotic interactions between the soil, plant and pathogen. Finally, the review will discuss the importance of carbon and nitrogen as energy and nutrient drivers of the biotic component of suppression, and will briefly discuss the role of phosphorus. Over all the review aims to highlight areas where knowledge gaps exist and further research is required.

1.2 Australian agro ecological zones and farming systems

The Australian cropping regions (Figure 1.1) cover an area of 11 916 000 hectares and in an average season produces AU$4 680 million per annum of wheat alone. The semi-arid regions of southern Australia cover approximately 25% of the Australian cropping area and annual wheat production is estimated to produce AU$1 170 million (Murray et al. 2009). The semi-arid regions of southern Australia are within a Mediterranean climate characterised by hot, dry summers and cool, wet winters. Crops are generally grown during the wetter winter months i.e. sown in autumn to early winter and harvested from mid spring to early summer (Murray et al. 2009).

Farming systems in these regions have been evolving towards minimum tillage systems which lead to an increase in the prevalence of soil-borne diseases, especially Rhizoctonia root rot (MacNish 1983; Rovira et al. 1983). Murray et al. (2009) estimate that root and crown fungi diseases in the southern agricultural region of Australia are costing AU$117 million per annum, of which AU$38 million is attributed to Rhizoctonia root rot. Such an increase in Rhizoctonia root rot in response to conservation tillage has been reported not only in the southern and western semi-arid dry land regions of Australia (Neate 1984; MacNish 1985a) but also in the Pacific North West of America (Weller et al. 1986). It is estimated that in the southern agricultural region of Australia over a 10 year period, for three-quarters of the time about 50% of the wheat crop is affected by Rhizoctonia root rot (Murray et al. 2009).This frequency and substantial loss of production due to Rhizoctonia root rot is driving research into alternative, largely non-cultural solutions to control Rhizoctonia root rot in farming systems, such as early sowing, rotation crop choices and summer weed control as discussed later in this review. However, there has been relatively little research investment into biological disease suppression.

Figure 1.1: Agro-ecological zones of the Australian cropping belt.
(http://www.grdc.com.au)

1.2.1 The Upper Eyre Peninsula cropping region

This thesis will focus specifically on soils from the Upper Eyre Peninsula, a dry land cropping region in South Australia (Figure 1.2). The Upper Eyre Peninsula is reported to produce approximately 40% of the annual wheat production in South Australia (Holloway et al. 2001). Regional reports estimate that yield losses due to Rhizoctonia root rot are up to 60% (Crouch et al. 2005) in some instances. Rhizoctonia root rot is a problem that continually plagues farmers in this region.

Soils in this region tend to be markedly alkaline, often with high calcium carbonate contents, are naturally infertile and have been termed “hostile” to plant growth (Coventry et al. 1998). These high concentrations of calcium carbonate result in fixation of nutrients essential to plant growth, particularly phosphorous (Holloway et al. 2001) via the formation of insoluble complexes (Bertrand et al. 2003). It is this combination of hostile soils, low crop production and large yield losses due to soil-borne diseases that is a key driver of the need for investigation into disease suppression as a means of Rhizoctonia root rot control for sustainable farming systems in the region.

Figure 1.2: Location of upper Eyre Peninsula within the agroecological zone of South Australian mid north, Lower Yorke Eyre. (www.grdc.com.au)

1.3 Management of Rhizoctonia root rot

In South Australia Rhizoctonia root rot disease was reported as a problem as early as the 1930's in the light textured low organic matter sandier soils (Kerr 1955). Conventional cultivation (tillage) practices were historically used as a means to control Rhizoctonia root rot. Although cultivation was not completely effective, Rhizoctonia root rot was reported as an increasing problem in cereal crops with the adoption of conservation tillage practices (MacNish 1985a; Pumphrey et al. 1987). During cultivation mycelia are broken mechanically, disrupting the hyphal network (Neate 1984; Rovira 1986). Fragmentation of the hyphae also impacts translocation of nutrients towards points of infection. A decrease in nutrient supply due to disruption of the hyphal network, renders the fungus less able to meet the high-energy requirements for the infection process (Boswell et al. 2002). If unable to complete the infection process, the pathogen will not cause disease in a plant, so by disrupting the mycelial network, tillage will lower disease levels in cultivated paddocks compared to those under conservation tillage practices.

In the 1980's, a modified direct drilling (MDD) technique was developed involving a modified drill point on the seeding rig that cultivated soil directly below the seed during direct drill seeding (Jarvis et al. 1986; Roget et al. 1996). The concept was to break up hyphae present below the seed as well as to loosen the soil (Neate 1984). This allows for improved root penetration through the zone of high pathogen load during seedling growth (Kelly et al. 2000; Schillinger et al. 2006), and hyphal disruption removes the link between the pathogen infection points and its energy source (MacNish et al. 1996). In semi-arid dry land farming regions of southern and western Australia this method has been be successful in lowering but not eradicating the incidence of Rhizoctonia root rot in wheat (Jarvis et al. 1986; Roget et al. 1996).

For many soil-borne diseases, rotation with a non-susceptible host is an effective means of controlling pathogens. Several authors have reported the lack of success in using this method with *Rhizoctonia solani* (Schillinger et al. 2006). They hypothesised this lack of success is due to the wide host range of *R. solani*, implying pathogen inoculum levels do not decrease when alternative crops are planted. There were numerous anecdotal reports describing a decrease in the incidence of bare patch in the wheat crop following a canola crop. Recently published results show that this effect is real, that is a single crop of canola decreases the amount of *Rhizoctonia solani* AG8 inoculum in the soil (Gupta et al. 2010a). This shows promise as a management technique; however in the second wheat crop after the canola, the amount of *Rhizoctonia solani* AG8 inoculum in the soil increases to quantities similar to those observed before the canola crop, therefore the incidence of bare patch may be severe again in subsequent seasons (Gupta et al. 2010a). This suggests that canola might only offer a temporary solution. Perhaps in low rainfall areas a more economically feasible solution which allows continuous cereal cropping would be a better option for the control of Rhizoctonia root rot.

Removal of the summer weed (green bridge) food source for pathogens, using a period of chemical fallow offers a limited level of protection from incidence of disease in direct drill systems. Chemical (herbicide) fallow treatments reduce organic material available to *Rhizoctonia solani*, which in turn lowers the natural inoculum levels of *R. solani* by removing its hosts. For this management technique to be effective the length of this fallow period needs to commence at least two weeks before seeding (Roget 1987; Schillinger et al. 2006). Unfortunately in semi-arid dry land systems predicting the break

of season to accomplish both an acceptable length of fallow and ideal seeding time can be difficult and is not often practical.

A combination of modified direct drilling and chemical fallow has been trialled and it is reported that this can be as effective if not more effective than conventional farming practices (normal cultivation) in reducing, but not eliminating, Rhizoctonia bare patch (Kelly et al. 2000). However, a more sustainable and long term control option might be the development of biologically suppressive soils, as discussed in the following section of the literature review.

1.4 Biological Suppression of Soil-borne root diseases

1.4.1 What is soil-borne root disease suppression?

The most widely used definition in relation to suppression of soil-borne root diseases is "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." (Weller et al. 2002). A range of terminology has arisen in relation to this complex concept and the following is a brief description of the major terms that have been used in the literature.

Suppression is often classified or referred to as either 'general' or 'specific'. General soil-borne disease suppression is said to be a function of total microbial activity in soil rather than a specific set of organisms (Mazzola 2002a), and is considered not transferable between soils (Weller et al. 2002). Alternatively specific suppression, has been described as the development of a specific organism, or a specific set of organisms that have antagonistic effects on a pathogen (Mazzola 2002a), which are transferable between soils (Weller et al. 2002).

The opposite of a suppressive soil is one termed conducive (Weller et al. 2002), where there is a large incidence of disease under the conditions present in that soil. However, these terms are not definitive since soils are dynamic and will vary in their ability to suppress or support disease at any point in time.

Other studies define or refer to suppression as 'long-standing' or 'induced' where long-standing suppression relates to a physical condition inherent in a soil. Induced

suppression relates to crop monoculture with a susceptible host, where a soil conducive to disease becomes suppressive over time (Cook et al. 1994; Weller et al. 2002).

Similar terminology is used to differentiate natural suppression from induced suppression. Naturally suppressive soils are those where the suppressive activity is related to soil physical and chemical (abiotic) characteristics influencing soil biology and often is unaltered by cropping practices (Schroth et al. 1982). In this case, induced suppression is dependent on cropping practices and has little to do with soil type.

van Bruggen et al. (2000) described suppression as a point at which an ecosystem is said to be stable and healthy. A similar point has been made by Cook et al. (1994) , who stated that there is no natural equivalent to rotation as used in agriculture. Natural ecosystems are accustomed to growth of the same species year after year. These views define disease suppression as a point similar to that, which would be found in nature where man has not disturbed the natural vegetation. The premise here is that after a period of time, plant species will adapt and persist despite the presence of a pathogen in the soil.

Disease suppression, for the context of work described in this thesis was considered as a state of balance within a soil, where the biological community (biotic component) has reached equilibrium so that even though the pathogen may be present, disease does not develop, unless the equilibrium is disturbed. Many factors contribute towards this equilibrium which will take time to develop. The management of critical factors to decrease the length of the time period before the development of disease suppression is an area of importance and interest to both farmers and researchers alike. Production agriculture by its very nature causes annual disturbances to the soil-plant system and so maintaining a state of equilibrium in soil is not necessarily straightforward.

1.4.2 Hypothesised mechanisms of disease suppression

Several mechanisms of action have been proposed in relation to biotic control of soil-borne fungal pathogens. Modes of direct action (antagonism) on a pathogen can be classified as antibiosis, competition or exploitation (Baker 1968).

Antibiosis is described as “antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds, or other toxic substances” (Fravel 1988).

Competition between a pathogen and other microbes within a soil can be for either or both nutrients and space. Theoretically this would result in populations of both the pathogen and antagonist decreasing as the carrying capacity (ability to support / carry populations) decreases (Paulitz 2000).

Exploitation, in relation to biotic control, refers to predation or parasitism of a pathogen by other organisms within the soil community such as mycoparasites (Baker 1968), and leads to pathogen inoculum decreasing as the population of antagonists increases (Paulitz 2000). Mycoparasitism has been reported to rely on lytic enzymes (e.g. chitinase and gluconases) in order to degrade cell walls of pathogenic fungi (Chet et al. 1990).

Abiotic factors on the other hand can act indirectly or directly on suppression. Soil physical characteristics can act directly on the disease organism, for example adverse edaphic conditions such as poor soil structure and drainage or extreme alkalinity may or may not favour pathogen growth and development resulting in higher or lower levels of pathogen inoculum respectively. Indirect abiotic influences are generally mediated via the soil biological component (Mazzola 2002a).

It is not likely in the complex soil environment for any one of the factors to be acting alone in the development of disease suppression, or that any one organism alone can control a pathogen by antagonism. Thus the food web dynamics, the interactions of organisms as well as the edaphic environment all play an integral role in soil-borne disease suppression (Sanchez-Moreno et al. 2007). The following section attempts to clarify some of the complexities and interactions that must be considered when looking at disease suppression.

1.4.3 Suppression is the outcome of a complex set of interactions

The four main components that interact to influence soil-borne disease suppression are the plant, the pathogen and soil abiotic and biotic factors (Figure 1.3). Although the interactions are discussed separately in the following sections, in reality they will not act independently and at any point in time, one or more of the interactions may be acting to support disease suppression.

It is important to note that while specific examples from high input horticultural systems will be discussed in the following sections there is a limited capacity to extrapolate from

these high input horticultural systems to low input dry land farming systems. It is also important to highlight that different *Rhizoctonia solani* AG groups are genetically isolated non-interbreeding populations with different environmental preferences and host ranges, that is they should be viewed as though they are different species.

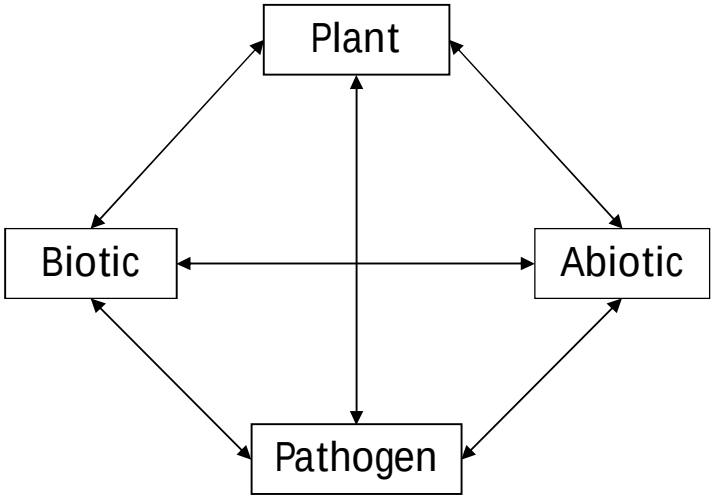


Figure 1.3: Bi-directional interactions between plant-pathogen and soil biotic and abiotic factors that influence disease suppression.

1.4.3.1 Abiotic – Biotic interactions in soil

Soil abiotic characteristics like organic matter, pH, clay content, water holding capacity and temperature are known to exert influences on microbial community structure and microbial activity (van Elsas et al. 2002). Indeed, an examination of take-all incidence in wheat in a variety of soils, concluded that disease prevalence was related to the abiotic capacity of each soil to support suppressive biota rather than conduciveness to the take-all pathogen (Duffy et al. 1997).

The abiotic edaphic environment controls nutrient availability for organisms living in the soil therefore any limitations in available nutrients will impact the size and function of microbial communities, especially if essential nutrients are limiting (Hamel et al. 2006). Competition for nutrients could be a hindrance to disease suppression if nutrient limitations prevent the selection and maintenance of microbial communities and their associated activities linked to disease suppression. For example; environmental conditions are known to influence microbial regulation of gene expression (activity) which is critical when suppression is linked to antibiotic production (Weger et al. 1995).

Conservation tillage practices alter a variety of soil characteristics and commonly, via residue inputs, increase carbon, nitrogen and moisture retention in soil. Over time these increases in carbon and nitrogen lead to an increase in both microbial biomass and activity (Wiseman et al. 1996; Pankhurst et al. 2002). Conservation tillage systems compared to conventional tillage systems have an increased proportion of fungi relative to bacteria, and in some cases this can be attributed to specific fungal species, for example *Trichoderma* spp. (Wiseman et al. 1996; Pankhurst et al. 2002).

As the abiotic environment of the soil changes, some organisms will be favoured and others inhibited resulting in shifts of the community structure. Ultimately these shifts may increase antagonistic activity towards a pathogen and enhance biologically based soil-borne disease suppression, or decrease antagonistic activity resulting in a more conducive soil. A schematic of the main factors involved in these interactions as discussed in the previous paragraphs (Figure 1.4) highlights the pivotal role of organic matter in these interactions.

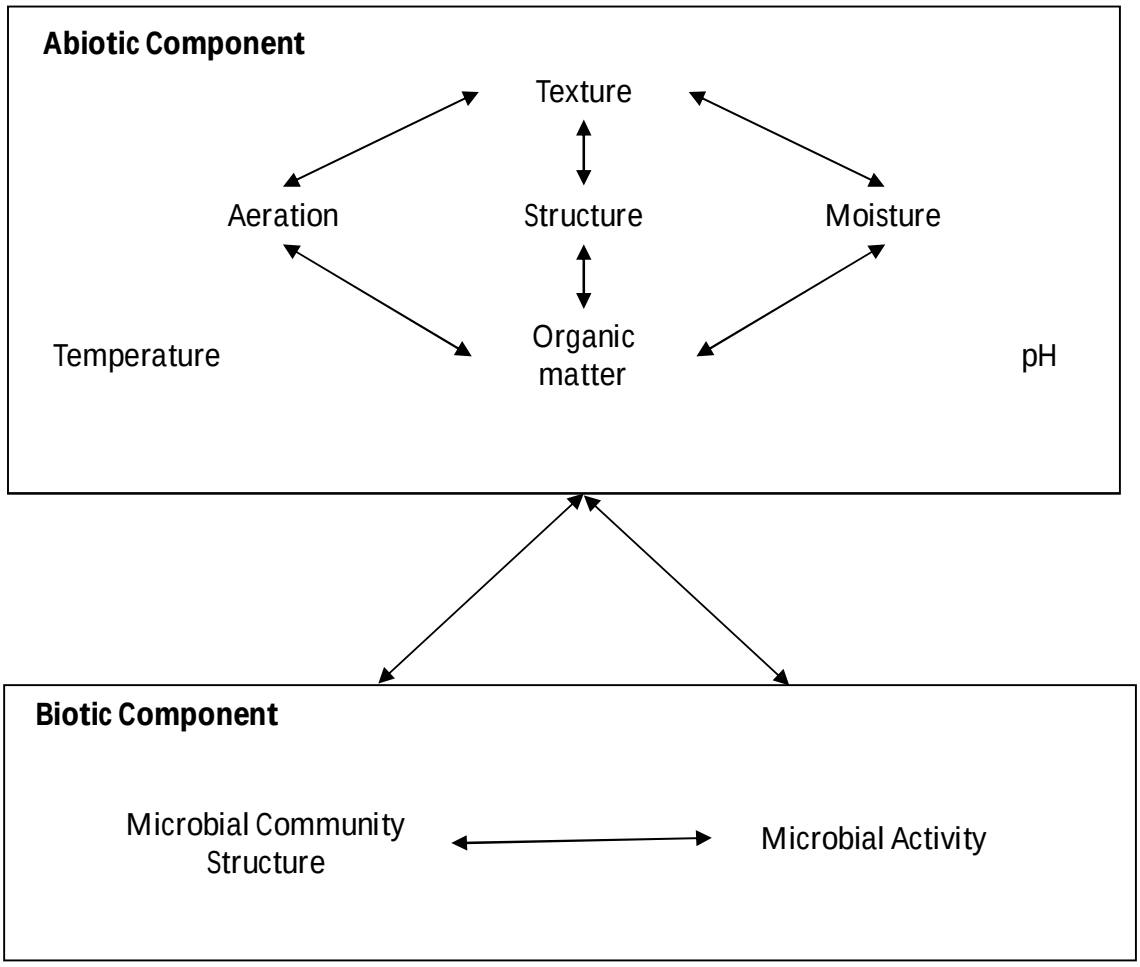


Figure 1.4: Generalised diagram of abiotic-biotic interactions in soil showing the central role of organic matter

1.4.3.2 Plant – Soil Biotic interaction

The main source of energy for soil micro-organisms comes from the release of root exudates as highlighted in Figure 1.5. Many factors are known to influence quality and quantity of these exudates, including: plant species, plant growth stage, soil abiotic characteristics, oxygen concentration, root disease or injury, plant nutritional status, microbes present and nitrogen source for the plant (Mahmood et al. 2005). Amount of exudates have been linked proportionally to plant biomass, thus all factors influencing plant biomass will in turn impact the quantitative release of root exudates (Hamel et al. 2006). Plant genotype plays an important role in determining plant biomass, thus different genotypes are likely to release different quantities of root exudates (Bowen et al. 1999). If these exudates then positively impact on microbial communities and activities, it may be a plausible strategy to use crop rotation to select for organisms linked

to disease suppression (Gu et al. 2003). In order to put this into practice a more detailed understanding of the genotypic specific influences would be required.

Different plant species are known to release different types of root exudates. These qualitative differences are said to select for specific microbes and, or microbe communities as well as to modify their antagonistic potentials (Gu et al. 2003). This ability of a plant species to select for a specific set of biological communities can lead to enhanced disease suppression and thus alteration of a soils natural suppressive ability (Mazzola et al. 2002b; Garbeva et al. 2004).

Some bacterial and fungal groups and / or specific species are known to have plant beneficial effects. For example *Pseudomonas* species are well documented plant growth promoters (Weger et al. 1995) and a shift in their populations after a succession of wheat crops on apple orchard soils has been linked to induced suppression against *R. solani* AG5 and AG6 (Mazzola et al. 2002b). Whilst, *Pantoea agglomerans* and *Exiguobacterium acetlicum* are also plant growth promoters, and have been found to be major contributors to suppression of *R. solani* AG8 found from the Avon site in southern Australia (Barnett et al. 2006).

Plant health has been described Cook (2000) as a list of ideals which do not differ much from human health, that is genetically “perfect”, optimum nutrition at every stage of growth and development, protection from physical harm, avoidance of exposure to harmful chemicals and avoidance of or protection against pests and diseases. Cook (2000) also discussed that plant health management is a framework by which to overcome a succession of limiting factors which will prevent the plant from reaching its full genetic potential. That is, plant health is compromised by the most limiting factor, and when that one limiting factor is removed the next limiting factor will prevent the plant from reaching its full genetic potential. The concept of plant health is important to integrate when attempting to interpret or understand the complexity of interactions that contribute to the final expression of disease suppression to Rhizoctonia root rot.

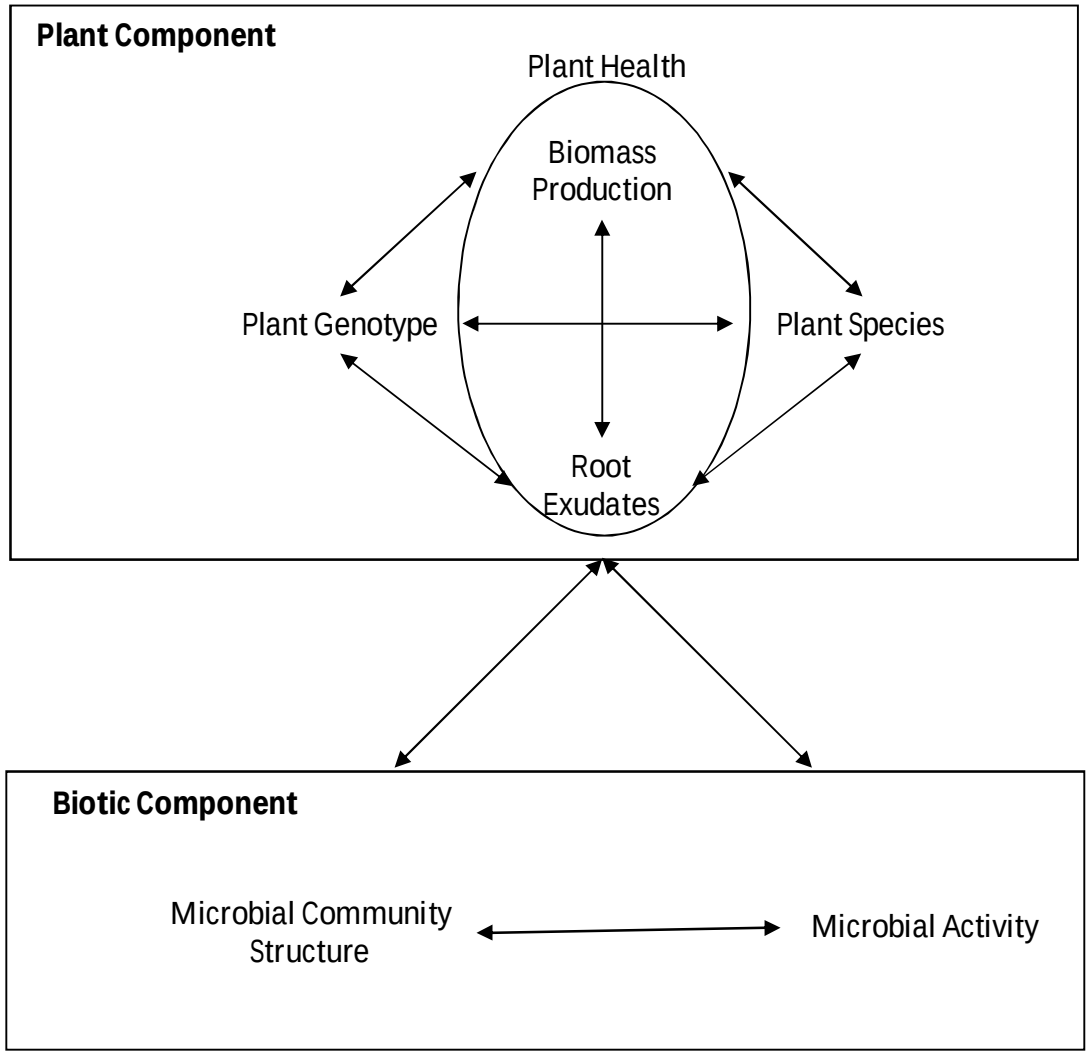


Figure 1.5: Generalised diagram of plant – biotic interaction highlighting the importance of plants as an energy source for the biological component

1.4.3.3 Soil biotic – Pathogen interaction

Pathogen-soil biota interactions include direct antagonism between the organisms in the soil with a pathogen (Figure 1.6), as mentioned earlier (Section 1.4.2). Many bacterial species are known to produce antifungal compounds and although these compounds may be effective in-vitro, their ability to be as effective in vivo on a heterogeneous soil environment must be considered.

Competition for space on organic material that is colonised by other organisms could prevent *R. solani* from inhabiting or accessing its nutrient source and thus prevent the successful completion of its life cycle (Papavizas et al. 1980). Examples can be found amongst other fungi and bacteria species; for example, under iron (Fe^{3+}) limiting conditions, *Pseudomonas* species that are capable of producing siderophores are known

to outcompete other organisms for iron. When this competition alters a pathogens' function, its ability to infect and cause disease will in turn be lowered (Weger et al. 1995).

Parasitism of pathogen inoculum is a mechanism by which the biotic component can lower pathogen levels within a soil. Parasitism may also influence a pathogens' ability to access and infect plant roots. An example of this is the parasitism of *Rhizoctonia* species by certain *Trichoderma* isolates, whereby *Rhizoctonia* mycelia are prevented from infecting roots. Furthermore, as they cannot then propagate this results in lowering of *Rhizoctonia* inoculum in the soil (Hoitink et al. 1999).

Soil macro fauna can also play an important role in the biotic-pathogen interaction. Previous studies on the effects of earthworms on Rhizoctonia disease have shown that in calcareous sandy soils like those found on the EP, both *Aporrectodea rosea* and *A. trapezoids* can reduce Rhizoctonia disease (Stephens et al. 1993; Stephens et al. 1994). Several mechanisms were proposed to contribute to this reduction in disease severity due to the presence of *A. trapezoids:* i) the worms may have disturbed the mycelial network in a similar manner to cultivation ii) the worms may have increased the concentration of plant available nitrogen iii) by ingesting the hyphae the worms would have parasitised the pathogen iv) worm castings may have produced an unfavourable environment for the pathogen to survive in (Stephens et al. 1993).

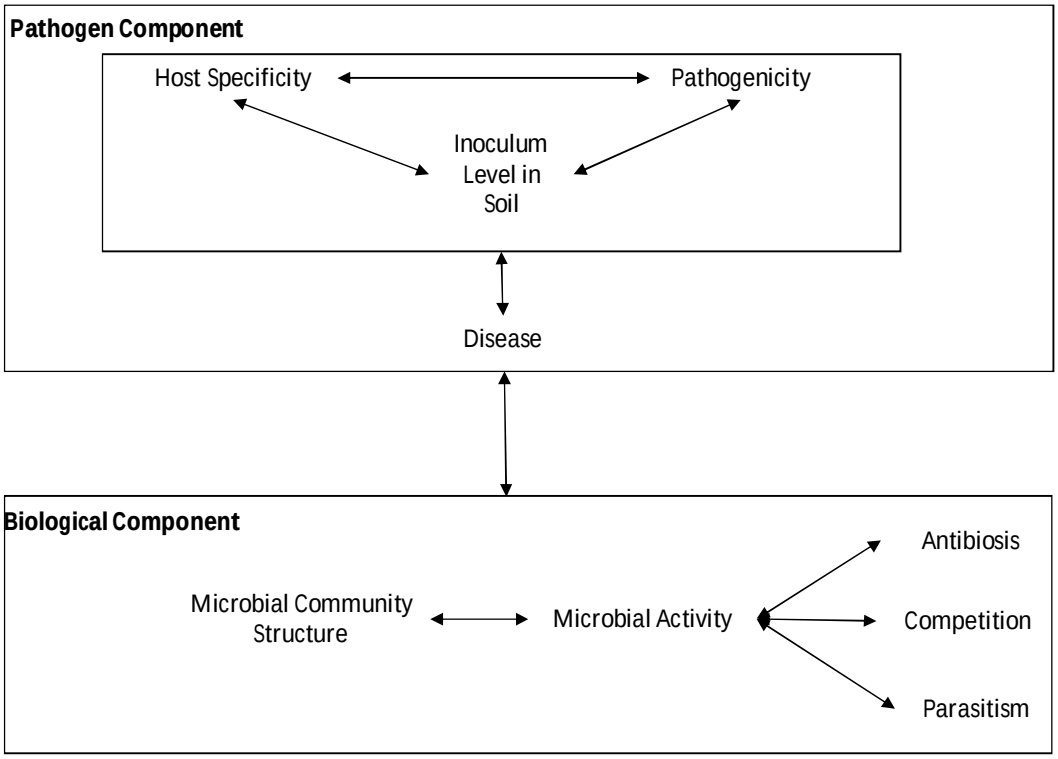


Figure 1.6: Generalised diagram of pathogen-biotic interactions

1.4.3.4 Plant - Pathogen interactions

One important factor to consider specifically relating to the pathogen *Rhizoctonia solani* AG8 and the incidence and severity of Rhizoctonia root rot disease is that there is not a good correlation between the amount of pathogen in the soil and the amount of disease expressed in the plant. This is likely to be due to a number of factors including localised soil and environmental conditions as discussed in Section 1.4.3.6.

The pathogen has to have the capacity to reach the plant before infection can occur It has been shown from work done on *R. solani* infection in radishes that there is a threshold distance away from the plant at which *R. solani* has the ability to cause infection. This threshold was dependent on a variety of external factors, for instance in the presence of *Trichoderma viride,* a known antagonist of *R. solani*, the pathogen needed to be closer to the plant in order to cause infection (Paulitz 2000).

Host susceptibility to the pathogen is critical for infection and most species found in agricultural rotations are known to be susceptible to *R. solani* AG8 (Neate 1984). Australian research has shown that there are different ranges of susceptibility between genotypes of the same species. There was a pronounced difference between two wheat

classes (hard white wheat and soft white wheat), with hard white wheat in monoculture and in rotation with barley consistently showing higher levels of Rhizoctonia disease when compared to the soft white wheat variety (Schillinger et al. 2006). This observation could be related to the plant effects on microbial selection as well as purely on host susceptibility.

The damage to roots after pathogen attack results in leaking or an increased release of root exudates. If these exudates preferentially select for suppressive organisms, then pathogen attack will lead to an increase in suppressive organism populations. This proposed sequence of events would help to explain the general increase in disease incidence reported before the development of disease suppression.

Figure 1.7 summarises the factors interacting in regard to plant and pathogen interactions.

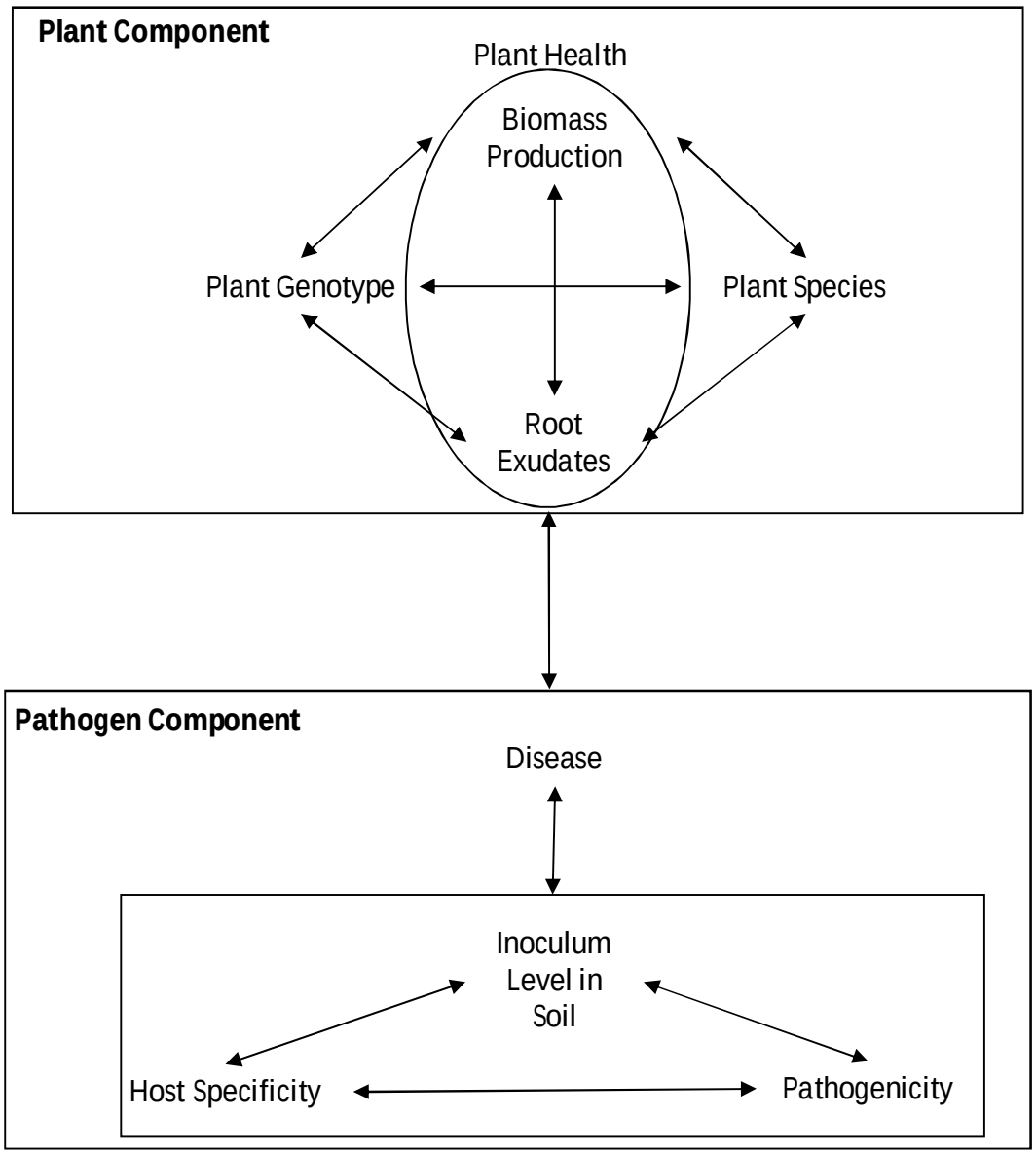


Figure 1.7: Generalised summary diagram of pathogen-plant interaction

1.4.3.5 Plant – Soil abiotic interaction

Any abiotic characteristics of soil causing plant stress and adversely impacting on plant health will increase a plants susceptibility to infection and thus disease. Under conditions of continual stress, any ability a plant might have had to compensate or recover from infection and disease will be lost, due to the plant expending energy on adapting to the stress rather than investing in defence mechanisms against disease (Schoeneweiss 1985).

Incidences of bare patch caused by *Rhizoctonia solani* are reported to increase in compacted soils. Two reasons behind this increase have been proposed, the first is that the density of pathogen hyphae increase, the second is that root growth is restricted due

to compaction (Glenn et al. 1990; Gill et al. 2004). Restricted root growth decreases a roots ability to pass quickly through the layer of maximum pathogen load. This in turn increases the chance of infection and reduces the chance for compensatory root growth (Gill et al. 2004). Roots restricted due to soil compaction in no-tillage systems also accumulate deleterious *Pseudomonad* spp., which have detrimental effects on plant growth (Watt et al. 2003), Figure 1.8 highlights the main plant- soil abiotic interactions.

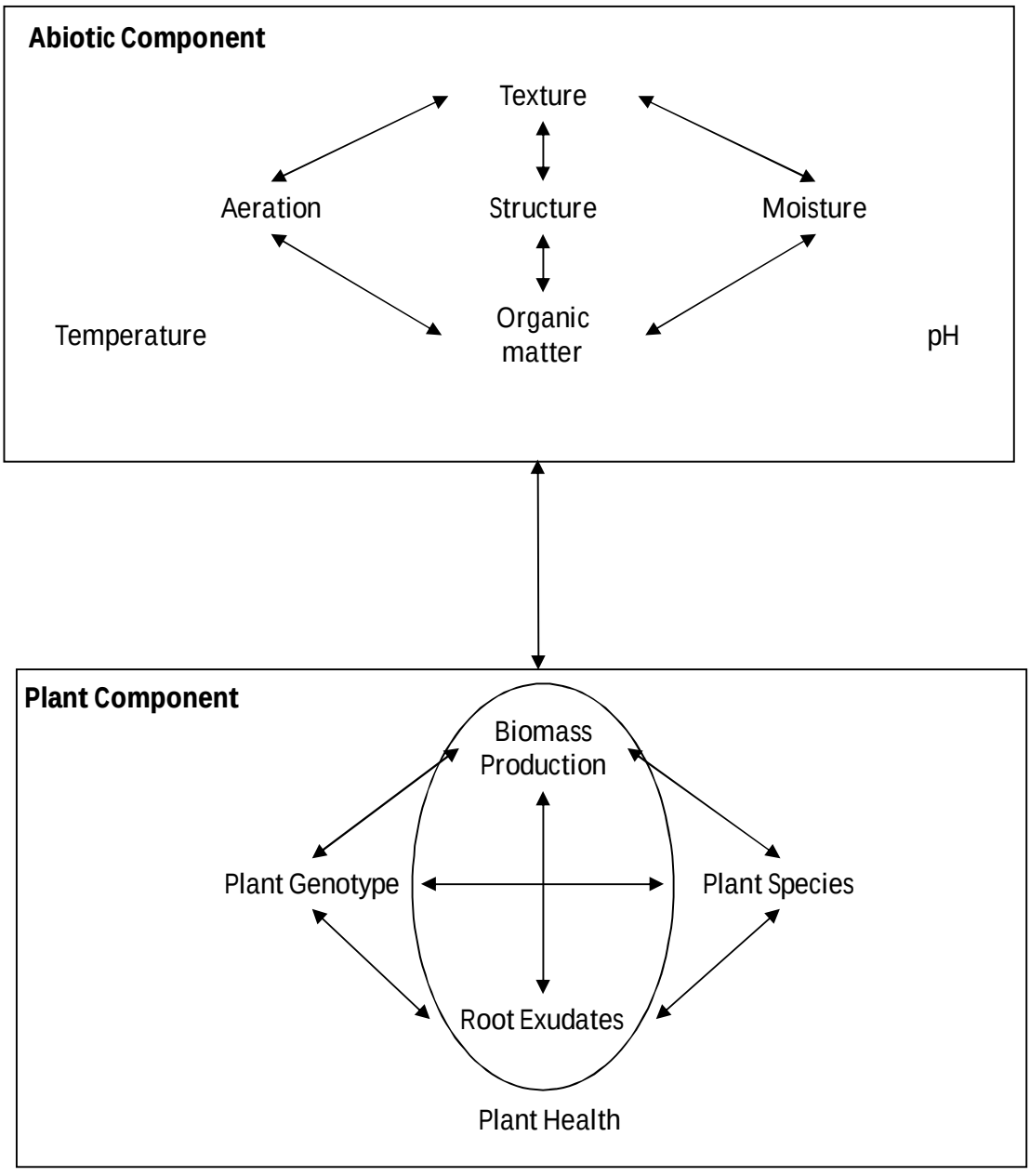


Figure 1.8: Generalised diagram of the soil abiotic-plant interaction

1.4.3.6 Pathogen –Soil abiotic interaction

Pathogen growth and virulence can be directly impacted by soil abiotic characteristics (Figure 1.9) (Gill et al. 2000; Mazzola 2002a). Depending on localised edaphic conditions, soils can shift along a continuum from suppressive to conducive towards *R. solani* (MacNish 1985b). This observation was seen when monitoring bare patch dynamics (time and space) over several seasons in Western Australia. Patches behaved differently, some expanded, others remained the same, some appeared while others disappeared depending on localised edaphic conditions (MacNish 1985b; MacNish 1996). In the U.S.A. similar trends in patch dynamics were observed, supporting this hypothesis that localised edaphic conditions impact on expression or suppression of disease (Cook et al. 2002).

Soil type has a large influence on both the spread of *R. solani* and the incidence of disease. On a large scale, it is more common to observe higher levels of Rhizoctonia bare patches in sandy soils. An observation which has been supported by glass house trials using a range of soils with differing amounts or proportions of sand (Gill et al. 2000). Additionally *Rhizoctonia solani* AG8 has the ability to quickly switch from a resting phase to a fast growing saprophytic phase (in the absence of a host) and then to a pathogenic phase (Neate 1984).

Studies on compacted soils have shown that the levels of Rhizoctonia root rot are greater than on a non-compacted soil and an increase in bulk density is reported to have increased the volume of soil explored by *R. solani* (Harris et al. 2003). Alternatively compaction is said to restrict fungal and root growth through a soil thus concentrating the pathogen and plant roots in this zone. This corresponds to greater disease incidence and slower root growth in compacted soils (Glenn et al. 1990; Gill et al. 2004; Peixoto et al. 2006). The restricted root growth also decreases the ability for compensatory growth after infection has occurred (Gill et al. 2004).

Soil moisture content plays a critical role in pathogen life cycles, for *R. solani* there is a reduction in competitive saprophytic ability where moisture contents are high (above 60% moisture holding capacity). Soil moisture is said to remain at a higher level for longer under compacted conditions, which may compound the effect of compaction on lowering saprophytic growth of *R. solani* AG8 (Glenn et al. 1990).

Aeration is important to all soil organisms and oxygen and carbon dioxide concentrations both have significant influences on community structure. *R. solani* is particularly sensitive to relatively high levels of carbon dioxide (Glenn et al. 1990). Any conditions which increase levels of carbon dioxide e.g. water logging have the potential to decrease levels of *R. solani* inoculum, which subsequently may decrease incidence of disease.

In lower ambient temperatures it is reported that levels of root damage caused by *R. solani* are greater than higher ambient temperatures (Smiley et al. 1993; Gill et al. 2001a). At lower temperatures plant growth will be slower which would expose roots to higher densities of pathogen inoculum for longer. Microbial activity under low temperatures will also be slower which may decrease levels of general suppression contributing to increases in disease commonly observed under cooler conditions.

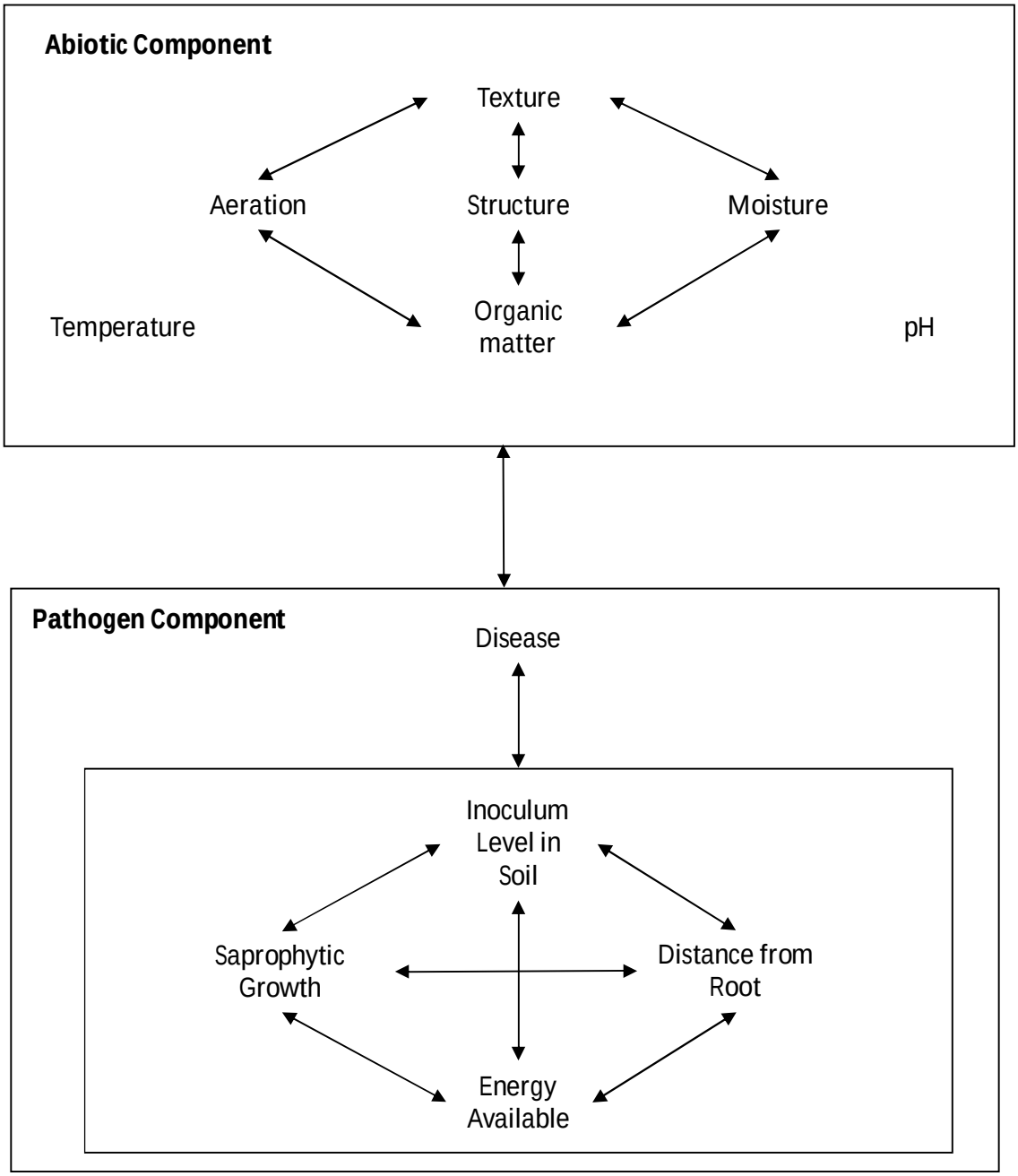


Figure 1.9: Generalised diagram of the soil abiotic-pathogen components interactions

1.5 Residues and application of nitrogen and phosphorus fertilisers – effects on biological disease suppression

1.5.1 Organic matter input from plant residues

In South Australian low fertility dry land cropping systems under conservation tillage systems, the long-term input of carbon into soil from retained plant residues, particularly cereal stubbles, appears to be the main driver of disease suppression (Roget et al. 1999). Increases in organic matter inputs lead to increases in soil microbial populations. This in

turn increases overall soil microbial activity and general disease suppression. Along with a general increase in microbial populations, there is a possibility to selectively increase specific populations of suppressive organisms such as those mentioned earlier in this review.

Retention of crop residues, leads to increases in both soil carbon and nitrogen over time, with an accompanying increase in microbial biomass and activity (Pankhurst et al. 2002). Increased microbial biomass results in greater immobilisation of carbon and suggests a higher proportion of more labile carbon than would be found in conventional tillage systems (Balota et al. 2003). Cereal crop residues in general have a wide C:N ratio, which may limit the quantity of available carbon and hence influence the biotic community structure and function (Hoyle et al. 2006). Thus crop residues may also have the ability to change the disease suppressive community structure and function, not just biomass. Since retention of wheat residues appear to be essential in the development of disease suppression towards Rhizoctonia root rot (Roget et al. 1999), it may be that there is a specific characteristic of wheat straw or wheat roots which alters the microbial community.

Nutrient concentration of soil organic matter and the rate at which nutrients become available are critical to both microbial activity and community structure (Hoitink et al. 1999). Organic matter is a critical factor controlling size and structure of a microbial community (Brant et al. 2006). Soil microbial populations will be driven by organic matter quantity and quality including; the nature of the initial organic materials, rate of decomposition, microbial population structure and environmental factors that may affect the decomposition of organic matter.

Rate of organic matter break down and turnover is regulated by a range of factors, for example, quantity and quality of residues, microbial community structure and any factors which will affect microbial activity, especially soil abiotic parameters (Bertrand et al. 2007). However, the interaction between microbial community structure and activity with organic matter is extremely complex (Figure 1.10). Organic matter quantity and quality are drivers for community structure, activity and population numbers. While community structure, activity and population numbers determine the breakdown, thus quantity and quality of decomposition products (Piovanelli et al. 2006).

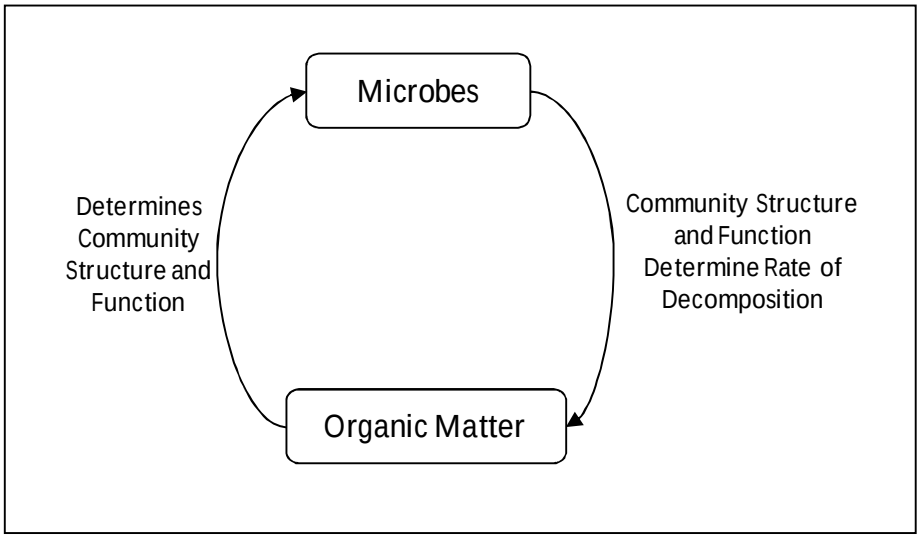


Figure 1.10: Relationships between organic matter decomposition and microbial community structure and function

1.5.2 Nitrogen and phosphorus fertilizer inputs

1.5.2.1 Nitrogen fertilizer

There are opposing points of view about the effects of mineral nitrogen on disease suppression. In farming systems of South Australia, an increase in available nitrogen during the non-cropping period (summer and early autumn) has been associated with a decrease in disease suppression of fungal diseases in the winter cereal crop (Gupta et al. 2006). However MacNish (1985a) found that increasing nitrogen fertilizer applications decreased the incidence of Rhizoctonia root rot. Whether the differences are due to compensatory growth by the plant, influences on the soil biotic component or due to the timing of nitrogen application in the soil is unclear. Glass house studies investigating the effect of increasing nitrogen application (as ammonium nitrate) also showed no consistent relationship between rate of nitrogen application and Rhizoctonia disease (Wall et al. 1994). This interaction warrants further investigation as N fertiliser applications tend to increase under intensification of farming systems, such as is occurring on the upper Eyre Peninsula.

The rate of carbon decomposition in soil is integrally related to the availability of mineral nitrogen. Raiesi (2004) found that increasing nitrogen fertilizer rates in winter wheat increased both carbon decomposition and microbial biomass carbon. An increase in microbial activity as organic matter decomposition increased. If general microbial activity

relates to general suppression, then increases in general microbial activity associated with increased nitrogen application should correspond to an increase in general disease suppression.

Form of nitrogen available to plant roots has been linked to different quantities of passive sugar efflux from roots. Studies suggest that nitrogen in the form of ammonium, increases root sugar efflux and microbial activity in the rhizosphere when compared to nitrate nutrition (Mahmood et al. 2005). If suppressive organisms respond to genotype specific exudates, an increase in exudate efflux may increase populations of suppressive organisms and speed up the development of disease suppression. This genotype specific response could be a management tool for farmers to encourage the development of suppression by planting specific genotypes. The effects of the hostile edaphic conditions in Eyre Peninsula soils on the response of roots to nitrate and ammonium in soil and subsequent infection by *Rhizoctonia solani* are unknown and warrant some investigation. A large proportion of fertilizer used is as urea or di-ammonium phosphate (DAP) and whether the conversion to nitrate is rapid or slow would determine the dynamic of exposure of both the pathogen and the plant roots to ammonium.

In-vitro studies on *R. solani* with ammonium and nitrate, found that ammonium was used as a nitrogen source until day 6 of incubation when ammonium became limiting and nitrate became the preferred nitrogen source. This trial included some known fungi antagonistic to *R. solani*, including *Trichoderma* spp. and found that in general total nitrogen usage (ammonium and nitrate) was significantly higher by the antagonistic fungi when compared to the pathogenic fungi (Celar 2003). In terms of competition, if these conditions occur in the field, antagonistic fungi would have a competitive advantage over pathogenic fungi. If this holds true, so long as the antagonistic fungi are present in adequate population sizes they should have the ability to out compete pathogenic fungi resulting in some level of disease suppression.

1.5.2.2 Phosphorus

Current research on the direct effects of phosphorus on disease expression or disease suppression of Rhizoctonia root rot is limited. This is an area of specific interest for soils of the Eyre Peninsula because these soils commonly have extremely high in calcium carbonate contents. This results in the formation of insoluble calcium-phosphorus

complexes (Bertrand et al. 2003). Studies on two non-calcareous soils, one from a moist tropical forest in Costa Rica and the other tropical pasture in Mexico showed that addition of P increased microbial activity after a 30 or 20 day period respectively (Cleveland et al. 2002; Galicia et al. 2004). In highly calcareous soils like those from the EP, the fixation of phosphorus is known to result in decreased plant growth. It is therefore reasonable to assume that fixed P in these soils will also be limiting the soil microbial populations and is likely to decrease the soil microbial population growth and activity resulting in a decrease in general disease suppression. The combination of compromised plant health as well as a decrease in soil microbial populations and activities due to P limitation may help to explain the persistent problem of Rhizoctonia root rot experienced on the Eyre Peninsula.

1.6 Conclusions and thesis aims

This literature review highlights the complexity of interactions involved in soil-borne disease suppression and emphasises the need for greater understanding of the drivers for disease suppression to Rhizoctonia root rot, as well as the mechanisms behind this disease suppression. All the work in this thesis is focused on the hostile soils from semi-arid farming systems of the Upper Eyre Peninsula in South Australia and the overall aim was to assess the potential of these soils to biologically suppress Rhizoctonia root rot in wheat.

The following chapter (Chapter 2) details the methods that were used throughout the work and only brief additional information is provided in succeeding chapters. Some preliminary investigation was undertaken (Chapter 3) to characterise the conditions suitable in Eyre Peninsula soils for the bioassay used to assess suppression.

The first major study (Chapter 4) investigates expression of Rhizoctonia root rot in relation to interactions between the abiotic-biotic components in soils from the Upper Eyre Peninsula, compared to a known suppressive (Avon) soil (Roget 1995) from another semi-arid region in South Australia. The hypothesis tested was that inherent abiotic constraints in Eyre Peninsula soils could prevent the biotic component of suppression from functioning in a general or specific manner to suppress Rhizoctonia root rot (Chapter 4).

Secondly, a study investigated the impact of increasing carbon inputs to some of these soils in development and expression of disease suppression (Chapter 5). The hypothesis was that inherent low organic matter content in these soils and low inputs from agricultural production may pose a limitation to the development and/or expression of disease suppression and that addition of carbon would induce an increase in either general or specific disease suppression.

The final studies (Chapters 6, 7 & 8) aimed to elucidate the interaction of key fertiliser inputs (nitrogen and phosphorus) on disease suppression of Rhizoctonia root rot. The hypothesis was that availability of nitrogen and phosphorus in these soils may affect the expression of disease suppression by constraining the plant as well as the pathogen and other soil organisms. This work also investigated the interactions between additions of mineral nutrients and increasing carbon inputs and subsequent effects on disease suppression of Rhizoctonia root rot.

Chapter 2
General Methods

2. General Methods

2.1 Soil sampling and preparation

A selection of soils from the Eyre Peninsula was used for the duration of the PhD, Table 2.2 summarises their locations and collection times as well as key abiotic characteristics. These soils were collected from farmer paddocks by random sampling of the top 10cm across the paddock. All soils were air dried and then passed through a <2mm sieve. A sub-sample of this soil was then sent to a commercial testing laboratory for analysis for extractable nitrate-N, ammonium-N (Searle 1984), Colwell available potassium and phosphorus (Colwell 1963), percent calcium carbonate as determined by Martin et al. (1955), percent organic carbon (Walkley et al. 1934) and micronutrients (Diethylene triamine penta acetic acid**,** D.T.P.A. Cu, Zn, Mn, Fe (Rayment et al. 1992)) results are summarised in Table 2.2.

2.2 Estimation of water holding capacity

Water holding capacity (WHC) was estimated using a 1 meter column technique (Marshall et al. 1979). Four replicates of each soil were used. An open ended core was used for each replicate. The core was placed onto a porous pressure plate which was one meter above a water line, and each core was filled with dry soil. Samples were saturated with reverse osmosis water every hour for 8 hours, after which they were left to drain for 48 hours. The moist soil was then placed into oven proof containers, weighed (soil wet weight) and then dried in an oven at 100°C for 24 hours. After drying, samples were reweighed (soil dry weight) and these values used to calculate the estimated water holding capacity (WHC). Estimated water content at 100% water holding capacity was calculated using the following equation:

$$\frac{\text{(wet soil weight – dry soil weight)}}{\text{dry soil weight}}$$

The average of all four replicates per soil was calculated and this value used as the WHC for each soil in all the experiments undertaken during this PhD (Marshall et al. 1979).

Table 2.1: Estimated moisture content at 100% water holding capacity determined using a 1m column technique for all the soils used in this thesis.

Soil	Water Holding Capacity H_2O g/g dry soil
Streaky Bay	24
Minnipa	16
Avon	20
Kimba	21
Mount Damper	18
Mudamuckla	12
Port Kenny	31
Lock	20

Table 2.2: Summary of key abiotic characteristics of soils used in this thesis, including GPS locations.

Soil	SB1	SB2	Minnipa	Avon	Lock	Mudamuckla	Kimba	Mt Damper	Port Kenny
GPS Coordinates S	32° 48 956'		32° 49. 797'	34◦14'	33° 28. 646'	32 11.058'	33° 10.110'	33° 04.159'	33° 07.182'
GPS Coordinates E	134° 10.175'		135° 08.695'	138° 18'	135° 47.058'	134° 04.770'	136° 23.51'	135° 03.584'	134° 42.935'
Collection Date	March 2006	Aug 2006	March 2006	April 2006	March 2006	March 2006	March 2007	March 2007	March 2007
Extractable macronutrients (mg/kg)									
NO_3-N	33	49	13	16	21	11	22	17	15
NH_4-N	4	4	3	2	5	1	3	5	13
P (Colwell)	37	48	29	68	67	40	54	33	47
K	411	403	550	639	344	293	863	506	685
S	13.9	12.6	6.4	8.0	9.4	7.7	7.5	7.6	15.3
Extractable (DTPA) micronutrients (mg/kg)									
Cu	0.25	0.20	0.47	0.49	0.68	0.37	0.72	0.26	0.24
Zn	1.03	1.32	1.12	1.22	3.44	1.02	1.74	1.22	1.31
Mn	6.9	5.6	5.9	5.1	8.7	3.7	6.7	6.6	6.9
Fe	3.07	4.28	4.07	3.32	4.14	1.72	4.24	5.49	5.35
Electrical Conductivity (EC1:5)									
(dS/m)	0.278	0.208	0.149	0.177	0.172	0.151	0.181	0.168	0.257
pH $_{(H2O)}$	8.5	8.4	8.6	8.6	8.5	8.7	8.5	8.5	8.6
Calcium Carbonate (%)	80	73	3	9	29	16	4	1	20
Organic Carbon (%)	1.94	2.25	1.20	1.95	1.73	1.12	2.02	1.40	2.62

2.3 Rhizoctonia inoculum preparation

Rhizoctonia inoculum was prepared based on the methods described by Barnett et al. (2006). Millet seeds colonised by *Rhizoctonia solani* AG8 were individually placed in the centre of a full strength potato dextrose agar plate (PDA, Difco). Plates were then placed into a laboratory incubator at 25°C and left for 3 days (sub-culture 1) after which they were removed. Plugs 10mm in circumference were cut with a potato corer in the fungal growth, each plug was removed and inverted into the centre of a fresh full strength PDA plate (sub-culture 2). These plates were incubated at 25°C for 4 days, after which were used as *Rhizoctonia solani* AG8 inoculum in bioassay pots. The rate of infested agar plug addition per pot was altered depending on the experiment, and will be mentioned in each experimental section.

2.4 Potting set up, growth conditions and sampling

2.4.1 Potting up and pathogen incubation

The potential suppression bioassay used 300ml plastic pots. Each pot had 250g dry weight of soil. Wet weight and soil moisture content for all bioassay pots will be discussed in detail in the following Chapter (Chapter 3.1: The effect of soil moisture content on severity of Rhizoctonia root rot disease in two soils from the Upper Eyre Peninsula, South Australia). For those pots that were inoculated with the pathogen, half the weight of required soil (moist) was placed into the pot. The *Rhizoctonia* infested agar plugs were evenly placed on top of this soil, and then covered with the remaining weight of soil see Table 2.3 for moist soil weight details. These pots were covered and placed into a controlled environment room (CER, at 15°C, with a 12 hour day / night regime) for two weeks to allow the pathogen colonisation of the soil.

Table 2.3: Summary of the amount of wet soil and water used per pot for 250g equivalent dry weight for each soil type used in this thesis. 100% Field capacity estimated using the 1m column technique (FC), 75% field capacity (75%FC) was the amount of moisture added to each pot per soil type for all bioassay experiments, wet weight (WW) the weight of moist soil per pot for 250g equivalent dry weight of each soil type at 75% FC, that is the amount of moist soil added to each pot for the bioassay experiments.

Soil	FC	75% FC	WW
Streaky Bay	24	18	268
Minnipa	16	12	262
Avon	20	15	265
Kimba	21	16	266
Mount Damper	18	14	264
Mudamuckla	12	9	259
Port Kenny	31	23	273
Lock	20	15	265

2.4.2 Surface sterilisation and pre-germination of wheat seeds

Wheat seeds (*Triticum aestivum*, c.v. Yitpi) were surface sterilised by washing in 1% sodium hypochlorite solution for 10 minutes, rinsing with reverse osmosis (R.O.) water three times, followed by a quick wash with 70% ethanol rinsing with R.O. water again and finally washing with 2% sodium thiosulphate for 5 minutes with a last rinse in R.O. water (Miche et al. 2001; Barnett et al. 2006). Surface sterile seeds were placed between moist filter paper and incubated at 25°C for 24 hours before planting.

2.4.3 Planting seedlings, growth conditions and sampling

Seven surface sterile, pre-germinated wheat seedlings were planted per pot. Seedlings were placed equidistant from one another and covered with 25g of wet soil. Once the seedlings emerged they were thinned to 5 per pot and the soil surface covered with about 20 grams of polythene beads to reduce evaporation. Pots were returned to controlled conditions at 15°C and 12 hour day night regime where they were kept watered to weight for 28 days until sampling.

At sampling, the root and soil mix was removed from each pot and washed with water to remove all adhering soil particles. Shoots were cut off at the base of the stem and dried in an oven for four days at 60°C after which dry weight was recorded. Roots were stored in a freezer prior to being rated for disease severity. Following disease rating, roots were dried in an oven for four days at 60°C after which dry weight was recorded.

2.4.4 Plant dry weight assessments and disease severity measures

Plant root and shoot dry weights were recorded as described in Section 2.4.3. For each experiment the average root or shoot dry weight per pot for all experimental replications was used in the statistical analysis.

Disease severity for Rhizoctonia root rot was measured based on the methods of Barnett et al. (2006). This was done by counting the total number of seminal roots, those seminal roots truncated by Rhizoctonia root rot before 10cm (the height of the 300ml plastic pot), those roots with infection but not truncated and used to calculate percentage root infection as follows

$$\text{Percentage root infection} = \frac{\text{No. truncated roots} + (\text{No. Infected roots}/2)}{\text{No. Seminal Roots}} \times 100$$

The number of infected root which are not truncated is divided in half as the roots are not truncated so do not have an equal disease value as the truncated roots, but the infection on these roots still needs to be taken into account as they impact on plant health and with time they would become truncated.

Root score was a visual assessment based on the methods of MacDonald et al. (1983) where 0 indicates no disease, and 5 the highest disease severity.

The percent root infection method was used in this thesis in addition to root score as it provides more detail than the 0-5 rating scale and it distinguishes between root health (as measured by root score) and specific root infection by *Rhizoctonia solani* AG8.

2.5 Experimental design and statistical analysis

All experiments were set out as randomised block designs. The number of replications varied and will be summarised in each experimental section.

Data was analysed in GenStat Tenth Edition using ANOVA. Comparisons between treatment means were made using the lsd at 0.05. Different letters have been used to indicate which treatments were significantly different from each other throughout this thesis.

Specific data for some experiments was analysed in PRIMER-E (Clarke et al. 2006) and this will be discussed in the relevant data chapters.

2.6 Micro-organism assessment

2.6.1 Microbial biomass carbon and nitrogen

Microbial biomass carbon was estimated using the fumigation-extraction method (Brookes et al. 1985). For each container treatment, two subsamples of 10 grams of soil were wet to 75% of field capacity and incubated for 14 days. The first subsample (control) was extracted with 40ml 0.5M K_2SO_4. At the same time, the second subsample was placed in a desiccator with chloroform, and fumigated for 7 days, after which samples were extracted using 40ml 0.5M K_2SO_4. All extracts were then analysed using absorbance spectrophotometery for ninhydrin-positive nitrogen compounds (NPC) at an absorbance of 570nm (Joergensen et al. 1990). Microbial biomass C was estimated using the flush of NPC, that is (fumigated NPC – control NPC) x 21 (Amato et al. 1988; Sparling et al. 1993).

2.6.2 DNA analysis of specific organisms

Sub-samples of soil (this will be described in more detail in each data chapter) were taken to the SARDI (South Australian Research and Development Institute) Root Disease Testing Services for quantification of DNA of selected pathogens using real time PCR (Ophel-Keller et al. 2008). The pathogens assayed were *Rhizoctonia solani* AG-8 (Whisson et al. 1995) *Gaeumannomyces graminis* var. *tritici* (Herdina et al. 1996) and the beneficial organisms *Exiguobacterium acetylicum*, *Pantoea agglomerans*, *Microbacterium*, *Trichoderma* Group A, (sect. *Pachybasium*) and Group B (sect. *Trichoderma* see Figure 2, described by Kullnig-Gradinger et al. 2002).

2.6.3 Carbon source utilization profiles

This method of fingerprinting the microbial community structure was based on the methods of Campbell et al. (2003). Briefly, whole moist (8.5% WHC) soil was sieved into a

deep welled 96 well plate, and placed into an airtight container with open water sources to maintain air moisture. This was then left to equilibrate in a dark controlled environment room over night. Disposable 96 well plates were made with 150 micro litres of agar per well. The agar was made with creosol red, an indicator dye. Once set, plates were transferred to a desiccator to avoid contamination by atmospheric CO_2.

A range of water soluble carbon sources which were standardized and relevant to Australian soils (Gupta et al. 2008) were previously prepared and stored in the freezer (Table 2.3). The day of the micro-respiration procedure, the sources required were removed from the freezer and thawed. The concentration of the carbon sources was designed to deliver 30 mg of C per g soil as sugars and 15 mg of C per g soil as amino acids.

Creosol red plates were read using a Maxline Microplate Reader with an adjusted filter position at 590nm. Carbon was delivered into the deep welled plates, a rubber gasket fitted carefully into the plate holes and then the creosol red plates inverted carefully onto this rubber gasket. Samples were placed in a warm (25 Deg C) dark room and left to incubate for 5 hours. After which the creosol red plates were removed and immediately re-read.

A series of standards were done where, known carbon dioxide concentrations were incubated with creosol red plates. This standard curve was then used to work out the carbon dioxide evolution for each carbon source. Carbon source, carbon dioxide evolution was calculated by (final creosol red plate reading – initial creosol red plate reading) = value of colour change attributed to carbon dioxide evolution. Using the equation for the standard curve, this was extrapolated into a value of carbon dioxide evolution. Data for each plate was standardized for the average well colour development of that plate. The treatment means of CO_2 evolution per carbon source were compared using ANOVA in Genstat. Multivariate analysis on the CO_2 evolution data for all carbon sources was done using PRIMER-E (Clarke et al. 2006).

Table 2.4: List of carbon sources relevant to Australian soils (Gupta et al. 2008) added to soils in this thesis to evaluate Carbon Source utilization profiles. The concentration of the carbon sources was designed to deliver 30 mg of C per g soil as sugars and 15 mg of C per g soil as amino acids.

1	Arabinose
2	Fructose
3	Galactose
4	Glucose
5	Xylose
6	Maltose
7	Sucrose
8	Raffinose
9	Hydroxy-L-proline
10	Glycine
11	Asparagine
12	Valine
13	Serine
14	Alanine
15	Lysine
16	Arginine
17	Glutamine
18	Aspartic
19	Methionine
20	Cysteine
21	Histidine
22	Tryptophan
23	Leucine
24	Phenylalanine
25	Fumaric acid
26	Malic Acid
27	Malonic Acid
28	Oxalic Acid
29	Succinic acid
30	Tartaric Acid
31	Mannose

Chapter 3
Preliminary bioassay experiments

3. Preliminary Bioassay Experiments

3.1 The effect of soil moisture content on severity of Rhizoctonia root rot disease in two soils from the Upper Eyre Peninsula, South Australia

3.1.1 Introduction

Previous studies on Rhizoctonia root rot have shown that moisture content is important in the development of disease (Gill et al. 2001a; Gill et al. 2001b). Since soils from the Eyre Peninsula had not been used previously in bioassay experiments, an initial bioassay was undertaken to test the impact of soil water content on disease severity.

The main aim of this bioassay was to optimize soil water content for plant growth and disease severity.

3.1.2 Materials and methods

Soils from Streaky Bay and Minnipa were used in a pot bioassay experiment. The bioassay was carried out as described in (Chapter 2, Section 2.4) except for the amount of water added. Using the estimated water holding capacity as 100%, the experimental treatments were (1) 55% of WHC (2) 65% of WHC (3) 75% WHC (4) 85% WHC and (5) 95% WHC. Each water treatment had a Rhizoctonia inoculated treatment (+) and a non-inoculated treatment (-). All treatments were replicated 5 times. Data was analysed in Genstat using AVONA to compare between treatment means.

3.1.3 Results

For both Minnipa and Streaky Bay soils, increasing moisture content decreased the percent root infection (PRI) and root score measures of Rhizoctonia root rot and increased root and shoot dry weights (Table 3.1). The treatments without added Rhizoctonia inoculum did not have significant disease severity measured as either PRI or root score with all moisture contents and the root and shoot dry weights were approximately double the root and shoot dry weights for treatments with added *Rhizoctonia* (Table 3.1).

In the Minnipa soil, disease severity measured as percent root infection decreased with increasing moisture content until 75% WHC where it remained relatively constant and there was no statistical difference in measured PRI between 75%, 85% and 95% WHC (Table 3.1). It appears that with lower moisture contents plant growth is restricted as root and shoot dry weight in the non-inoculated treatments, generally increased with increasing moisture content, specifically root dry weights were not significantly different between 65% and 75% WHC and again between 85% and 95% WHC, while shoot dry weight continued to significantly increase with increasing moisture content (Table 3.1).

For the Streaky Bay soil, increasing moisture content in the + *Rhizoctonia* treatments significantly decreased percent root infection in the 85% and 95% WHC treatments compared to the 55%, 65% and 75% WHC treatments (Table 3.1). In the non-inoculated treatments, increasing WHC did not significantly alter root dry weights but did increase shoot dry weight from the 55% WHC treatment in all treatments above 75% (Table 3.1).

From this data set, 75% of water holding capacity was chosen for all bioassay experiments carried out during this PhD and described in this thesis. 75% of water holding capacity was chosen as it maximised percent root infection for both soil types used in this bioassay.

Table 3.1: Percent root infection (PRI), root score (0 -5 scale, where 0 = no disease and 5 = maximum disease) and root (RW) and shoot (SW) dry weights (mg) for plants grown in the Minnipa and Streaky Bay soils. Treatments were based percentages of estimated water holding capacity (WHC) measured by a 1m column technique taken to be 100%WHC. Treatments were; (1) 55% of WHC (2) 65% of WHC (3) 75% WHC (4) 85% WHC and (5) 95% WHC. Different letters show significant differences between treatment means using the lsd $_{(0.05)}$. ns represents results which were not significantly different at P=0.05.

Minnipa Soil	PRI	Root Score	RW (mg)	SW (mg)
+ *Rhizoctonia solani* inoculum				
55 +	95 a	4.3 a	46 d	52 d
65 +	89 b	3.5 b	62 ab	95 c
75 +	80 c	3.2 b	70 bc	130 b
85 +	82 c	3.0 b	78 c	135 b
95 +	79 cd	3.1 b	86 d	186 a
Lsd $_{(0.05)}$	6	0.7	11	21
No *Rhizoctonia solani* inoculum				
55 -	0	0.0	85 c	113 e
65 -	0	0.0	102 b	136 d
75 -	1	0.0	107 b	171 c
85 -	1	0.0	131 a	218 b
95 -	0	0.0	137 a	241 a
Lsd $_{(0.05)}$	ns	ns	9	14

Streaky Bay Soil	PRI	Root Score	RW (mg)	SW (mg)
+ *Rhizoctonia solani* inoculum				
55 +	96 a	4.6 a	42 c	74 c
65 +	94 a	4.4 a	44 c	124 b
75 +	92 a	4.2 a	50 c	141 b
85 +	68 b	2.7 b	72 b	150 ab
95 +	60 c	2.0 c	93 b	175 a
Lsd $_{(0.05)}$	8	0.5	19	30
No *Rhizoctonia solani* inoculum				
55 -	0	0.0	111	148 c
65 -	1	0.0	131	181 bc
75 -	0	0.0	147	207 ab
85 -	0	0.0	150	223 a
95 -	0	0.0	144	214 ab
Lsd $_{(0.05)}$	ns	ns	ns	41

3.1.4 Discussion

Overall these results showed that disease severity is greater in dry soil compared to wetter soil which supports evidence from previous studies on soils from Western Australia (Gill et al. 2001a; Gill et al. 2001b).

The lower root and shoot dry weights for plants grown in the non-inoculated pots in the low moisture content treatments suggests that plants grown in both Minnipa and Streaky Bay soils were suffering moisture stress when grown in the low WHC treatments. In combination with the increase in disease severity seen in plants grown in the inoculated pots in these low moisture content treatments the results suggests that the moisture stressed plants are likely to have had an increased susceptibility to *Rhizoctonia solani* AG8 attack. This observation supports the results of other studies which have reported that plants grown in conditions of moisture stress have an increased susceptibility to pathogen attack and therefore disease (Schoeneweiss 1985; Gill et al. 2001b). The combination of decreased plant growth and increased disease susceptibility indicates that the concept that any decrease in plant health (in this case moisture stress) will increase the susceptibility of plants to disease (Cook 2000).

Although no microbial parameters were measured in this work the decrease in disease severity with increasing moisture content may have been related to microbial factors. It is possible that in this experiment as soil moisture increased, populations and activities of soil organisms may have increased therefore increasing competition within the rhizosphere and limiting the ability of the pathogen to cause disease on the seedling roots. Had microbial parameters been measured and they indicated there was an increase in microbial activity with increasing moisture content, it would support previous work done in Western Australia on where it was observed that increasing moisture content decreased disease severity of *Rhizoctonia solani* AG8 by increasing the soil microbial activity (Gill et al. 2001b).

Inoculation of the bioassay pots with pathogen involves exposing the plants to the pathogen, which for all treatments resulted in high disease severity i.e. above 60% root infection. As expected the inoculated treatments had lower plant dry weights than the non-inoculated treatments. The decrease in root dry weight can be attributed to smaller root systems due to pathogen attack, while the decrease in shoot weights is likely to be

linked to smaller root systems being less able to provide the water and nutrients required for the shoot growth.

3.2 The effect of varying pathogen load on severity of Rhizoctonia root rot disease in two soils from the Upper Eyre Peninsula, South Australia

3.2.1 Introduction

There have been no previous published studies with Rhizoctonia inoculum in bioassays using soils from the Eyre Peninsula. Studies in Western Australia linked sandier soils to increased disease severity (Gill et al. 2000).

The main aim of this preliminary experiment was to establish a rate of pathogen inoculum which would result in high disease severity for Eyre Peninsula soils.

3.2.2 Materials and methods

Soils from Streaky Bay and Minnipa were used in a pot bioassay experiment. The bioassay was carried out as described previously (Sections 2.3, 2.4 and watered to weight at 75% water holding capacity as described in Section 3.1) except for the number of Rhizoctonia plugs used. The experimental treatments were 1 plug, 2 plugs and 3 plugs of Rhizoctonia inoculum in each pot. Plugs were cut from full strength agar with a 10mm corer. All treatments were replicated 5 times. Data was analysed in Genstat using AVONA to compare between treatment means.

3.2.3 Results

Overall, in both Minnipa and Streaky Bay soils increasing the pathogen inoculum increased the severity of Rhizoctonia root rot. Disease severity measured on roots grown in Minnipa soil for +1 and +2 plugs of pathogen inoculum was lower than disease severity measured on roots grown in the Streaky Bay soil (Table 3.2). By +3 plugs of pathogen inoculum there was little difference in the severity of disease on roots grown in either soil (Table 3.2). In the Streaky Bay soil, +1 plug of pathogen did not result in disease severity different from +3 plugs of pathogen suggesting that Streaky Bay soil is much more conducive to Rhizoctonia root rot than the Minnipa soil (Table 3.2).

Root dry weights were similar for all pathogen loads despite the different disease severity on plants grown in both Minnipa and Streaky Bay soils (Table 3.2). Shoot dry weight in from plants grown in the Minnipa soil decreased with increasing pathogen load (and increasing disease severity) (Table 3.2). Shoot dry weight from plants grown in the Streaky

Bay soil was higher in the +1 plug treatment than in the +2 or +3 plug treatments (Table 3.2).

Table 3.2: Percent root infection (PRI), root score (0-5 scale where 0 = no disease and 5 = maximum disease) and root (RW) and shoot (SW) dry weights (mg) for plants grown in the Minnipa and Streaky Bay soils. Experimental treatments were +1, +2 or +3 agar plugs of *Rhizoctonia solani* AG8 inoculum. Different letters show significant differences between treatment means using the lsd $_{(0.05)}$. ns represents results which were not significantly different at P=0.05.

Minnipa Soil	PRI	Root Score	RW (mg)	SW (mg)
+1	54 c	1.68 c	126	159 a
+2	68 b	2.32 b	107	125 b
+3	80 a	2.88 a	97	123 b
Lsd $_{(0.05)}$	11	0.42	ns	25
Streaky Bay Soil	PRI	Root Score	RW (mg)	SW (mg)
+1	76	2.58 b	84	142 a
+2	76	3.26 ab	75	94 b
+3	83	3.65 a	78	102 b
Lsd $_{(0.05)}$	ns	0.79	ns	15

3.2.4 Discussion

These preliminary results would suggest that the Streaky Bay soil would fall closer toward the conducive end of the disease suppression scale as all rates of pathogen load result in high disease severity whereas the Minnipa soil has increasing measures of disease severity for all pathogen loads so would be closer to the suppressive end of the scale.

One possible explanation for the greater disease severity in the Streaky Bay soil across all pathogen loads may be due to the sandier texture of this soil. Previous work in Western Australia has linked sandier textured soils to increased disease caused by *Rhizoctonia solani* (Gill et al. 2000).

An alternative possibility is that the Streaky Bay soil has the potential to be suppressive but that expression of disease suppression in the Streaky Bay soil is less than it is in the Minnipa soil. Providing that disease suppression is microbial in nature the difference in response of disease severity to increasing pathogen loads between Streaky Bay and Minnipa soils could be due to a lack of specific suppressive organisms in the Streaky Bay soil or perhaps some limitation suppressive activity of these suppressive organisms preventing them from being suppressive towards Rhizoctonia root rot.

Another explanation for the higher disease severity in the Streaky Bay soil could be due to deficiencies of P, Zn and Cu (Coventry et al. 1998; Holloway et al. 2001; Bertrand et al. 2003) caused by the formation of insoluble complexes with calcium carbonate, which was extremely high in the Streaky Bay soil. Previous studies investigation the interactions between Zn and Rhizoctonia root rot indicated that increasing Zn application to soil decreased Rhizoctonia root rot (Thongbai et al. 1993). Since Zn is one of the nutrients tied up during the formation of insoluble calcium carbonate complexes in highly calcareous soils, it is possible that a deficiency in Zn alone may be having an effect on the severity of Rhizoctonia root rot in the Streaky Bay soil used in this experiment. It is more likely that the formation of insoluble calcium carbonate complexes tied up several nutrients, therefore the effects of multiple nutrient deficiencies on the plant may have been predisposing it to disease severity. For example plants grown in the Streaky Bay soil were smaller, less healthy and likely to be less resilient thus would in turn be more susceptible to pathogen attack than those grown in the Minnipa soil.

Since Minnipa soil had high infection with 3 plugs per pot and low infection with one plug per pot. The moderately high infection measured at 2 plugs per pot was considered ideal as it was not too high or too low, thus it was decided that two pathogen plugs per pot would be used in future bioassay experiments.

Chapter 4
Biotic and abiotic constraints in Eyre Peninsula agricultural soils influence soil-borne disease suppression to *Rhizoctonia solani* AG-8

4. Biotic and abiotic constraints in Eyre Peninsula agricultural soils influence soil-borne disease suppression to *Rhizoctonia solani* AG8

4.1 Introduction

Soils on the upper Eyre Peninsula are reported to have hostile physico-chemical characteristics which are known to cause reduction in crop productivity (Coventry et al. 1998). The nature of this soil hostility includes low organic carbon contents (Rovira 1992; Jeffery et al. 1995 McKenzie et al. 2004) and chemical constraints in surface soils such as high amounts of calcium carbonate which can result in fixation of nutrients like phosphorus, zinc and manganese (Coventry et al. 1998; Holloway et al. 2001), and may cause bicarbonate induced plant growth depressions (Alhendawi et al. 1997). The negative effects of the abiotic constraints in the soil on plant, and in particular root growth (Alhendawi et al. 1997; Holloway et al. 2001 Kopittke et al. 2005), may increase the susceptibility of the plants to soilborne pathogen diseases. Expression of disease suppression is described as a complex of interactions between the soil abiotic and biotic components (Mazzola 2007). It is likely that in this upper EP region one or both factors may be limiting the development and expression of disease suppression due to the hostile nature of the soils. Expression of disease suppression occurs on a sliding scale where expression of disease suppression is not necessarily an “on” or “off” phenomenon.

The overarching hypothesis is that, if these constraints such as low organic carbon, nutrient fixation due to high calcium carbonate contents and bicarbonate induced plant growth depressions are directly affecting plant productivity then it is likely that they are reducing growth, activity and diversity of organisms in soil, including those involved in disease suppression.

The main aim of this work was to investigate the expression of disease suppression to *Rhizoctonia solani* AG8 in selected soils from the upper Eyre Peninsula, a major farming region in southern Australia. Also, to determine the relative importance of biotic and abiotic soil constraints in the plant-soil-pathogen interactions which result in expression of disease.

Two bioassay experiments were undertaken in sequence (Figure 4.1). The first experiment tested the following hypotheses:

1. Suppression of *Rhizoctonia solani* AG8 in soils from three EP locations is absent or reduced compared to a soil from another agricultural region in SA which is suppressive to soil-borne diseases.
2. Biotic components that suppress *Rhizoctonia solani* AG8 are not present or not functional in these EP soils.
3. These EP soils have biotic components that suppress *Rhizoctonia solani* AG8 but suppression is constrained by abiotic components (physical and chemical characteristics of the soils).

The second experiment also addressed the above hypotheses but focussed on the biotic component (microbial community structure as described by PFLA) of soils from three locations on EP and tested the following hypotheses:

1. Microbial community structure of soils from three locations on EP will be similar to each other but differ from that of a soil from another agricultural region in SA which is suppressive to soil-borne diseases.
2. Microbial community structure may rapidly change in response to changes in the soil abiotic matrix.

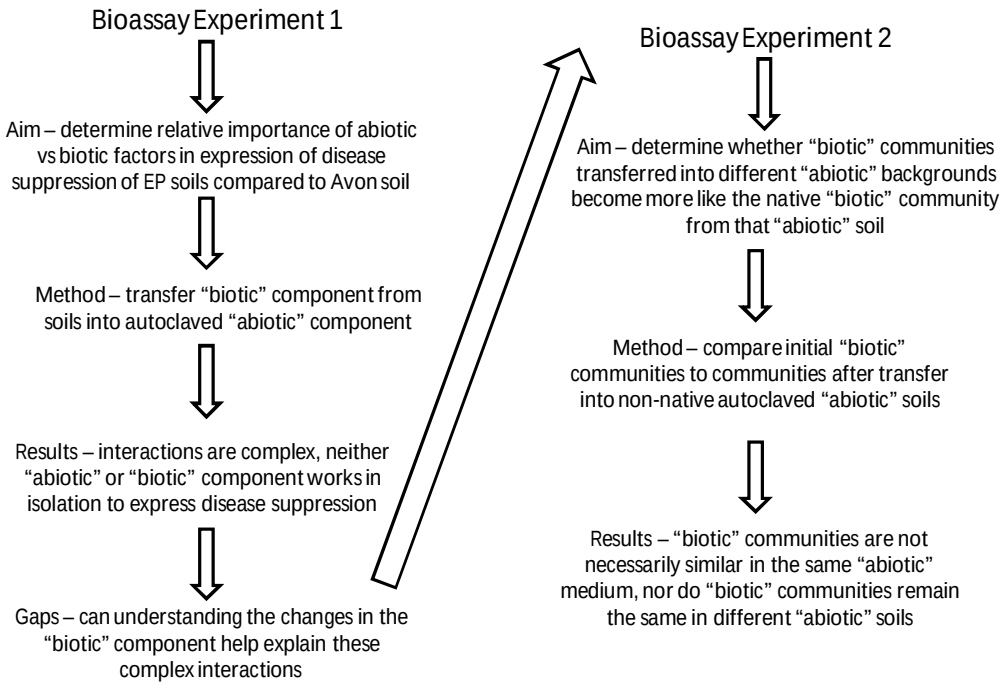


Figure 4.1: Generalised structure of two bioassay experiments conducted in sequence

4.2 Materials and Methods

4.2.1 Soil preparation

Soil collection, descriptions and analysis are summarised in Materials and Methods, Section 2.1. Soils used in the first bioassay experiment were Avon (A), Minnipa (M), Mudamuckla (Mu) and Streaky Bay (SB). Soils used in the second bioassay experiment were Avon (A), Mudamuckla (Mu), Streaky Bay (SB) and Lock (L).

All soils for the abiotic component of the study were autoclaved (1 hour, 121°C) twice, 24 hours apart, before the experiment.

4.2.2 Inoculum preparation

Two types of inoculum were used in this study, a "rhizo-biology" inoculum from each soil (which had not been autoclaved) and the pathogen *Rhizoctonia solani* AG8. The "rhizo-biology" inoculum consisted of dried 14 day old wheat roots plus surrounding rhizosphere soil. It was obtained by growing wheat in a sterile 50ml plastic tube filled with approximately 50g of fresh (non-sterile) soil, planted with 8 surface sterilised and pre-germinated wheat seeds, and maintained at 75% field capacity for 2 weeks in a controlled environment room (15°C and 12 hour day night regime). The soil and root mix was removed intact from each tube, dried at 15°C for 10 days. Root material with some adhering soil particles was gently separated from the rest of the soil, cut into pieces of approximately 5mm and used as the "rhizo-biology" inoculum. The inoculum was added at 1% by weight of air dry soil. For all soils reference to the "rhizo-biology" inoculum was made with a lower case letter (in the first bioassay Avon (a), Minnipa (m), Mudamuckla (mu) and Streaky Bay (sb) and in the second bioassay Avon (a), Mudamuckla (mu), Streaky Bay (sb) and Lock (l)) in order to distinguish biotic from abiotic factors.

Rhizoctonia solani AG8 inoculum was added as two 10mm agar plugs from a *R. solani* colony grown for 4 days on ¼ Potato dextrose agar (PDA). Preparation as described in Materials and Methods, Section 2.3.

4.2.3 Experimental conditions

Both experiments were run in 300ml plots, in a controlled environment room and soil moisture between 50% and 75% soil of water holding capacity, as described in the Materials and Methods, Section 2.2.

4.2.4 Experimental design and statistical analysis

The bioassay experiments were set out as a randomised block design where every treatment was replicated four times. There were two controls, the disease control was autoclaved soil inoculated with pathogen only and the healthy control was autoclaved soil without addition of pathogen or "rhizo-biology". The first bioassay experiment tested Minnipa, Mudamuckla, Streaky Bay and Avon soils.

First bioassay experimental treatments were:

(1) inoculation of autoclaved soil from each location with "rhizo-biology" specific to that same soil,

(2) inoculation of autoclaved soil from each location with Avon "rhizo-biology" known to suppress Rhizoctonia disease,

(3) inoculation of autoclaved Avon soil (known to support suppressive organisms) with "rhizo-biology" specific to soil from each other location, and

(4) inoculation of autoclaved Eyre Peninsula soils, with "rhizo-biology" from each location on the Eyre Peninsula.

The second bioassay experiment treatments were the same as the first bioassay but with different soils (Lock, Mudamuckla, Streaky Bay and Avon).

Pathogen inoculum was added to all of these treatments, as described previously.

For each treatment, data was collected for disease severity as percent root infection and root score and plant dry weight as root and shoot dry weights as described in Section 2.4.4.

Percent root infection, root score, root and shoot dry weight data was analysed in GenStat Tenth Edition using ANOVA. Comparisons between treatment means were made using the lsd at 0.05.

4.2.5 Phospholipid Fatty Acid Extraction and Analysis

Phospholipid fatty acid extraction from soils, fractionation and quantification were based on the technique described by Bardgett et al. (1996). A standard qualitative bacterial methyl ester mix was used to identify separated fatty acid methyl esters, by chromatographic retention time and mass spectral comparison. For each sample abundance of individual fatty acids was expressed on a dry weight of soil basis. Nomenclature of fatty acids was based on fatty acids as described by Frostegard et al. (1993).

Rhizo-biology PLFAs were extracted from a subsample of the rhizo-biology inoculum before it was added to the experimental treatments. PLFAs extracted from biotic and abiotic treatment combinations were taken from sacrificial pots after incubation for 14 days in the CER under the same conditions as the experimental treatments. This time was chosen as it was equivalent to time for incubation with pathogen inoculum before seeds were sown.

PLFA data was analysed using transformed log (x+1) PLFA patterns in PRIMER-E software (Clarke et al. 2006) and plotted using non-metric multidimensional scaling (MDS). A 2D stress <0.2 was considered to represent a good reflection of the overall microbial community structure. PERMANOVA was used to determine the significant differences between treatments (Clarke 1993).

4.3 Results

4.3.1 Bioassay experiment 1

4.3.1.1 Soil physico-chemical (abiotic) properties

All four soils used in the experiment were strongly alkaline having a pH_{H2O} between 8.5-9.00 and moderately saline (Hazelton et al. 2007). They differed in carbonate content (Table 2.2), with Streaky Bay considered extremely calcareous, Mudamuckla strongly calcareous, Avon moderately calcareous and Minnipa weakly calcareous. The Streaky Bay

and Avon soils had a similar organic carbon content, with the Minnipa soil being about 30% lower, and Mudamuckla considerably (60%) less (Table 2.2). There were no apparent macro- or micro-nutrient deficiencies in any of the soils used for the study, although Mudamuckla and Streaky Bay had extractable copper concentrations close to the critical limit of 0.2 mg/kg for soils from this region (King et al. 1975).

4.3.2 Plant dry weight and root disease assessments

Shoot and root dry weights for plants in the healthy control treatments were higher in the Avon soil than any of the EP soils, notably roots from plants grown in the extremely calcareous soil from Streaky Bay had the lowest dry weight (Figure 4.2).

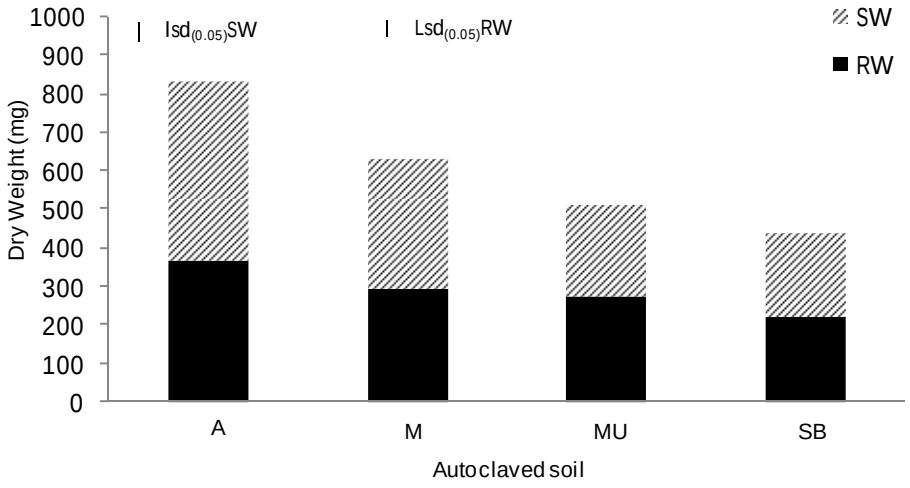


Figure 4.2: Wheat root (RW) and shoot (SW) dry weights (mg) after 28 days growth from the healthy control treatments which was autoclaved soil from the known suppressive soil Avon (A) and the Eyre Peninsula soils Minnipa (M), Mudamuckla (MU) and Streaky Bay (SB).

Plants from the disease control treatment exhibited high levels of root disease (more than 70% root infection with *R. solani*) for all soils, with roots in Minnipa soil having significantly higher percentage root infection with *R. solani* than Streaky Bay (Figure 4.3). Although root disease score for plants across all soils was not significantly different the data followed the same trend as the percentage root infection data (Figure 4.3). Despite the similar Rhizoctonia root disease severity and root dry weights, the shoot dry weight of

plants from the Avon disease control was significantly higher than that of plants in any of the EP soils (Figure 4.3).

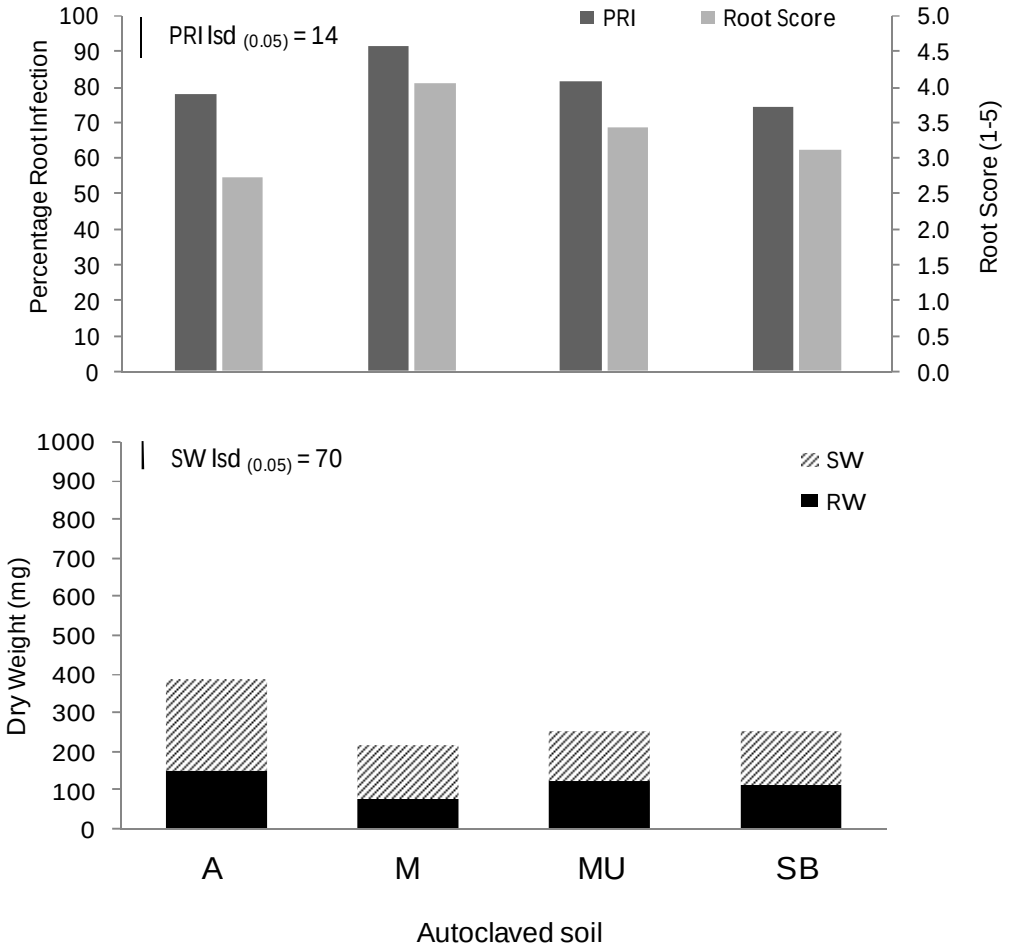


Figure 4.3: Percentage root infection (PRI) and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in the four test soils; the known suppressive Avon soil (A) and the Eyre Peninsula soils Minnipa (M), Mudamuckla (MU) and Streaky Bay (SB). For the disease control treatment which was autoclaved soil inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

Disease severity, assessed by Rhizoctonia root score, for plants grown in Avon soil inoculated with Avon "rhizo-biology" was lower than for plants from either Minnipa or Streaky Bay soils inoculated with their own "rhizo-biology" (Figure 4.4). Percent root infection for the Avon soil inoculated with Avon "rhizo-biology" was 50% compared to more than 60% in the other treatments. Root and shoot dry weights were significantly higher for plants from the Avon soil inoculated with its own "rhizo-biology" compared to those from any of the EP soils inoculated with their own "rhizo-biology" (Figure 4.4).

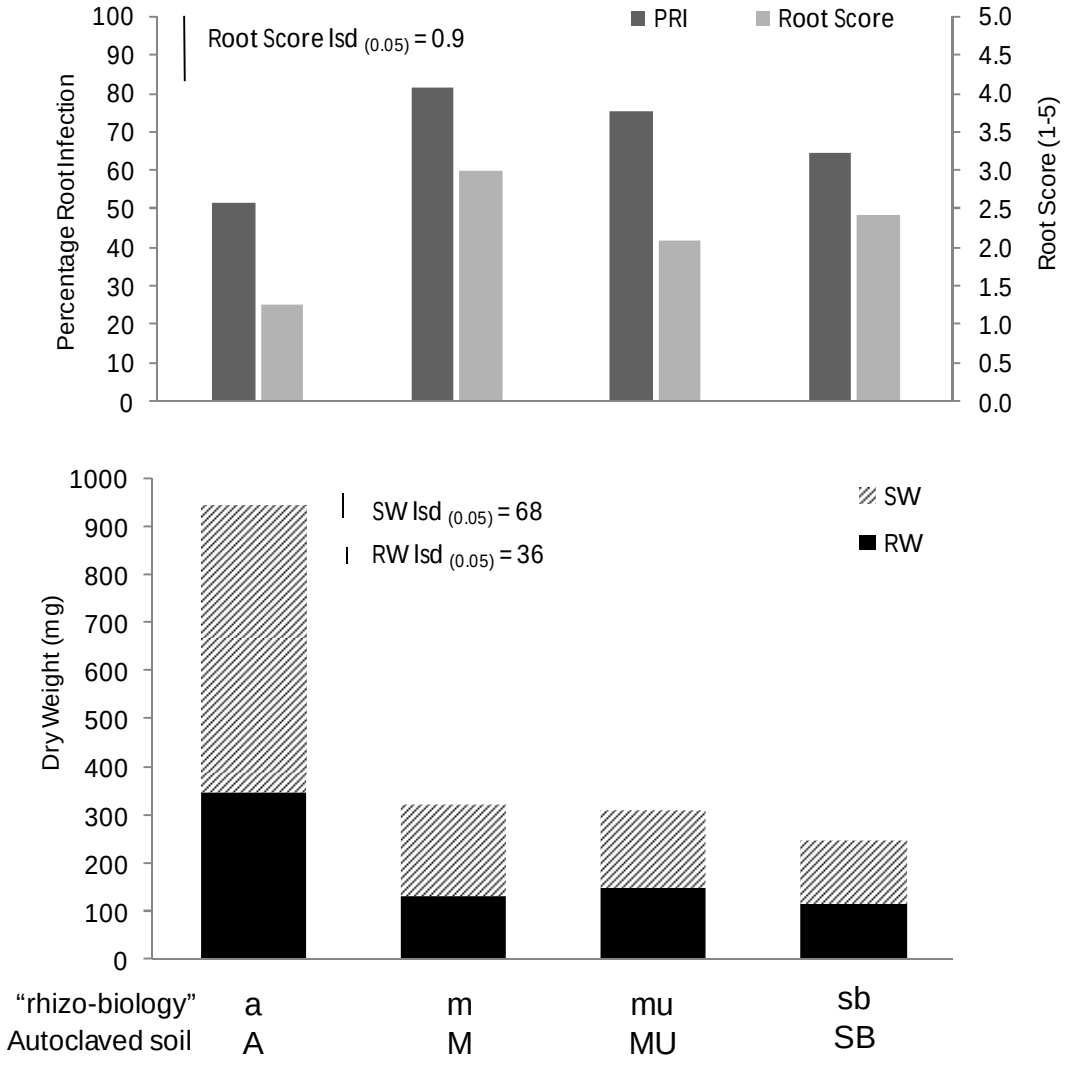


Figure 4.4: Percentage root infection (PRI) and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots containing the four test soils; the known suppressive Avon soil (A) and the Eyre Peninsula soils Minnipa (M), Mudamuckla (MU) and Streaky Bay (SB), for each autoclaved soil inoculated with its own "rhizo-biology" inoculum. All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

Transfer of the suppressive Avon "rhizo-biology" into Avon, Mudamuckla or Streaky Bay soils lowered the Rhizoctonia root score of plants grown in these soils relative to the disease controls, while in the Minnipa soil, root score remained the highest (Figure 4.5). Again, root and shoot dry weights were lower for plants from the three EP soils inoculated with Avon "rhizo-biology" compared to plants from Avon soil inoculated with its own "rhizo-biology" (Figure 4.5).

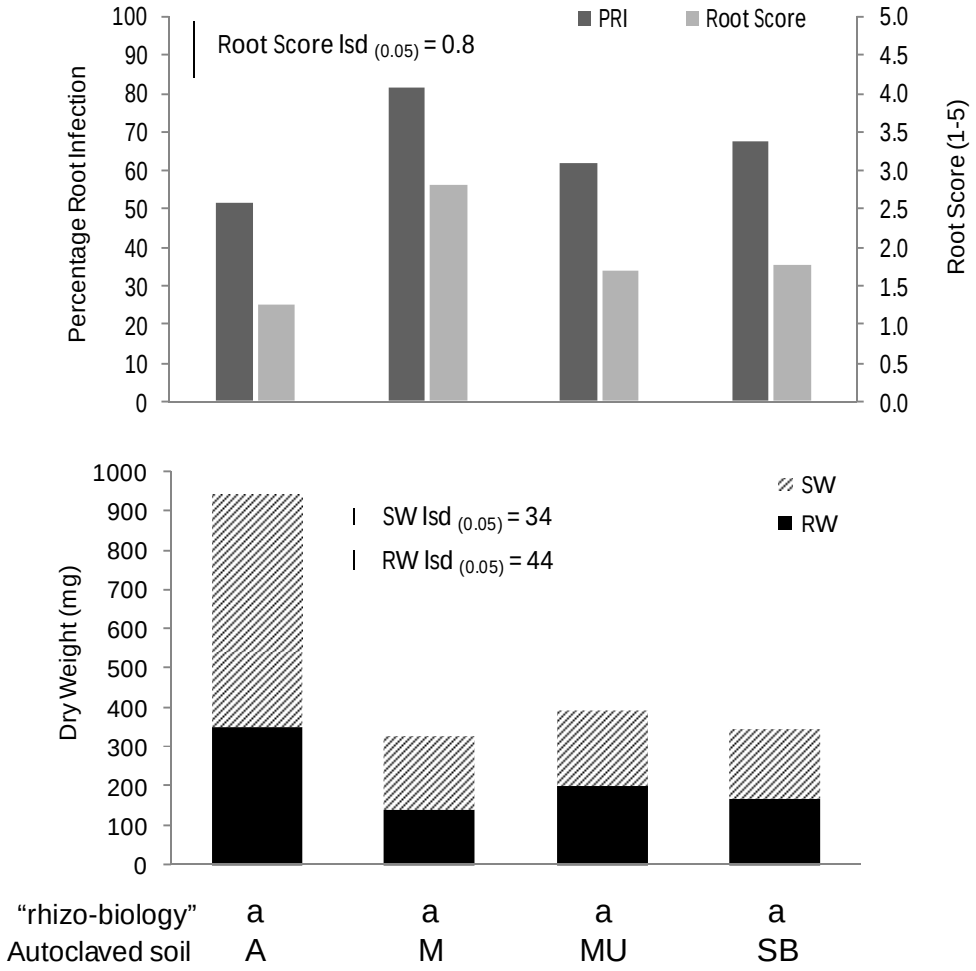


Figure 4.5: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots containing the four test soils; the known suppressive Avon soil (A) and the Eyre Peninsula soils Minnipa (M), Mudamuckla (MU) and Streaky Bay (SB), inoculated with the Avon soil (a) "rhizo-biology". All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

Disease severity (Rhizoctonia root score and PRI) for plant roots from the disease control and from where Minnipa and Mudamuckla "rhizo-biology" had been inoculated into autoclaved Avon soil was higher than the disease severity for plant roots where Avon and Streaky Bay "rhizo-biology" had been inoculated into Avon soil (Figure 4.6). Root and shoot dry weights for the disease control plants were lower than those of plants from the treatments inoculated with "rhizo-biology" (Figure 4.6). Avon soil inoculated with Avon "rhizo-biology" resulted in plants with a higher shoot weight than where the Avon soil had been inoculated with "rhizo-biology" from any of the three EP soils; and a higher root

dry weight than the Avon soil inoculated with Minnipa and Mudamuckla "rhizo-biology" (Figure 4.6).

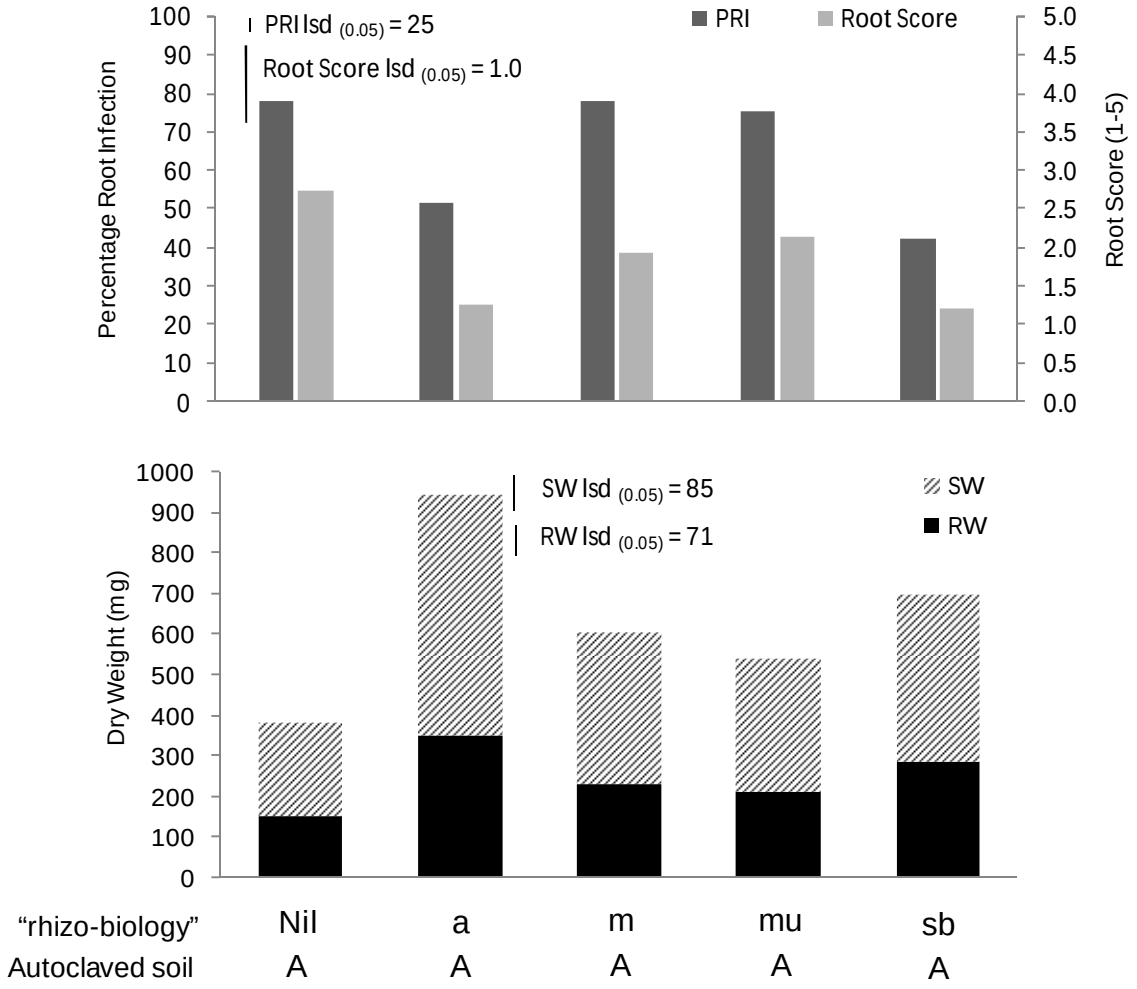


Figure 4.6: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots containing the known suppressive Avon soil (A) inoculated with "rhizo-biology" from the Avon soil (a) and the Eyre Peninsula soils Minnipa (m), Mudamuckla (mu) and Streaky Bay (sb) and the disease control (nil "rhizo-biology"). All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

Percentage root infection, and to some extent root score, tended to be higher for all "rhizo-biology" inoculations into the Minnipa soil compared to the Mudamuckla and Streaky bay soils (Figure 4.7). Minnipa and Streaky Bay "rhizo-biology" inoculated into either Mudamuckla or Streaky Bay soils generally resulted in lower disease severity (especially assessed by root score) than for the disease control or Mudamuckla "rhizo-

biology" inoculated into either Mudamuckla or Streaky Bay soils (Figure 4.7). Root and shoot dry weights tended to be lower in the Streaky Bay soil, except for when it was inoculated with the Minnipa "rhizo-biology" which resulted in higher root and shoot dry weights (Figure 4.7) and a corresponding lower root disease score (Figure 4.7). Minnipa and Streaky Bay "rhizo-biology" inoculated into Mudamuckla soil resulted in plants with higher root and shoot dry weights than the disease control (Figure 4.7). While, the Minnipa soil had similar root and shoot dry weights for plants regardless of the "rhizo-biology" inoculum and lower dry weights in the disease control (Figure 4.7).

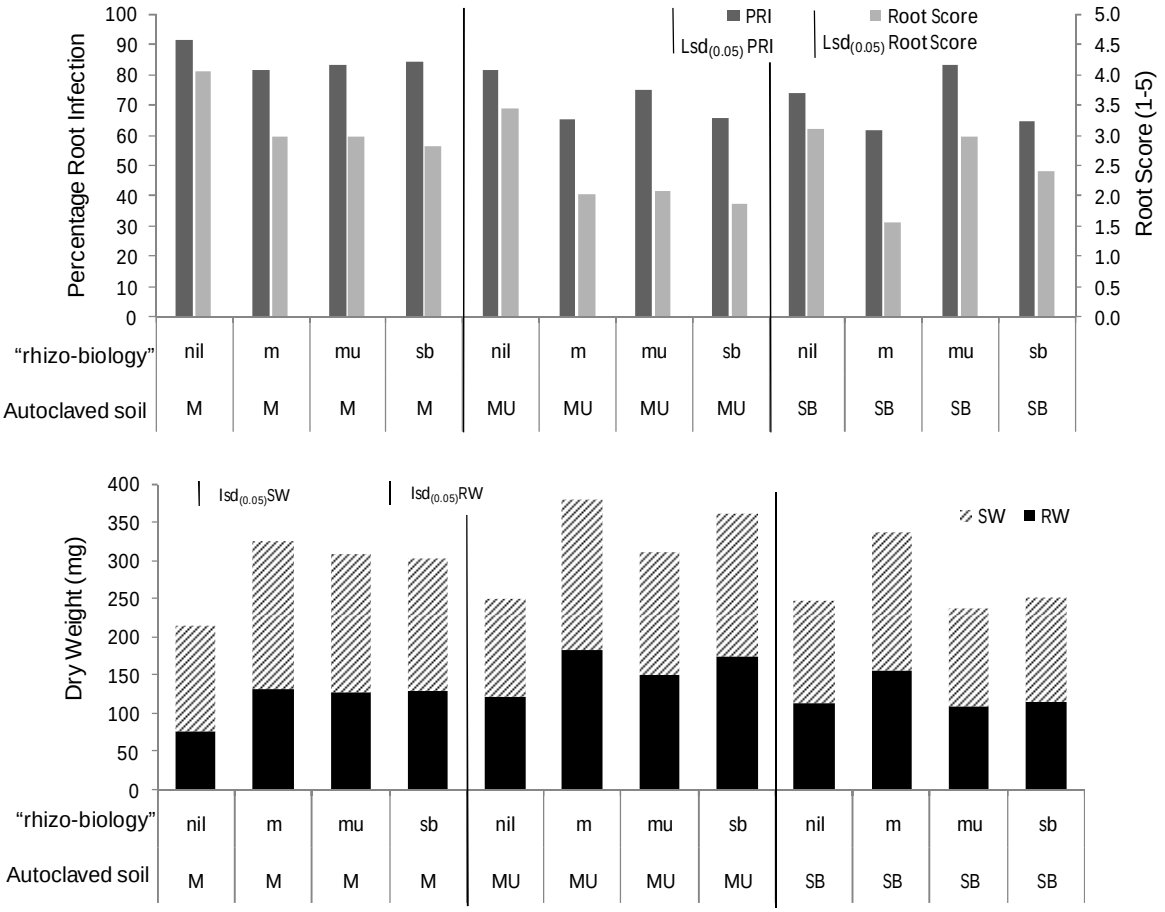


Figure 4.7: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots containing the Eyre Peninsula soils Minnipa (M), Mudamuckla (MU) and Streaky Bay (SB), inoculated with each Eyre Peninsula soils "rhizo-biology" (m, mu and sb) and the disease control (nil "rhizo-biology") for each soil. All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

4.3.3 Bioassay Experiment 2

Abnormality in root growth was observed for the plants grown in Mudamuckla soils in this second bioassay experiment and thus disease could not be assessed so the data is not reported. Dry matter for plants in the Avon soil was lower in this experiment (Table 4.1) compared to previous data although PRI and root score were similar and indicative of suppression of Rhizoctonia root rot. The SB2 soil was not the same batch as the first bioassay (it was collected in the growing season, see SB2, Chapter 2) and caused greater suppression than the first batch, in fact similar to the Avon soil, although dry matter production was less than in Avon soil (Table 4.1). The Lock soil with a PRI of 59 was less suppressive than Avon or SB2 but still supported better growth than SB2.

Table 4.1: Percent root infection (PRI), root score and root (RW) and shoot (SW) dry weights (mg) from 28 day old wheat plants grown in each autoclaved soil (Avon, Lock and Streaky Bay 2 (SB2)) inoculated with that soils own "rhizo-biology" inoculum (avon, lock and streaky bay 2 (sb2)). All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum.

Soil	"rhizo-biology"	PRI	Root Score	RW (mg)	SW (mg)
Avon	avon	43	1.6	158	222
Lock	lock	59	2.3	133	202
SB2	sb2	44	1.7	159	224

Of more interest in this bioassay were the PLFA analyses of the biotic components taken as the rhizospheres of plants grown in the soils and then following inoculation of those rhizosphere soils (biotic components) into abiotic matrices. Fatty acid analysis on the "rhizo-biology" inoculum shows that the Mudamuckla "rhizo-biology" is different from Avon and Streaky Bay "rhizo-biology" at a P value of 0.032 for the Bray Curtis similarity (Figure 4.8). The main discriminatory fatty acids were C18:2 ω6c (ectomycorrhizal fungi), C17:0 (Gram positive bacteria), C15:0 (non-specific) and C16:1 ω7c (Gram negative bacteria) and C 20:2 ω6c, C20:3 ω6c (protozoa) together contributing 90% of the difference between Mudamuckla compared to Avon and Streaky Bay "rhizo-biologies".

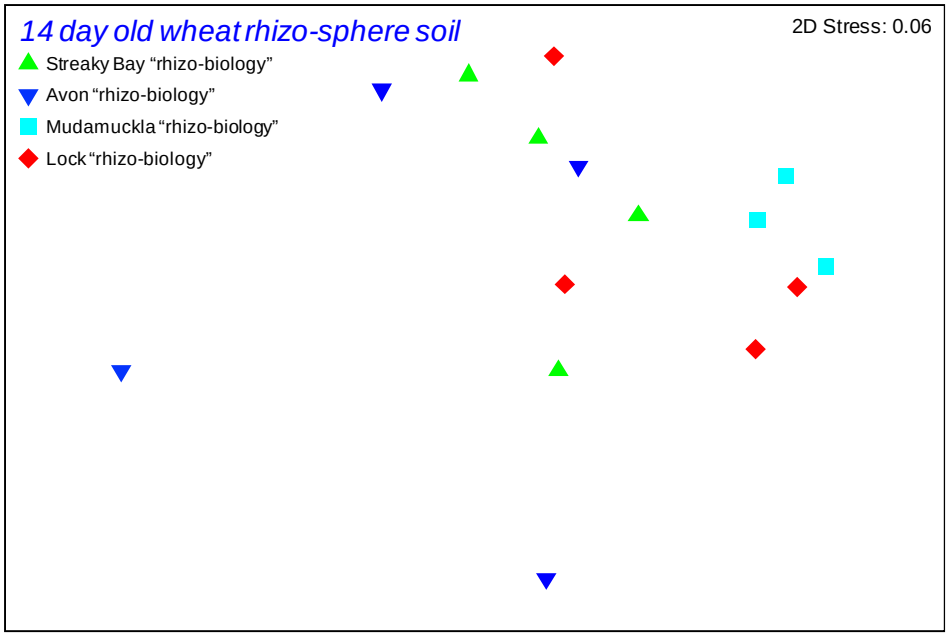


Figure 4.8: Bray Curtis similarity for phospholipid fatty acid extraction from the "rhizo-biology" inoculum, which was rhizo-sphere soil from wheat plants that had been grown in each of the test soils Streaky Bay 2, Avon, Mudamuckla and Lock for 10 days.

Data from analysis of the fatty acids shows that Avon soil inoculated with avon "rhizo-biology" and Lock soil inoculated with avon "rhizo-biology" were different from Avon "rhizo-biology" inoculum (Figure 4.9). However, disease severity for Avon soil inoculated with avon "rhizo-biology" and Lock soil inoculated with avon "rhizo-biology" were similar for PRI and root score, while disease severity in the Streaky Bay soil inoculated with avon "rhizo-biology" was lower (Figure 4.10). Correspondingly, root and shoot dry weights were lower in the two treatments with higher disease severity and higher in the Streaky bay soil inoculated with avon "rhizo-biology" which had lower disease severity (Figure 4.10).

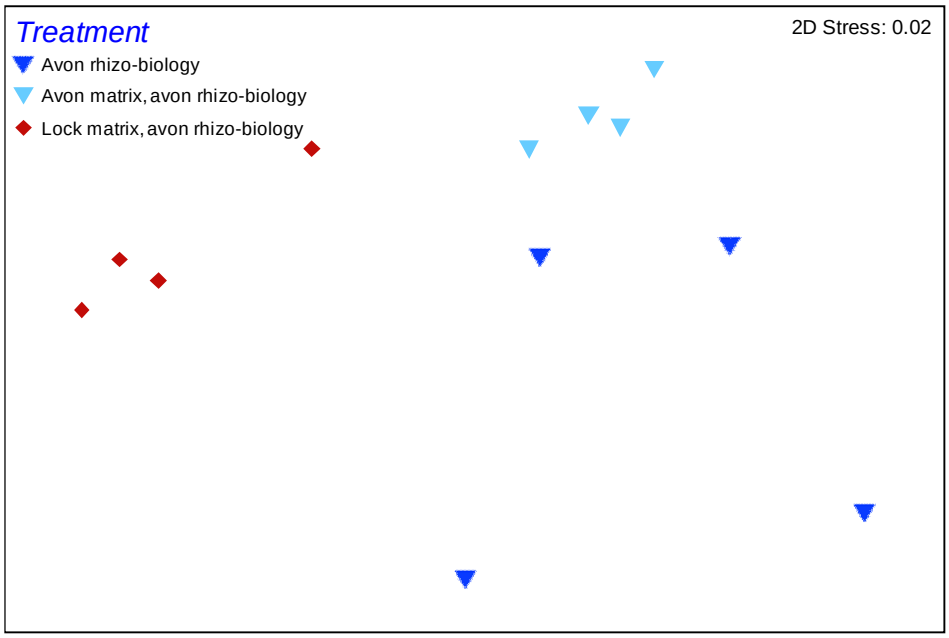


Figure 4.9: Bray Curtis similarity for phospholipid fatty acid extraction from the "rhizo-biology" inoculum for the avon test soil, the Avon matrix with avon "rhizo-biology" and Lock matrix with avon "rhizo-biology" extracted two weeks after inoculation with the avon "rhizo-biology" inoculum.

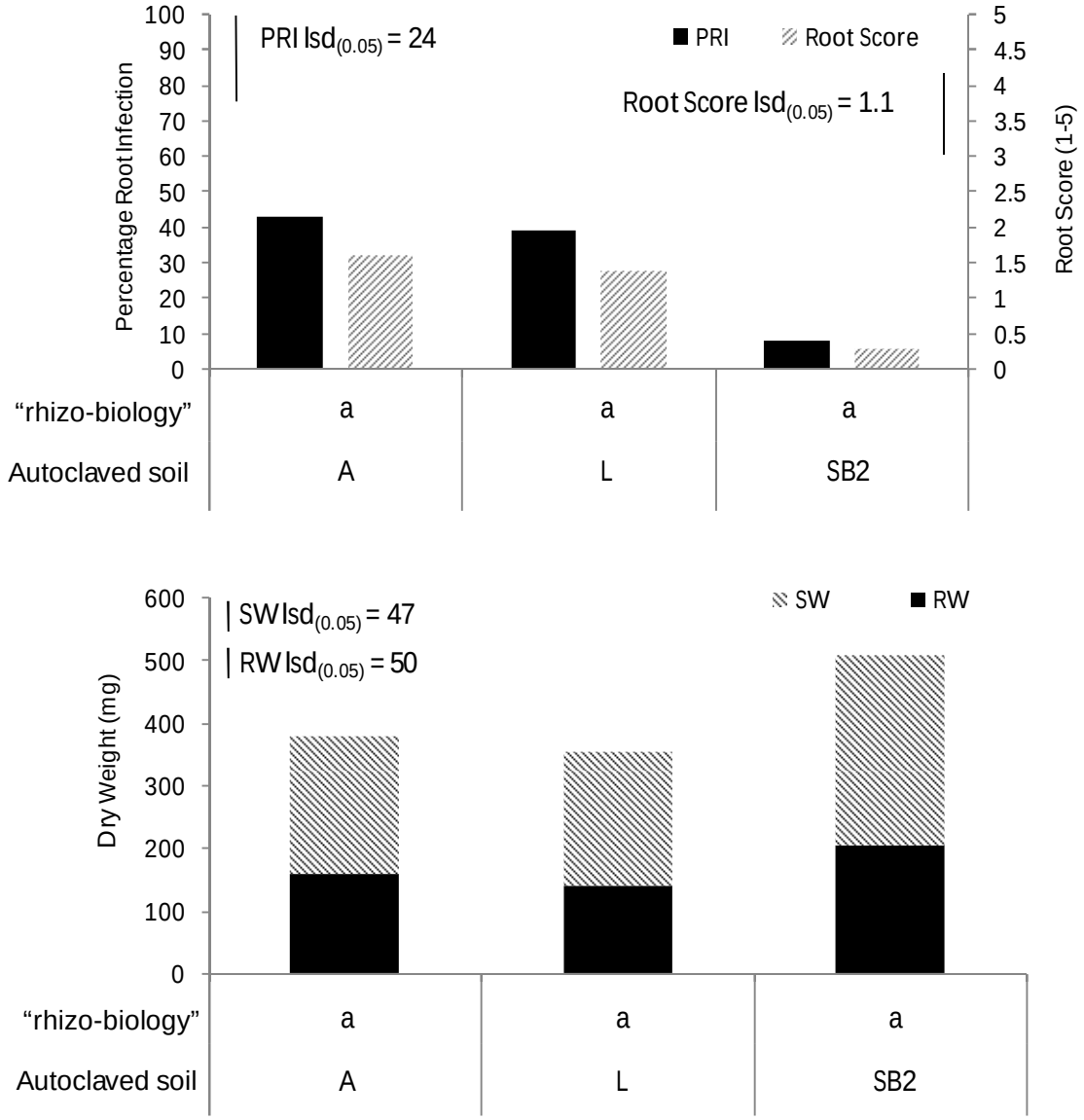


Figure 4.10: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 from the three test soils; the known suppressive Avon soil (A) and the Eyre Peninsula soils Lock (L) and Streaky Bay (SB2), for each test soil inoculated with the Avon soil (a) "rhizo-biology".

The initial avon "rhizo-biology" inoculum had different PLFA similarity from the Avon soil inoculated with avon "rhizo-biology" and Avon soil inoculated with lock "rhizo-biology" (Figure 4.11). The community profiles from Avon soil inoculated with avon "rhizo-biology" and Avon soil inoculated with lock "rhizo-biology" were also different from one another (Figure 4.11). Despite these differences in microbial community fatty acid profiles, the disease severity (PRI and Root score) as well as root and shoot dry weights for Avon soil

inoculated with avon “rhizo-biology" and Avon soil inoculated with lock “rhizo-biology” were not different from each other (Figure 4.12). Although the fatty acid profile for Avon soil with streaky bay “rhizo-biology” was not different from the other treatments analysed (Figure 4.11), the disease severity for this treatment and the Avon soil with mudamuckla “rhizo-biology” was higher for both PRI and root score than the Avon soil with either avon or lock “rhizo-biology” inoculum (Figure 4.12). As the disease severity of these treatments increased, the root and shoot dry weights were correspondingly lower than those from the avon and lock “rhizo-biology” treatments (Figure 4.12).

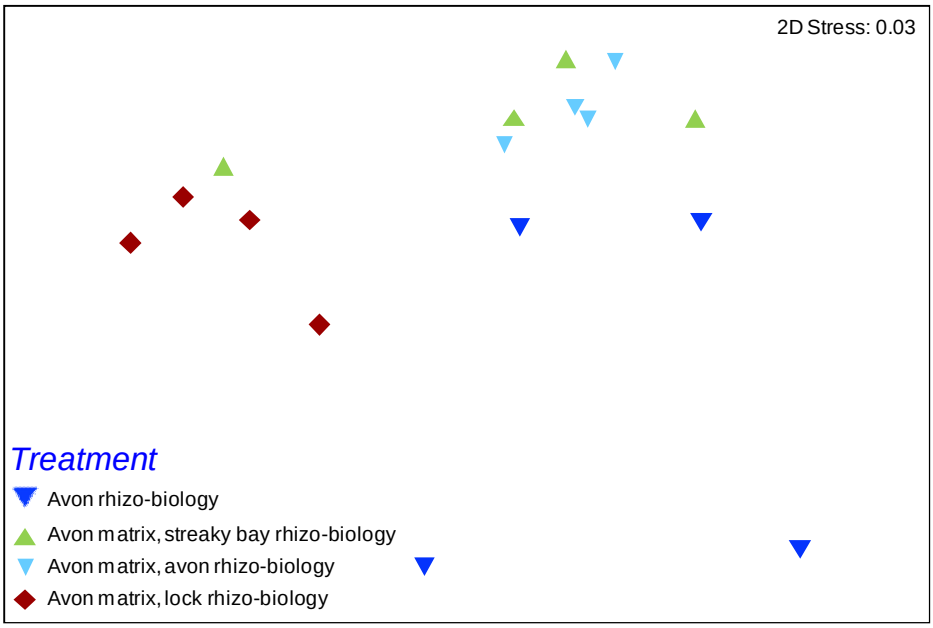


Figure 4.11: Bray Curtis similarity for phospholipid fatty acid extraction from the “rhizo-biology” inoculum for the avon test soils, the Avon matrix with streaky bay, avon and lock “rhizo-biology” extracted two weeks after inoculation with each of the “rhizo-biology” inoculums.

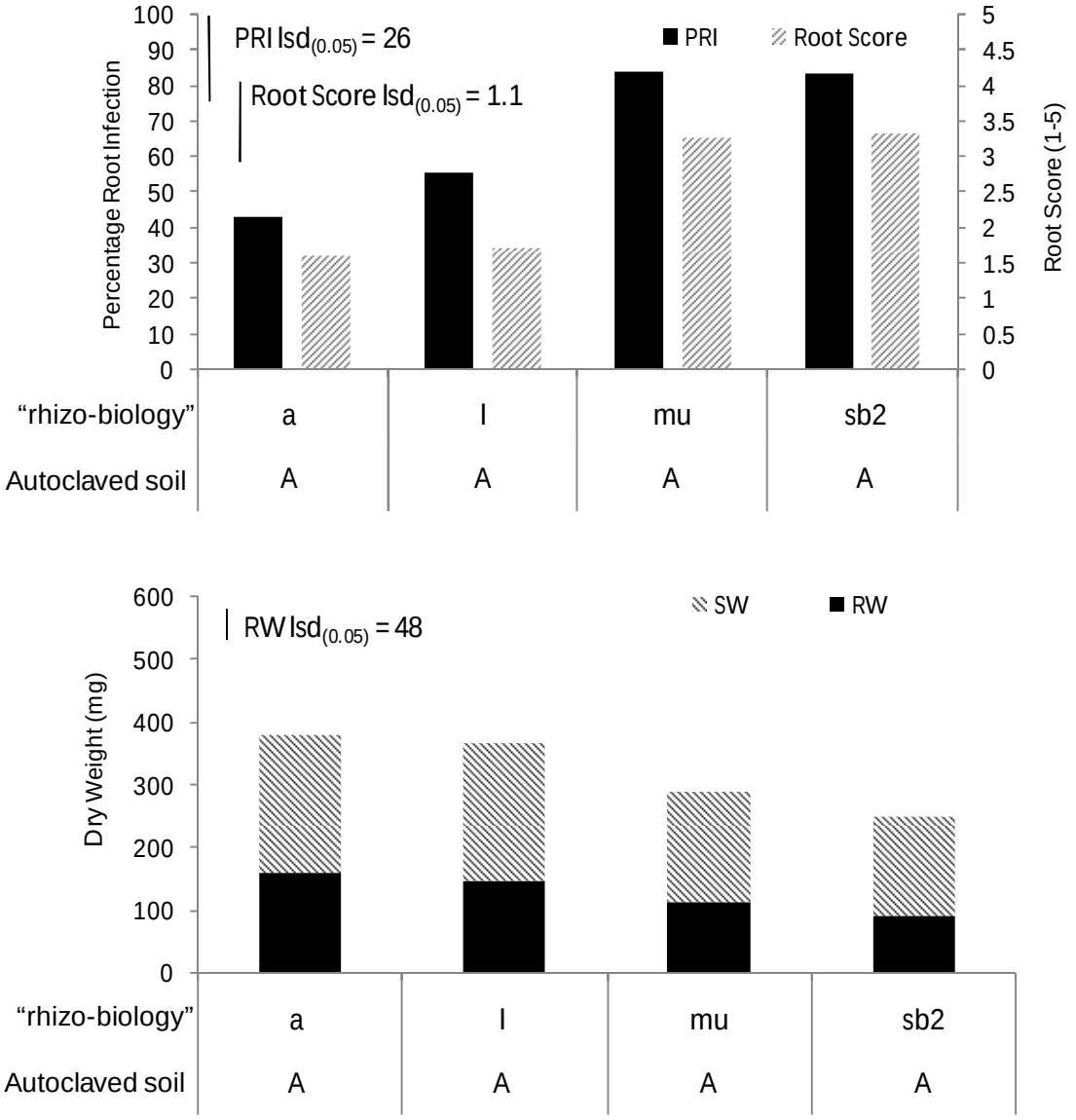


Figure 4.12: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root (RW) and shoot (SW) dry weights (mg) after 28 days growth in pots inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 from the four test soils; the known suppressive Avon soil inoculated with "rhizo-biology" from the Avon soil (a) and the Eyre Peninsula soils Lock (l), Mudamuckla (mu) and Streaky Bay 2 (sb2).

For each Lock and Streaky bay 2 soils inoculated with "rhizo-biology" from each of the EP soils, there was no significant interaction between soil and "rhizo-biology" for all the parameters measured, but for all parameters the main effect of soil was significant (Figure 4.13). Streaky bay 2 soil had lower average disease severity than Lock soil, and had higher average root and shoot dry weights than the Lock soil (Figure 4.13).

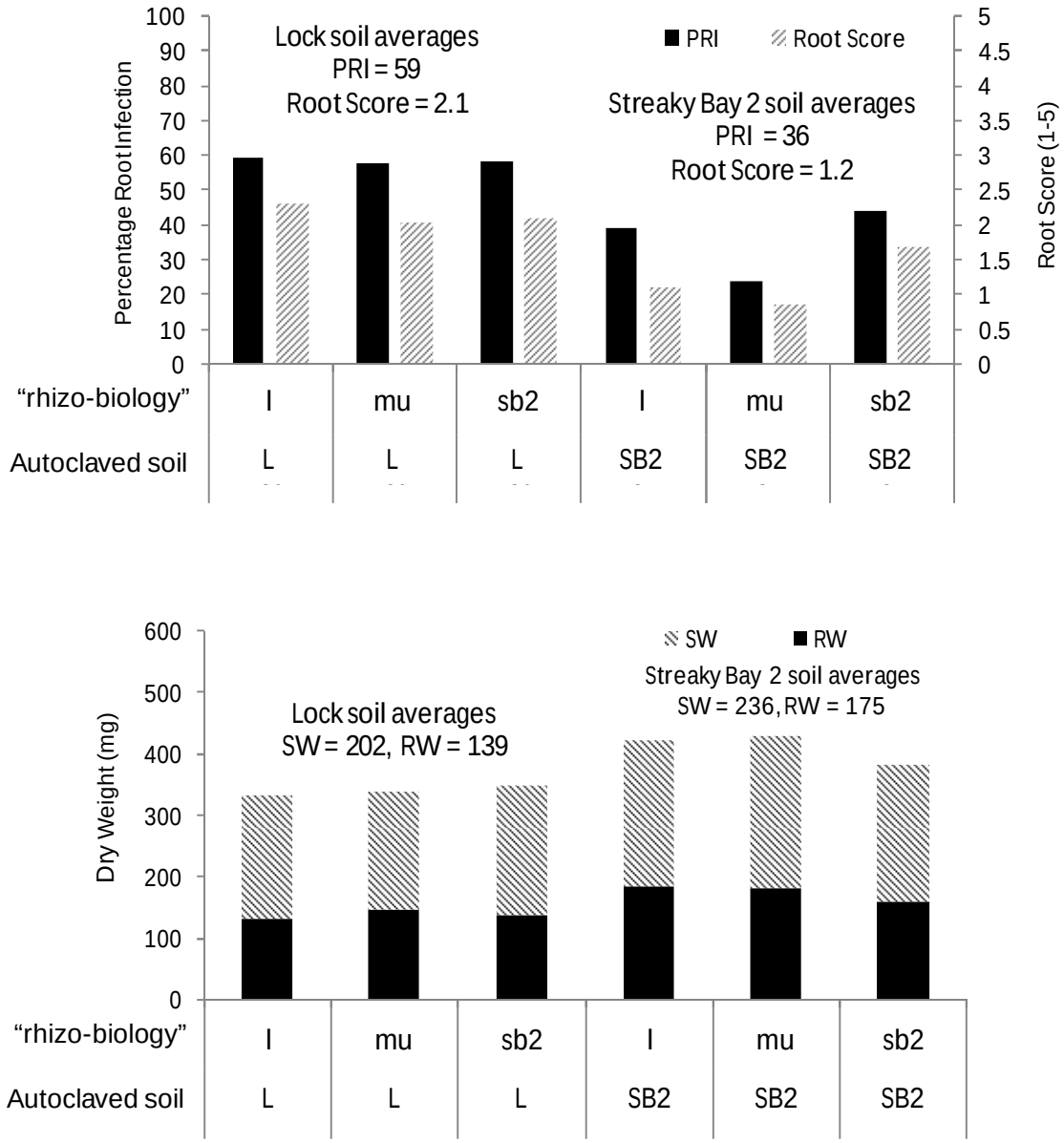


Figure 4.13: Percentage root infection (PRI), and root score (1-5 scale) for wheat roots and root and shoot dry weights (mg) after 28 days growth in pots inoculated with *Rhizoctonia solani* AG8 from the Eyre Peninsula soils Lock (L) and Streaky Bay (SB2), inoculated with each Eyre Peninsula soils lock (l), Mudamuckla (mu) and Streaky Bay 2 (sb2) "rhizo-biology" .

4.4 Discussion

Overall the results from the first bioassay indicate that the assessed Eyre Peninsula soils were not as suppressive towards Rhizoctonia root rot as the Avon soil. However, the level of disease suppression depended on the specific soil abiotic and biotic conditions for each scenario tested. There are differences between the three soils with respect to the major interactions that are limiting expression of disease suppression, as discussed below. Furthermore, these results suggest some biotic potential to develop biological suppression to *R. solani* even in the constrained abiotic environment of an extremely calcareous soil. These results highlight the complexity of biotic-abiotic interactions involved in the plant-pathogen-soil continuum.

Disease severity from each soils "rhizo-biology" inoculated into its own soil matrix suggest that though some level of disease suppression was observed in EP soils, the level of suppression was less than that observed in Avon soil. Although it is known that varying soil types have an inherently different expression of disease severity for the same amount of pathogen inoculum (Roget et al. 1999, Section 3.2). The disease control data from the first bioassay suggests that despite the differences in soil characteristics, the severity of disease was not limited by soil physical and chemical characteristics in any of the soils used. Differences in disease suppression can therefore be attributed to inoculation with "rhizo-biology", specifically differences in a) composition of microbial communities, b) functional ability of the microbial community (expression). The microbial community structure and function is likely to have been influenced by crop history, and the soil physical and chemical properties. Although, the crop history for all the soils used in this work was at least one season of wheat, it is probable that the wheat varieties were different. Previous studies of the effect of different wheat cultivars grown in the same soil found that *Pseudomonads fluorescens* populations and subsequent disease suppression varied dependent on which cultivar was sown (Mazzola et al. 2002b; Gu et al. 2003). Therefore it is reasonable to assume that there was in part some a differential effect of wheat variety on the microbial community structure in these soils. Soil physical characteristics are known to influence microbial community structure and function, and have been linked specifically to disease suppression. For example high clay and organic matter contents have been reported to decrease disease severity of *Fusarium* wilt indirectly, via biologically mediated disease suppression (Larkin et al. 2002). Soil chemical

properties, are known to influence microbial community structure, function and activity, including those involved in disease suppression (Garbeva et al. 2004; Garbeva et al. 2008). For example availability of Zinc is known to reduce disease severity of Rhizoctonia root rot (Thongbai et al. 1993). It is highly likely that in this bioassay, crop history influenced the soil microbial community structures, which were in turn, influenced by the effect of the individual soil physical and chemical characteristics which then via a complex of interactions contributed to the differences in disease severity observed.

Lower disease severity obtained from the transfer of the suppressive Avon "rhizo-biology" into each of the autoclaved EP soils, suggests that both Mudamuckla and Streaky Bay soils have abiotic characteristics able to support the expression of biological disease suppression. It is interesting here to consider one of the factors reputed to be a driver for disease suppression in soils, namely organic carbon (quantity and quality), and to note that organic carbon was lowest in the Mudamuckla soil, in fact only 0.6% compared to 1.7 % for Streaky Bay. Suggesting that the amount of carbon may not be as crucial as carbon type and its availability to the micro-organisms involved in disease suppression. Research on Western Australian soils found that although microbial community size was related to total amount of soil organic matter, the structure and function of the microbial community was influenced by both total amount of soil organic matter and the quality of the organic matter (Murphy et al. 2011). Perhaps in these soils, it is the quality and availability of the soil carbon that influence disease suppression rather than the total amount of organic carbon in the soil. It is not clear why the Minnipa soil did not support some disease suppressive activity by the Avon biota since it has apparently less abiotic constraints, at least to plant growth, than the other two EP soils and an organic carbon content higher than Mudamuckla. It is reasonable to assume that in this experiment organic carbon alone is not limiting at least the expression of biological disease suppression, implying that the two more calcareous soils from EP do have the potential to support organisms involved in biological disease suppression.

The PRI and root disease score of plants following inoculation of "rhizo-biology" from the Eyre Peninsula soils into the Avon soil suggest that Streaky Bay has a biotic component that is capable of suppressing Rhizoctonia root rot. It appears that despite the SB soil having (a) a biotic component capable of decreasing disease severity in the abiotic matrix

of another soil (Avon) and (b) an abiotic matrix able to support disease suppressive biota from another soil (Avon "rhizo-biology"), the SB abiotic and biotic soil components are unable to function together to suppress expression of the disease. One reason for this could be that the suppressive component of the SB biota was not constrained in the less hostile Avon soil due to increased plant health, growth and rhizo-deposition. Plant differences in rhizo-deposition are known to result in variations in rhizosphere communities through the release of secondary metabolites (Bais et al. 2006). It is assumed that plant health influences the quantity and quality of rhizo-deposition (via secondary metabolites). Therefore differences in health of the plants in Avon (healthy) versus EP soils (less healthy) may have affected the rhizo-deposition and modified the disease suppressive communities. It is known that healthier plants can withstand disease better than less healthy plants (Bais et al. 2006), so those plants grown in the Avon soil will be better able to cope with disease compared to those from the EP soils. Rhizo-deposition is also influenced by soil type (Bais et al. 2006). Thus, it is proposed that the combination of healthier plants and soil type differences are altering the expression of disease suppression via the secondary metabolites released during rhizo-deposition. The situation is different for the Avon biota that appear to exhibit strongly developed suppressive function so are able to suppress *R. solani* infection even in plants grown on the constrained abiotic matrix of the SB soil. Clearly it is not just the soil biotic and abiotic components alone that influence disease suppression, but also the interactions between the plant and the soil, as well as the plant and the pathogen.

Furthermore, irrespective of "rhizo-biology" treatments in the Minnipa soil, there was a greater disease severity than for any EP "rhizo-biology" in the other EP soils compared to the disease control, which contradicts other published data suggesting that Minnipa soil is disease suppressive (Cook et al. 2007). One possible reason for this may be the specific impact of Minnipa soil on rhizo-deposition and its influence on the rhizosphere micro-organisms. Soil type is known to impact rhizo-deposition of secondary metabolites (Bais et al. 2006). These metabolites can be linked to growth or inhibition of specific micro-organisms in the rhizosphere (Bais et al. 2006). Although no specific organisms or groups of organisms were quantified in the work. In this chapter, it is possible that rhizo-deposition of secondary metabolites was influenced by the abiotic characteristics of the Minnipa soil. This may then have had a negative impact on the ecosystem functioning of

the rhizo-sphere microorganisms, such that irrespective of the "rhizo-biology" treatments in the Minnipa soil expression of disease suppression was limited. Another reason for this may be that the amount of pathogen inoculum in the pot system was excessively high. The Minnipa soil disease control appeared to be the most conducive to Rhizoctonia root rot, this may have overwhelmed the ability of each "rhizo-biology" to express disease suppression.

The results from bioassay 2 suggest that the three EP soils investigated (Lock, Mudamuckla and Streaky Bay 2) had a similar level of expression of disease suppression to the Avon soil. Neither abiotic nor biotic characteristics in isolation appeared to be limiting expression of disease suppression. Expression of disease suppression after inoculation of "rhizo-biology" into each soil matrix, was sometimes altered and appears to be dependent on the specific combination of "rhizo-biology" and soil matrix.

As seen by the different PLFA profiles for each soil in this experiment, the formation of a microbial community structure is complex and involves many factors and processes. Characteristics such as soil structure, texture and soil pH (Garbeva et al. 2004; Mazzola 2007) directly influence microbial communities by provision of niche micro-habitats and differential supply of nutrients. Indirectly, soil type and crop history affects rhizo-deposition of secondary metabolites. It is known that secondary metabolites have an impact on the selection of rhizo-sphere organisms and therefore will impact microbial community structure (Bais et al. 2006; Mazzola 2007).

When the avon "rhizo-biology" was inoculated into different autoclaved soil matrices, the two week incubation period altered the PLFA profile compared to that from the initial Avon rhizo-biology. The original "rhizo-biology" consisted almost solely of rhizo-sphere soil, which is likely to have a community structure determined by the micro-environment of the root zone. The root zone is a nutrient and carbon rich zone which has higher microbial activity and diversity compared to the bulk soil (Bowen et al. 1999). Once the "rhizo-biology" was inoculated into bulk soil and incubated for two weeks, there was no rhizo-deposition. Thus the soil specific abiotic characteristics may have more effect on survival of the organisms from the initial "rhizo-biology" inoculum. For example by provision of micro-habitats for the organisms, as well as essential nutrients like carbon

and phosphorus although these will be in smaller concentrations compared than if there was a plant root present.

Conversely, when Avon soil was inoculated with Avon or Lock "rhizo-biology", the lock "rhizo-biology" did not become more like the Avon "rhizo-biology" during the two weeks incubation due to the characteristics of the Avon soil, nor were either like the initial Avon "rhizo-biology" inoculum. It appears that in these treatments the interaction between initial "rhizo-biology" inoculum and abiotic characteristics was more influential in the microbial community structure as shown by the PLFA profile. This data highlights the complexity involved in expression of disease suppression, and that interactions between the abiotic and biotic components of a soil system are specific to each combination of the soil matrix and soil biology. In some combinations it may be aspects of the soil matrix preventing the expression of disease suppression while in others it may be a lack of suppressive organisms within the soil biology.

Despite the differences in PLFA profiles (community structures) these treatments had similar amounts of disease severity. Marschner et al. (2003) demonstrated that despite differences in microbial community structure, there were not corresponding changes in enzyme activities nor were there changes in functional diversity. It is likely that the ability of different microorganisms to carry out the same function (functional redundancy) is one mechanism by which disease suppression is still expressed in these treatments despite the difference in community structures determined by this PLFA analysis. Another mechanism may be that expression of disease suppression is operating at different points along the pathogen infection pathway for example via resource competition, antagonism or parasitism (Baker 1968). Studies on suppression of Take-all in Montana soils showed that in one soil mycoparasitism was the main mechanism behind suppression of take-all, while in the other soil it was antibiosis and possibly siderophore production (Orlando et al. 1994). Although, the PLFA technique does not isolate all soil organisms specific to disease suppression, nor does it give an indication of the general microbial activity of the soil organisms. It is concluded from the work in this chapter that the via the combination of soil abiotic characteristics and the initial "rhizo-biology" inoculum, expression of disease suppression between the different soil matrices is operating at different points along the infection pathway in combination with functional redundancy.

Chapter 5
Soil-borne disease suppression: influence of carbon additions in three Australian soils on populations of specific organisms linked to suppression of Rhizoctonia root rot

5. Soil-borne disease suppression: Influence of carbon additions in three Australian soils on populations of specific organisms linked to suppression of Rhizoctonia root rot.

5.1 Introduction

Rhizoctonia solani AG8 causes root disease in semi-arid farming systems in Australia and North Western USA in cereal crops (MacNish 1983; Rovira 1992; Paulitz 2006). In Australia, *Rhizoctonia solani* AG8 is the main causal agent of Rhizoctonia root rot and is a severe production constraint for many of the dry-land cereal production regions (Rovira 1986; Rovira 1992). The Eyre Peninsula in South Australia is a cereal production region which due to its climatic and edaphic constraints is particularly vulnerable to Rhizoctonia root rot. In the northern part of the Eyre Peninsula (approx 1million ha in cereal production (PIRSA 2009)), it has been estimated that Rhizoctonia root rot could reduce yields by up to 60% (Crouch et al. 2005). Conservative estimations suggest production losses from Rhizoctonia in wheat of AU$35 million per annum for the cereal regions of Australia (Brennan et al. 1998).

At a long term field site at Avon, a climatic region of South Australia comparable to the Eyre Peninsula, a similarly high incidence of Rhizoctonia root rot declined over a period of 10 years in a long-term farming system experiment (Roget 1995). Further research demonstrated that this decline was due to biological disease suppression, where the soil microflora changed over time from a non-suppressive suite of organisms to one that could suppress Rhizoctonia root rot disease (Wiseman et al. 1996; Gupta et al. 1999a). Specific soil microorganisms (*Exiguobacterium acetylicum*, *Pantoea agglomerans*, *Microbacterium* spp.) have been isolated from this site and these can act in combination to decrease the incidence of Rhizoctonia root rot in bioassays (Barnett et al. 2006). The development of disease suppression is hypothesized to be driven by an increase in biologically available carbon inputs over time which alter the composition and activities of microbial community (Roget et al. 1999; Gupta et al. 1999a).

Organic carbon input additions are widely recognised in many agricultural systems as contributing to disease suppression for a variety of soilborne pathogens, including

Rhizoctonia solani (Bonanomi et al. 2007). Organic carbon values for EP soils are generally low (e.g. <1.5%), as are the organic carbon inputs (<1 t per ha per year) into these farming systems (Rovira 1992; Coventry et al. 1998). If as suggested above, disease suppression is biologically driven by increasing carbon inputs then, in many of the Eyre Peninsula soils, organic carbon may be the main factor limiting the development of disease suppression in these soils.

Although it is proposed that the main limitation for biologically mediated disease suppression in EP soils is the amount of organic carbon available for biological activities, it is important to recognise the complexity of the interactions between biotic and abiotic factors that result in disease suppression (Mazzola 2007). To date there have been only a few studies investigating the extent of disease suppression specifically in EP soils (Roget et al. 1999).

In this study it was hypothesised that addition of organic carbon sources representative of the main type of carbon inputs in agricultural systems to selected Eyre Peninsula soils would decrease the incidence of Rhizoctonia root rot via biologically mediated disease suppression. More specifically this study aimed to investigate if (i) the addition of either young root carbon (narrow C:nutrient ratio) or cut wheat-stubble (wide C:nutrient ratio) to the selected EP soils would decrease root disease severity, (ii) these two carbon sources would decrease root disease severity by different amounts and (iii) the disease incidence would be correlated to presence and abundance of specific organisms previously linked to disease suppression (as measured by specific DNA tests for these organisms).

5.2 Materials and Methods

5.2.1 Soil sampling and preparation

Three 'non-suppressive' soils were selected using data from a previous bioassay conducted to evaluate disease suppression in a range of Eyre Peninsula soils (Cook et al. 2007). Soil samples were collected from three farmer fields on the Eyre Peninsula, South Australia, which experiences a Mediterranean climate, with hot dry summers and cool moist winters. Soils were collected in March 2007 by random sampling from the top 10cm across each paddock and were then air dried and sieved through a 2mm sieve. A sub-

sample of this soil was then sent to a commercial testing laboratory (CSBP Soil Analysis Laboratory, 2 Altona Street, Bibra Lake, WA, 6163, Australia) for analysis for extractable nitrate-N, ammonium-N (Searle 1984), Colwell available potassium and phosphorus (Colwell 1963), percent calcium carbonate as determined by Martin et al. (1955) and percent organic carbon (Walkley et al. 1934), results are summarised in Table 5.1. The soil collection sites were located close to Kimba (33° 10.110'S, 136° 23.51'E), a slightly calcareous dark reddish brown loam fine sand (Jeffery et al. 1995), Mount Damper (33° 04.159'S, 135° 03.584'E), a non calcareous clay loam (Jeffery et al. 1995) and Port Kenny (33° 07.182'S, 134° 42.935'E), a calcareous grey-brown loam (Forward 1996). Field capacity for each soil was also determined using a 1 meter column technique (Marshall et al. 1979).

Table 5.1: Chemical characteristics of the selected Kimba, Mount Damper and Port Kenny soils, after collection, air drying and sieving through a 2mm sieve.

	Kimba	Mount Damper	Port Kenny
$pH_{(CaCl2)}$	7.6	7.6	7.7
Extractable Nitrate-N (mg/kg)	22	17	15
Extractable Ammonium-N (mg/kg)	3	5	13
Extractable Phosphorus (mg/kg)	54	33	47
Extractable Potassium mg/kg	863	506	685
Calcium Carbonate %	3.6	1.3	19.8
Organic Carbon %	2.02	1.40	2.60

5.2.2 Carbon addition in glass house

3.5kg of air dried soil from each location was weighed into 12 plastic containers (31cm x 13cm x 12cm) and watered by weight to 75% of field capacity. Experimental treatments (carbon additions) were then applied (four replicates), young root carbon, stubble carbon or no carbon addition (control). The addition of young root carbon was obtained by planting a high density (875 seeds/m^2) of surface sterilised wheat seeds (*Triticum aestivum*, cv.Yitpi) into each of the four containers filled with soil and growing plants for 28 days (Mazzola et al. 2000; Miche et al. 2001; Barnett et al. 2006). After 28 days plant shoots were cut off just above the soil level, removed, a subsample of roots was taken, then the remaining root-soil mixture in each container was broken up and mixed well.

This was repeated three times resulting in a total of four cycles for the growing wheat plants. The subsample of roots was oven dried and used to estimate the root biomass added during the four wheat growing cycles. The average amount of root biomass added in each treatment for all three soils over the four growing wheat cycles was 6.3g dry root/kg soil.

The stubble carbon treatment involved addition of above ground wheat stubble (collected from a paddock at Roseworthy in South Australia) cut to approximately 1cm-long pieces, at 5.6g dry stubble/kg dry soil and mixed into the soil of each container. Containers were watered to weight and after 28 days the soil was again mixed well in each container. These steps were carried out for four cycles.

The control treatment had no carbon addition but was watered to weight and mixed well every 28 days as described previously.

After the four growing cycles, all soil was left to air dry for five days before sub-samples of 200g were removed for quantification of DNA, and 100g for estimation of microbial biomass carbon and the rest used for the potential suppression bioassay.

5.2.3 Potential suppression bioassay

The bioassay involved the transfer of 750g dry soil from each container, into three replicate 300ml pots (3 reps of 250g dry soil) with added *Rhizoctonia solani* AG8 inoculum (three 10mm agar plugs on full strength potato dextrose agar). Pathogen inoculum was placed in the soil at approximately half the pot height (5cm) with plugs equidistant from one another. Each pot was watered to 75% field capacity, covered with a lid and incubated in a controlled environment room at 15°C. After two weeks, 7 surface sterile, pre-germinated wheat seeds (*Triticum aestivum*, c.v. Yitpi) were planted per pot. Seeds were surface sterilised by immersion in 1% sodium hypochlorite solution for 10 minutes, rinsing with reverse osmosis (R.O.) water three times, followed by a quick wash with 70% ethanol rinsing with R.O. water again and finally washing with 2% sodium thiosulphate for 5 minutes with a last rinse in R.O. water. Once the seedlings had emerged they were thinned to 5 per pot and the soil surface covered with about 20 grams of polythene beads to reduce evaporation. Pots were returned to controlled conditions at 15°C and 12 hour day/night regime where they were kept watered to weight for 28 days until sampling. At

sampling, the root and soil mix was removed from each pot and washed with water to remove all adhering soil particles. Shoots were cut off at the base of the stem, dried in an oven for four days at 60°C and average dry weight per plant calculated. Roots were stored in a freezer prior to being rated for disease incidence. Following disease rating, roots were dried in an oven for four days and dry weight per plant calculated.

Disease severity for Rhizoctonia root rot was measured based on the methods of Barnett et al. (2006). This was done by counting the total number of seminal roots, those seminal roots truncated by Rhizoctonia root rot before 10cm, and those infected with lesions but not truncated before 10cm. These counts were then used to calculate percentage root infection as follows

$$\text{Percentage root infection} = \frac{\text{No. truncated roots} + (\text{No. Infected roots}/2)}{\text{No. Seminal Roots}} \times 100$$

Disease severity assessment for take-all caused by *Gaeumannomyces graminis* var. *tritici* was estimated visually as percent seminal root infection.

Data was analysed as ANOVA, RCBD using GenStat Tenth Edition. Least significant difference (l.s.d.) at P=0.05 was used for comparison of treatment means.

5.2.4 Quantification of specific organisms

A sub-sample of air-dried soil was taken before the bioassay and sent to the SARDI (South Australian Research and Development Institute) Root Disease Testing Services for quantification of DNA of selected pathogens using real time PCR (Ophel-Keller et al. 2008). The pathogens assayed were *Rhizoctonia solani* AG8, *Gaeumannomyces graminis* var. *tritici* and the beneficial organisms *Exiguobacterium acetylicum*, *Pantoea agglomerans*, *Microbacterium*, *Trichoderma* Group A, (sect. *Pachybasium*) and Group B (sect. *Trichoderma* see Figure 2 as described by Kullnig-Gradinger et al. 2002). These tests are all available via SARDI (SARDI RDTS, GPO Box 397, Adelaide, S.A., 5001, Australia).

5.2.5 Microbial biomass carbon

Microbial biomass carbon was estimated using the fumigation-extraction method (Brookes et al. 1985). For each container treatment, two subsamples of 10 grams of soil

were wet to 75% of field capacity and incubated for 14 days. The first subsample (control) was extracted with 40ml 0.5M K_2SO_4. At the same time, the second subsample was placed in a desiccator with chloroform, and fumigated for 7 days, after which samples were extracted using 40ml 0.5M K_2SO_4. All extracts were then analysed using absorbance spectrophotometery for ninhydrin-positive nitrogen compounds (NPC) at an absorbance of 570nm (Joergensen et al. 1990). Microbial biomass C was estimated al. 1 using the flush of NPC, that is (fumigated NPC – control NPC) x 21 (Amato et al. 1988; Sparling et al. 1993).

5.3 Results

Addition of different carbon sources to the three soils had differing effects on microbial biomass (Figure 5.1), DNA levels of pathogens (Figure 5.2) and DNA levels of potentially suppressive bacteria and fungi (Table 5.2). Specifically, addition of young root carbon to all three soils increased the amount of DNA of the potentially suppressive bacteria Microbacterium and *Pantoea agglomerans* compared to the nil carbon treatment (Table 5.2). Stubble carbon increased the amount of DNA of Microbacterium and *Pantoea agglomerans* in Mount Damper and Port Kenny soils only (Table 5.2). Stubble carbon significantly increased the amount of DNA of *Trichoderma* group A in Mount Damper and Port Kenny soils; addition of young root carbon increased *Trichoderma* group A significantly only in Port Kenny soil. Microbial biomass carbon was higher in the young root carbon treatment than the Nil or stubble carbon treatment in all 3 soils (Figure 5.1) and there was a positive correlation between amounts of DNA for *Exiguobacterium acetylicum* (r=0.59), Microbacteria (r=0.61) and estimated microbial biomass carbon. The amount of DNA for pathogens *Rhizoctonia solani* AG-8 and *Gaeumannomyces graminis* var. *tritici* increased significantly in the young root carbon treatment but not in the stubble amended soils (Figure 5.2).

Table 5.2: Amount of DNA for *Exiguobacterium acetylicum, Pantoea agglomerans, Microbacterium* and *Trichoderma* group A and B ($log_{(10)}$ pg DNA / g soil) extracted from Kimba, Mount Damper and Port Kenny soils after four x 28 day cycle incubations in the glass house with either no carbon (Nil), young root carbon (YRC) or cut stubble carbon (Stubble). Different letters show significant differences between the treatment means at P=0.05, ns shows a non significant result at P=0.05.

Soil	*Exiguobacterium acetylicum*	*Microbacterium*	*Pantoea agglomerans*	*Trichoderma* Group A	*Trichoderma* Group B
		($log_{(10)}$ pg DNA / g soil)			
Kimba					
Nil	0.00	4.55 c	0.51 cde	1.90 cde	1.71 c
YRC	2.39	5.64 a	2.88 a	2.17 bcd	2.55 a
Stubble	0.17	4.71 c	1.25 bc	2.28 bc	2.00 bc
Mount Damper					
Nil	0.27	3.99 e	0.40 de	1.69 e	2.34 ab
YRC	2.66	5.55 ab	3.02 a	1.80 de	2.69 a
Stubble	0.40	4.65 c	1.50 b	2.42 b	2.52 a
Port Kenny					
Nil	0.25	3.99 e	0.12 e	0.54 g	0.36 de
YRC	1.09	5.40 b	0.99 bcd	1.25 f	0.23 e
Stubble	0.19	4.29 d	1.06 bcd	3.16 a	0.73 d
l.s.d. $_{(0.05)}$	ns	0.17	0.82	0.43	0.50

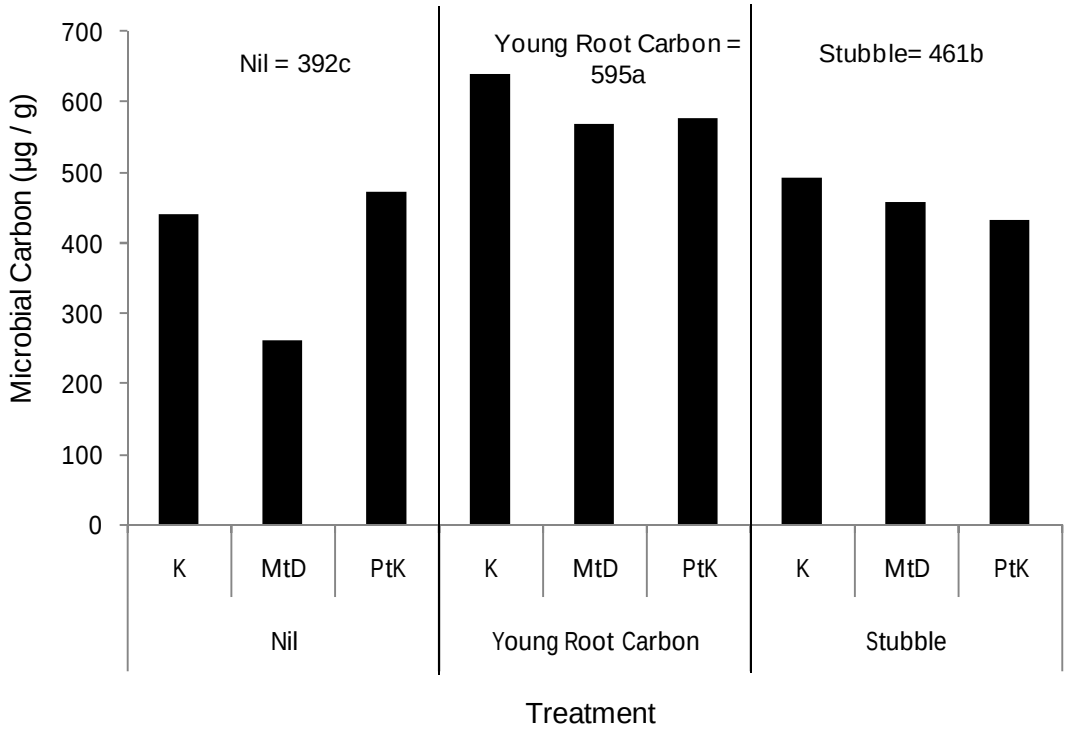
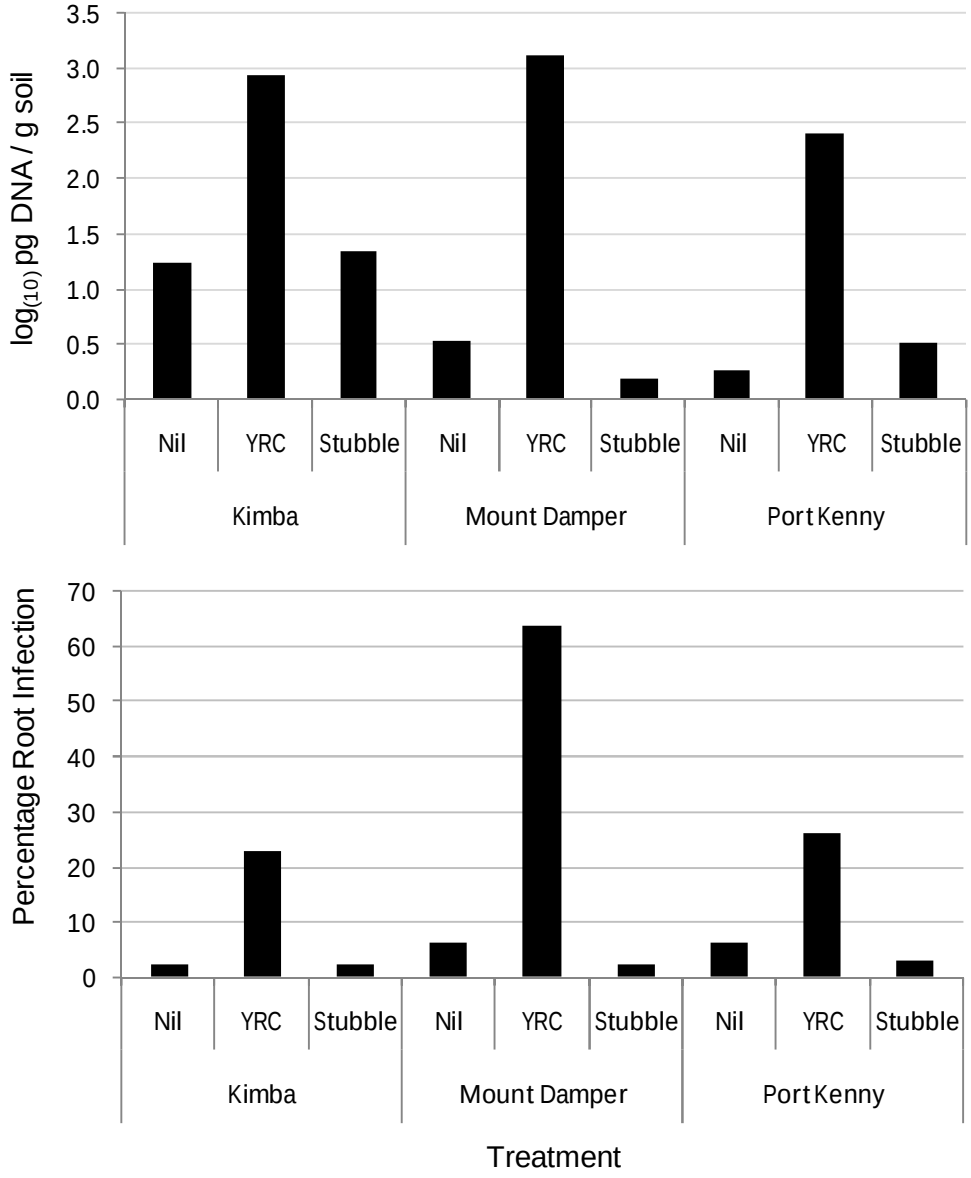


Figure 5.1: Microbial carbon (µg/g soil) extracted from Kimba (K), Mount Damper (MtD) and Port Kenny (PtK) soils after six months incubation in the glass house with either no carbon, young root carbon or cut stubble carbon. The main effect of carbon treatment is significant at p=0.05 ($lsd_{(0.05)}$ = 82), while soil type and the interaction between carbon treatment and soil type were not significant at p=0.05.

When carbon amended and control soils were tested in a bioassay with added Rhizoctonia inoculum, previous addition of either young root carbon or stubble carbon decreased the incidence of Rhizoctonia root rot in all three soils (Table 5.3). In treatments without added pathogen soils with young root carbon added had increased root infection by both *Rhizoctonia solani* AG8 and *Gaeumannomyces graminis* var. *tritici* (Figure 5.2). In the Kimba soil, Rhizoctonia root rot was higher in the young root carbon treatment than the stubble carbon treatment, while in the Port Kenny soil it was higher in the stubble carbon treatment than the young root carbon treatment (Table 5.3). The incidence of take-all infection was positively correlated (r=0.904) with the DNA amounts of *Gaeumannomyces graminis* var. *tritici* and was highest in the young root carbon treatment in all three soils (Table 5.3).

a) *Rhizoctonia solani* AG-8



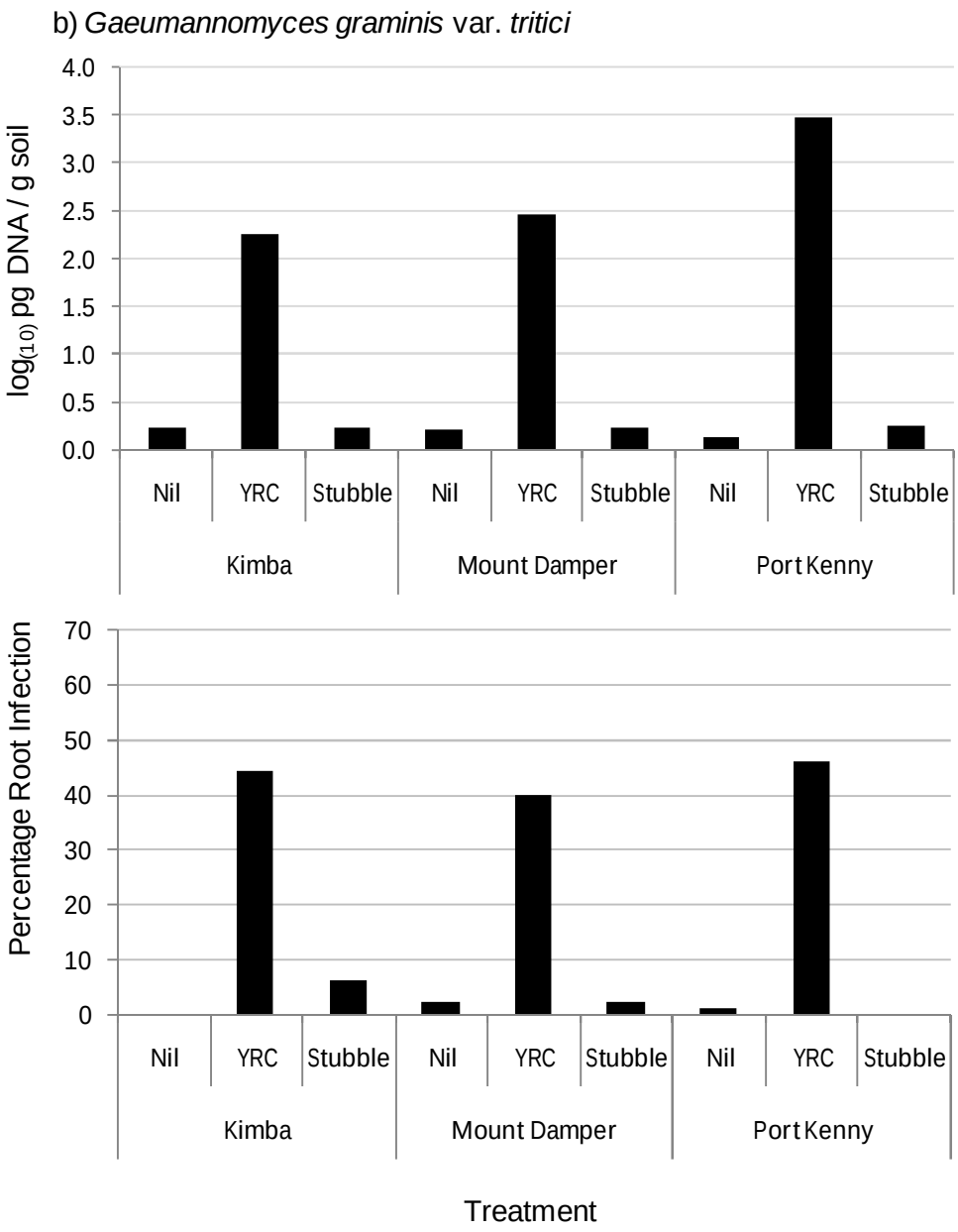


Figure 5.2: Amount of DNA for (a) *Rhizoctonia solani* AG8 and (b) *Gaeumannomyces graminis* var. *tritici* ($log_{(10)}$ pg DNA / g soil) extracted from Kimba, Mount Damper and Port Kenny soils after four 28 day cycles of incubation in the glass house and percentage root infection from the bioassay for treatments without added pathogen with either no carbon (Nil), young root carbon (YRC) or cut stubble carbon (Stubble). The interaction was not significant for the amount of *Rhizoctonia solani* AG8 DNA or percentage take-all root infection. L.s.d. $_{(0.05)}$ for the interaction of Rhizoctonia root infection is 11, and for *Gaeumannomyces graminis* var. *tritici* DNA is 0.42.

Root dry weights were lower in the nil carbon treatment where incidence of Rhizoctonia root rot was higher, compared to the stubble carbon treatment, while root dry weights

were lowest in the young root carbon treatments (Table 5.3). Shoot dry weights were similar in the nil carbon and stubble carbon treatment and lower in the young root carbon treatments and this was significant in two of the soils (Table 5.3). Root and shoot dry weight measurements were much lower for plants grown in the Port Kenny soil than for plants in the other two soils (Table 5.3).

Table 5.3: Percentage Rhizoctonia root infection with *Rhizoctonia* inoculation (+*Rhizoctonia* PRI) or without *Rhizoctonia* inoculation (-*Rhizoctonia* PRI), Percentage *Gaeumannomyces graminis* var. *tritici* infection (Take-all PRI), root and shoot dry weights per plant (+ Rhizoctonia inoculated pots) from a bioassay that was inoculated with *Rhizoctonia solani* AG8 from Kimba, Mount Damper and Port Kenny soils which had been pre-incubated for four x 28 day cycles in the glass house with either no carbon (Nil), young root carbon (YRC) or cut stubble carbon (Stubble). Different letters show significant differences for the treatment means at P=0.05.

Soil		+Rhizoctonia PRI	-Rhizoctonia PRI	Take-all PRI	Root Dry Weight (mg)	Shoot Dry Weight (mg)
Kimba						
	Nil	60 bcd	2 c	0 d	43 b	83 ab
	YRC	49 de	23 b	36 b	24 cd	29 c
	Stubble	36 f	2 c	8 c	56 a	86 a
Mount Damper						
	Nil	85 a	6 c	0 d	29 c	74 b
	YRC	65 bc	64 a	33 b	18 de	25 c
	Stubble	57 cd	2 c	1 cd	43 b	78 ab
Port Kenny						
	Nil	71 b	6 c	1 cd	24 cd	33 c
	YRC	39 ef	26 b	46 a	17 e	27 c
	Stubble	53 cd	3 c	0 d	27 c	36 c
l.s.d. $_{(0.05)}$		13	11	7	7	12

Details of the statistical significance of treatment effects on all the parameters measured are provided in Table 5.4

Table 5.4: F values and l.s.d. values for Amounts of DNA from *Exiguobacterium acetylicum*, *Microbacterium*, *Pantoea agglomerans, Trichoderma* group A, *Trichoderma* group B, *Rhizoctonia solani* AG8 and *Gaeumannomyces graminis* var. *tritici* and data for microbial carbon, percentage Rhizoctonia root infection without *Rhizoctonia solani* AG8 inoculation (-*Rhizoctonia* PRI), , percentage take-all root infection without Rhizoctonia solani inoculation (-Take-all PRI), percentage Rhizoctonia root infection with *Rhizoctonia solani* AG8 inoculation (+*Rhizoctonia* PRI), percentage take-all root infection with *Rhizoctonia solani* AG8 inoculation (+Take-all PRI) and root and shoot dry qeights per plant (mg) (+ Rhizoctonia inoculated pots) from a two way ANOVA analysis run through GenStat 10, for soil x carbon treatment main effects and their interactions.

	F value			l.s.d. $_{(0.05)}$		
	Soil	Carbon Treatment	Interaction	Soil	Carbon Treatment	Interaction
Exiguobacterium acetylicum	0.094	<.001	0.09	0.54	0.54	0.94
Microbacterium	<.001	<.001	<.001	0.10	0.10	0.17
Pantoea agglomerans	<.001	<.001	0.015	0.47	0.47	0.82
Trichoderma group A	0.003	<.001	<.001	0.25	0.25	0.43
Trichoderma group B	<.001	0.045	0.038	0.29	0.29	0.50
Microbial carbon	0.075	<.001	0.174	82	82	142
Rhizoctonia solani AG-8	0.003	<.001	0.128	0.43	0.43	0.74
-Rhizoctonia PRI	<.001	<.001	<.001	6.23	6.23	10.79
Gaeumannomyces graminis var. *tritici*	0.007	<.001	<.001	0.25	0.25	0.42
-Take-all PRI	0.542	<.001	0.348	4.25	4.25	7.37
+*Rhizoctonia* PRI	<.001	<.001	0.031	7.27	7.27	12.59
+Take-all PRI	0.105	<.001	0.009	4.18	4.18	7.23
Root Dry Weight per Plant (mg)	<.001	<.001	<.001	3.84	3.84	6.65
Shoot Dry Weight per Plant (mg)	<.001	<.001	<.001	6.68	6.68	11.57

5.4 Discussion

When Eyre Peninsula soils were amended with different sources of carbon, addition of young root carbon increased the amount of pathogen inoculum for *Rhizoctonia solani* AG8 and *Gaeumannomyces graminis* var. *tritici* in all three soils. Addition of young root carbon also increased the amount of DNA for the beneficial organisms *Microbacterium* and *Pantoea agglomerans* in two of the three soils. Conversely, the addition of stubble carbon significantly increased levels of *Trichoderma* group A in two out of the three soils, and *Trichoderma* A was elevated in the third soil.

The expression of disease severity after pathogen inoculation was lower in the carbon amended soils compared to the unamended soils. These observations support previous

reports on reduced soil-borne disease incidence following the addition of organic C amendments (Mazzola 2007).

The results suggest that populations of micro-organisms in the unamended Eyre Peninsula soils may be limited by insufficient carbon since microbial carbon and DNA levels of the potentially suppressive organisms *Microbacterium*, *Exiguobacterium acetylicum*, *Pantoea agglomerans* were higher in the young root carbon treatment than the stubble carbon treatment and lowest in the nil carbon control treatment. Supporting evidence for this is provided in previous studies on soil organism growth limitations, such as Demoling et al. (2007) who reported that bacterial growth in a range of Swedish soils was limited by lack of carbon for most soils they tested. In their work carbon was added as glucose, a more labile carbon source and evaluated after 48 hours (Demoling et al. 2007) compared to the young root carbon or stubble treatments in this work which were more complex carbon sources and evaluated after a much greater time period. Despite the differences in experimental technique the greater positive effect of the young root carbon amendment on increasing suppressive organisms seen in this work is likely to be due to the quality of C inputs i.e. lower molecular weight carbon compounds that are more readily available for microbial use (Bowen et al. 1999) and a narrower C:N and C:P ratio (e.g. C:N 60:1, C:P 330:1(Iqbal 2008) in the young root carbon treatment compared to more complex carbon compounds (Killham 1994) and wider C:N, C:P ratios (e.g. C:N 100:1, C:P 500:1 (Iqbal 2008) in the stubble.

The highly calcareous Port Kenny soil tended to have lower amounts of DNA for the quantified organisms compared to the Kimba and Mount Damper soils. These differences may be due to the highly calcareous hostile nature of this soil for plants and microorganisms (Coventry et al. 1998; Nannipieri et al. 2003). Highly calcareous soils are known to present nutrient constraints for plant growth (e.g. phosphorus and zinc) (Holloway et al. 2001; Bertrand et al. 2003), resulting in lower plant dry weights and smaller root systems. Similarly, these constraints may limit nutrient availability for the soil organisms (Nannipieri et al. 2003), including suppressive organisms resulting in overall lower levels of suppression. For example, lower plant dry weights in the Port Kenny soil would have resulted in less biologically available carbon inputs in the young root carbon treatment compared either Kimba or Mount Damper soils. Thus if biologically available carbon is the main driver for the development and function of a suppressive community,

the Port Kenny soil is likely to be less suppressive to Rhizoctonia root rot than Kimba and Mount Damper soils.

In contrast to the young root carbon treatment the addition of stubble had less effect on the microbial carbon and bacterial populations, but significantly increased some Trichoderma fungi in two out of the three soils. Since there was higher microbial biomass carbon, and in general higher DNA amounts of potentially suppressive microorganisms in soil amended with young root carbon it might be expected that during the potential suppression bioassay this treatment should have resulted in less Rhizoctonia root rot as there would potentially be more soil organisms to suppress the disease. However, amendment with young root carbon also increased the amounts of DNA for the pathogens *Rhizoctonia solani* AG8 and *Gaeumannomyces graminis* var. *tritici*. It is interesting to note that amounts of DNA for *Rhizoctonia solani* AG8 were significantly higher in the young root treatment compared to those in both the stubble carbon and nil carbon treatments. This observation accords with previous field scale research suggesting that the presence of weeds (i.e growing plants) prior to sowing of crops (sometimes called the "green-bridge") increases the incidence of Rhizoctonia root rot compared to a fallow period (Roget 1987). The young root carbon treatment roots may have simulated a similar effect to that of a "green bridge" by harbouring the pathogen, while the stubble and nil carbon treatments simulated the conditions of a well-managed 'clean' fallow and did not harbour the pathogen. The lack of difference in pathogen DNA between the stubble carbon addition and nil carbon amounts is probably a result of interactions between the pathogen and other soil microorganisms, for example, competition for nutrients and space (Baker 1968), antagonism (Fravel 1988) and predation (Curl 1988; Gupta et al. 1999b) Another reason for this lack of difference may be that stubble is a poorer host for Rhizoctonia than it is for other organisms e.g. *Trichoderma* species.

In the pathogen bioassay Rhizoctonia root infection was highest in the unamended control treatment for all three soils tested; this treatment had the lowest amounts of DNA for most of the isolated suppressive organisms as well as lower microbial carbon values than the two carbon amended treatments. Unexpectedly, the young root carbon treatment had higher root disease severity than the stubble carbon treatment for Kimba and Mount Damper soils. This is probably due to a number of factors, particularly the increase in amounts of DNA for *Rhizoctonia solani* AG8 and *Gaeumannomyces graminis*

var. *tritici*. This increase in *Rhizoctonia solani* AG8 DNA in the young root carbon treatment added an additional pathogen load to the inoculated bioassay pots and this may have overwhelmed the suppressive organisms. In addition, the development of take-all negatively impacted on plant health and root growth which is likely to have compounded the effects of the Rhizoctonia root rot. The second important factor was soil nutrient depletion during the carbon addition in the glass house period, which led to less healthy plants that were likely to be more susceptible to pathogen attack. In contrast, the young root carbon treatment in the Port Kenny soil had less Rhizoctonia root rot severity than the stubble carbon treatment. This is probably due to the development of take-all which complicated rating of these roots for Rhizoctonia root rot.

In the stubble carbon treatment, the presence of higher amounts of *Trichoderma* group A DNA is likely to have contributed to the lower disease incidence seen in this work as there are many reports of *Trichoderma* species as successful biocontrol agents (Henis et al. 1979; Harman et al. 2004). It is acknowledged that *Trichoderma* species alone would not be the only organisms involved in disease suppression in the stubble carbon treatment but would act in combination with other suppressive organisms. The lower pathogen load would also have contributed to the decrease in disease severity in this treatment.

Plant disease research can either be inoculum based or community based, the data described and discussed in this Chapter is an example of community based research. The aim of community based research is to understand the overall microbial community changes associated with a change in measured disease (Mazzola 2007; Gupta et al. 2008). In this experiment the community changes induced by the imposed treatments was investigated by monitoring five specific organisms potentially linked to disease suppression. The correlation between the changes in populations of specific organisms measured in this work and their direct impact on the amount of disease severity will need to be confirmed in an inoculum based experiment (causation). Some examples of inoculum based (biocontrol) experiments have previously been described by Bowen et al. (1999), Barnett et al. (2006) and Gupta et al. (2008).

Despite our focus on only the five specific suppressive organisms and the development of take-all in the young root carbon treatment which complicated the direct interaction between changes in the *Rhizoctonia solani* inoculum and suppressive microorganisms.

These results clearly demonstrate that the microbial communities of these selected Eyre Peninsula soils have the potential to develop higher levels of disease suppression following exposure to multiple additions of carbon inputs in the form of crop residues. It is likely that organic carbon inputs in the agricultural systems of the region, and more specifically labile carbon inputs are limiting factors for the development of disease suppression. Climatic influences on the Eyre Peninsula and other semi-arid regions throughout the world, particularly low and erratic rainfall coupled with high temperatures, may well restrict additions of carbon *per se* via less crop dry matter production and in particular biologically available carbon via less root growth (Rovira 1992; Sadras et al. 2004). These influences on carbon inputs will impede the development of disease suppression. Further work on identifying the potential in these farming systems to increase inputs of carbon and the nature of carbon addition in the field environment is recommended.

Addition of either young roots or cut stubble as a source of carbon to Eyre Peninsula soils resulted in an increase in organisms suppressive to soil-borne disease, and suppression of root disease when pathogen inoculum was added to these soils. This occurred despite the hostile and constrained nature of these soils and the fact that young roots increased the amount of pathogen DNA whereas stubble did not. This suggests that through changes in farm management systems which help manipulate the quantity and quality of carbon inputs it may be possible to increase the root disease suppressive ability of these hostile soils

Chapter 6
Effects of added mineral nitrogen (NO_3-N, NH_4-N) and phosphorus on Rhizoctonia disease suppression in two soils from the Eyre Peninsula

6. Effects of added mineral nitrogen (NO_3-N, NH_4-N) and phosphorus on Rhizoctonia disease suppression in two soils from the Eyre Peninsula

6.1 Introduction

It was suggested earlier (Chapter 4) that there may be certain abiotic constraints in soils from the EP (for example high calcium carbonate content that reduces phosphorus availability to plants by formation of insoluble calcium-phosphorus complexes (Bertrand et al. 2003), which are limiting the development and/or expression of disease suppression. It is probable that phosphorus may also be limiting for soil micro-organisms, and if this is the case, micro-organisms involved in suppression of Rhizoctonia root rot may be phosphorus limited. This could be another factor contributing to the observed lack of disease suppression in Eyre Peninsula soils. In addition to phosphorus the other major macronutrient routinely added as fertiliser is nitrogen. Information to date regarding the effects of nitrogen on disease suppression of Rhizoctonia root rot is contradictory. Briefly, as discussed in the literature review, some reported data supports the hypothesis that an increase in mineral nitrogen prior to the cropping period results in a loss of disease suppression (Gupta et al. 2006). Alternatively, in Western Australia application of mineral nitrogen fertilizers increased the severity of Rhizoctonia root rot (MacNish 1983; MacNish et al. 1996). The effects of nitrogen fertilizers in any form on disease suppression in EP soils are unknown.

Nutrient application can also alter host resistance and plant susceptibility to pathogen attack i.e. applied nutrients can directly impact plant health (Cook 2000) which has implications for the measurement of disease severity. The interactions between plant health, measurement of disease severity and nutrient application adds increased complexity to the evaluation of disease suppression and needs to be taken into account when interpreting results for nutrient-disease based experiments.

Further, results from the addition of carbon sources such as wheat stubble or young roots to selected EP soils (Chapter 5) indicate that in these soils low quantities of biologically available carbon may be also limiting the expression of disease suppression.

These observations on nutrient and biologically available carbon limitations lead to the following experiment which aimed to test the effect of the major nutrients (nitrogen and phosphorus) commonly added as agricultural fertilizers on disease suppression in two soils from the Eyre Peninsula (Minnipa and Streaky Bay 2), in the presence and absence of a biologically available carbon source (sucrose). The use of sucrose as a biologically available carbon source to magnify the expression of disease suppression has been reported previously and is why sucrose was selected for this experiment (Roget et al. 1999).

The overarching hypothesis of this experiment was that disease suppression in these selected EP soils was limited by certain abiotic constraints present in the soils. To test this, the most commonly limiting agricultural mineral nutrients (NH_4-N, NO_3-N and PO_4) were added as a balanced nutrient solution either with or without an available carbon source (sucrose). After the addition of *Rhizoctonia solani* AG8 inoculum, disease severity was measured on young wheat roots. The experiment tested whether the addition of mineral nutrients would alleviate the soil abiotic constraints, and result in a decrease in disease severity (thus an increase in disease suppression).

The first aim was to test if any of the macro nutrients such as nitrogen (ammonium or nitrate) and phosphorus (P) was limiting plant growth in the Minnipa and SB2 soils (healthy controls).

The second aim was to test the impact of the addition of these macro-nutrients on disease suppression. That is, to evaluate the severity of Rhizoctonia root rot, in the presence of mineral nutrients and the pathogen *Rhizoctonia solani* AG8.

The third aim was to test potential disease suppression. That is to evaluate the severity of Rhizoctonia root rot, with the addition of mineral nutrients, biologically available carbon (sucrose) and *Rhizoctonia solani* AG8 inoculum.

6.2 Materials and Methods

Minnipa and Streaky Bay 2 (SB2) soils were selected for this experiment. SB2 a highly calcareous soil and Minnipa a lower calcareous soil reported to be suppressive to Rhizoctonia root rot (Cook et al. 2007).

The experiment was carried out using methods described in detail the materials and methods Chapter 2. Briefly, soils were moistened with the appropriate nutrient solution treatment and mixed well. This moist soil was then placed into bioassay pots and kept moist at 75% WHC, *Rhizoctonia solani* AG8 inoculum was added at two plugs per pot and each treatment was replicated four times.

A summary of the concentrations and specific compounds used for the nutrient solutions is summarised in Table 6.1. All pots received Trace Element and Basic Macro Nutrient solutions and the four treatments were (i) nil (no additional macronutrients; (ii) ammonium-N, (iii) nitrate-N or (iv) phosphorus nutrient solutions (Table 6.1)

Table 6.1: Nutrient solutions added to Minnipa and Streaky Bay soils.

Trace Element Nutrient Solution	**mg/kg soil**
H_3BO_3	0.31
$MnSO_4.4H_2O$	0.31
$ZnCl_2$	0.01
$CuCl_2.H_2O$	0.01
MoO_3	0.01
Basic Macro Nutrient Solution	**g/kg soil**
$MgSO_4.7H_2O$	0.06
Ferric Citrate	0.01
Ammonium N Nutrient Solution	**100mgN/kg soil**
$CaCl_2$	0.07
K_2SO_4	0.06
$(NH_4)_2SO_4$	0.12
Nitrate N Nutrient Solution	**100mgN/kg soil**
$Ca(NO_3)_2$	0.15
KNO_3	0.06
Phosphorus Nutrient Solution	**50mgP/kg soil**
KH_2PO_4	0.02

6.2.1 Experimental design

A single experiment was conducted, where the first set of treatments (designated healthy controls) tested the effect of each nutrient solution combination on plant growth without addition of the pathogen. The second set of treatments tested the impact of nutrients on disease severity of Rhizoctonia root rot compared to a disease control which had no nutrient treatment added. The third set of treatments tested the effect of adding a biologically available carbon source (Roget et al. 1999) and was tested by adding sucrose

(at 1% of the dry weight of soil) to the nutrient treatments. The treatments are summarised in Table

Table 6.2: Summary of experimental treatments used in this bioassay, with macro nutrient solution treatments as mg per kg dry soil.

- *Rhizoctonia solani* AG8	+ *Rhizoctonia solani* AG8	
- Sucrose	- Sucrose	+ Sucrose at 1% dry weight
-*	-**	-
100mg NH_4-N*	100mg NH_4-N	100mg NH_4-N
100mg NO_3-N*	100mg NO_3-N	100mg NO_3-N
50mg PO_4*	50mg PO_4	50mg PO_4

* Healthy controls without the addition of pathogen

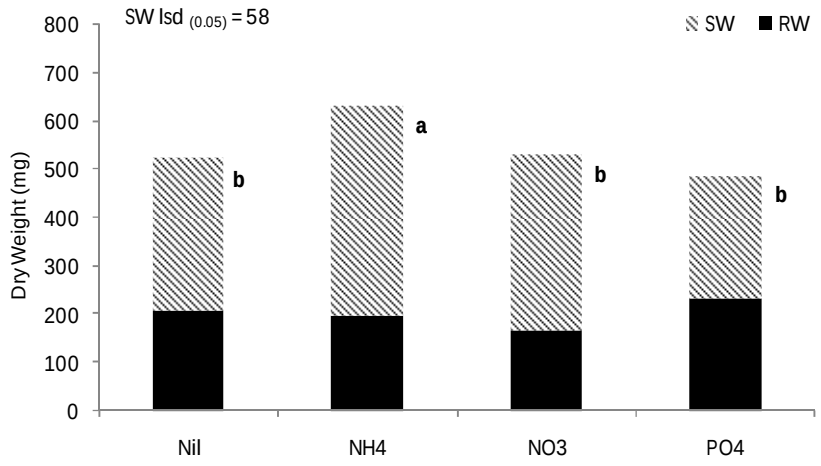
** Disease control, no nutrient solution and no pathogen or sucrose addition

6.3 Results

Overall, these results suggest that addition of nitrogen as either nitrate or ammonium and phosphorus as orthophosphate affected disease severity and thus disease suppression of Rhizoctonia root rot in bioassay conditions. Effects of the added nitrogen on disease severity differed depending on the form, while orthophosphate phosphorus addition increased disease severity.

The plant dry weight data for the healthy controls in Minnipa and SB2 soils suggest that nitrogen itself is not limiting plant growth in either of these soils, but that only nitrogen in the form of ammonium significantly increased shoot dry weight in both soils. In Minnipa and SB2 soils plants had greater shoot dry weights with NH_4-N addition compared to NO_3-N (Figure 6.1 a & b). Addition of orthophosphate to the SB2 soil increased root and shoot dry weights to about double that for all other treatments in that soil (Figure 6.1b) suggesting that availability of phosphorus is limiting root and shoot growth in the SB2 soil. Apart from the orthophosphate treatment, plants grown in the SB2 soil generally had lower plant dry weights than those grown in the Minnipa soil (Figure 6.1b).

(a) Minnipa soil



(b) Streaky Bay soil

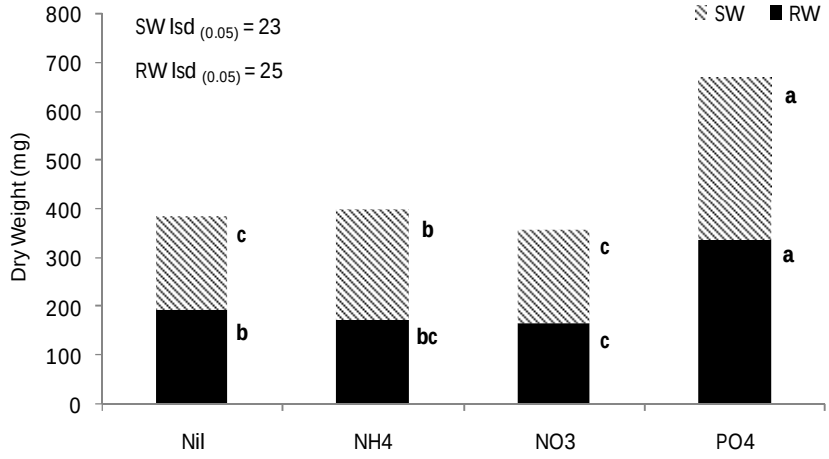


Figure 6.1: Root (RW) and shoot (SW) dry weight (mg) for control plants grown in (a) Minnipa and (b) Streaky Bay soil without addition of a nutrient solution (Nil) and addition of nutrient solutions (NH_4, NO_3 and PO_4) all treatments do not have the addition of pathogen inoculum (healthy controls).

In treatments without any added sucrose the effect of NH_4-N nutrient addition on disease severity of Rhizoctonia root rot was similar in Minnipa and SB2 soils. Addition of NH_4-N significantly decreased the severity of Rhizoctonia root rot compared to NO_3-N (Figure 6.2a, Figure 6.3a) and this decrease in disease severity corresponded to a significant increase in shoot dry weight (Figure 6.2b, Figure 6.3b). The addition of PO_4 significantly increased disease severity of Rhizoctonia root rot in the SB2 soil (Figure 6.3a), which did

not correspond to a decrease in root or shoot dry weights but rather a significant increase in shoot dry weight (Figure 6.3b).

Similar effects of the different from of added nitrogen on disease severity in the presence of a biologically available carbon source (sucrose) occurred independent of soil type.

In both soils, addition of sucrose with NH_4-N increased the severity of Rhizoctonia root rot compared to NH_4-N alone (Figure 6.2a, Figure 6.3a). The effect of addition of sucrose with NO_3-N was the opposite and there was a decrease in disease severity of Rhizoctonia root rot when sucrose and NO_3-N were added together compared to $N0_3$-N alone (Figure 6.2a, Figure 6.3a).

Shoot dry weights for plants grown in the Minnipa soil were significantly lower in the sucrose with NH_4-N or NO_3-N than in the NH_4-N or NO_3-N treatments alone (Figure 6.2b).

Disease suppression in the SB2 soil was greatest in the sucrose only and PO_4 with added sucrose treatments with a significant decrease in disease severity compared to the disease control and PO_4 treatments without sucrose (Figure 6.3a).

Root and shoot dry weights for the disease control, NH_4-N, NO_3-N and Nil nutrient treatments with added sucrose were all similar, while root and shoot dry weights for the PO_4 with sucrose treatment were the highest (Figure 6.3b). Shoot dry weight for the PO_4 treatment without sucrose was greater than the PO_4 with sucrose treatment while root dry weights were not different between the treatments (Figure 6.3b).

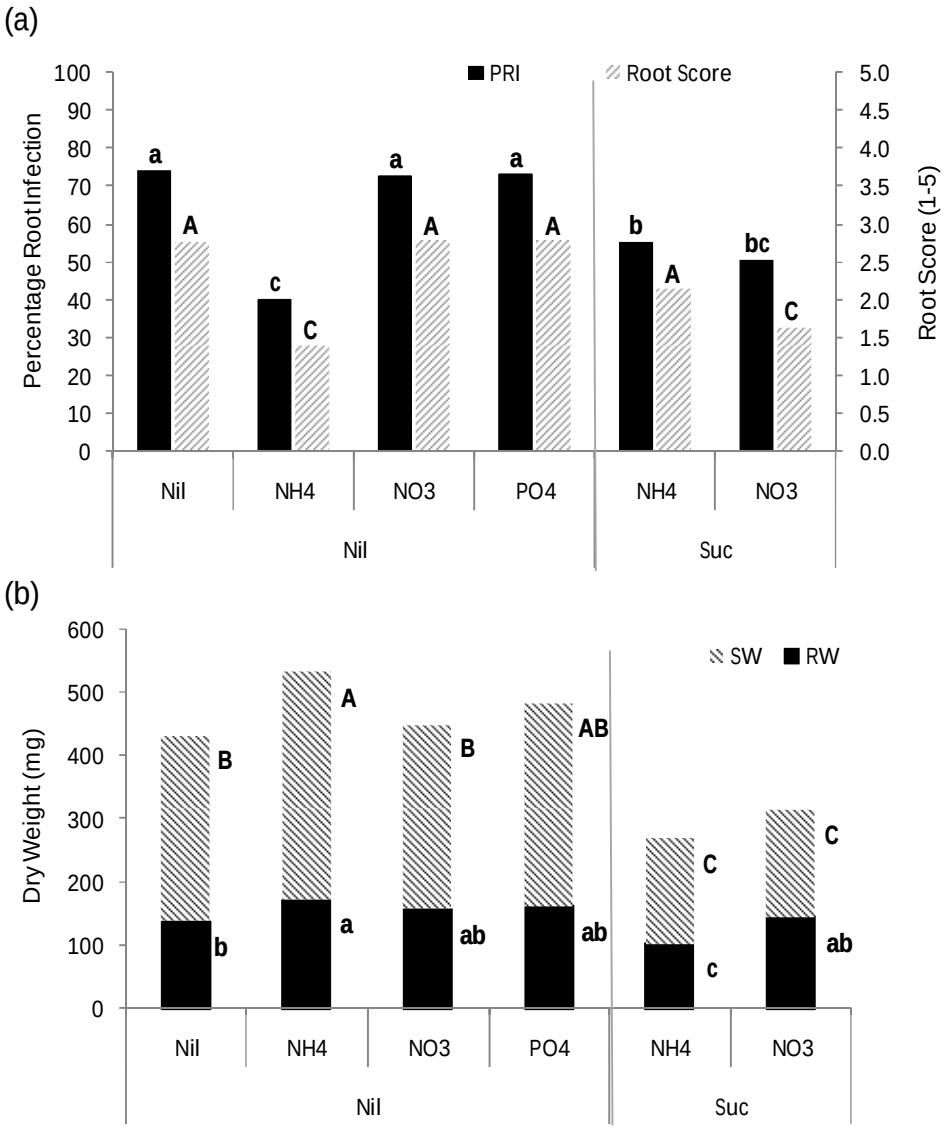
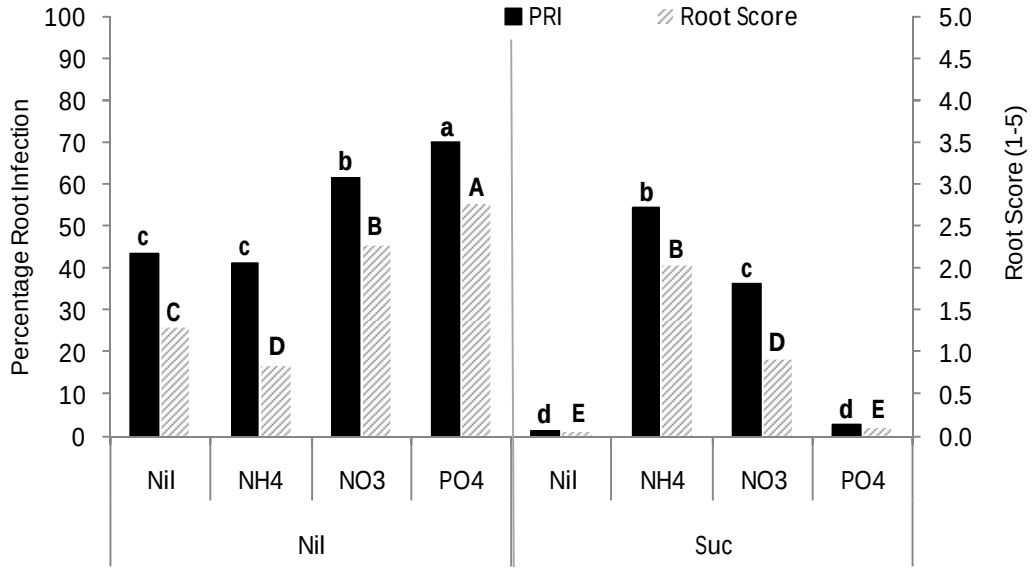


Figure 6.2: (a) Percent root infection (PRI) and Root Score for *Rhizoctonia solani* AG8 (b) root (RW) and shoot (SW) dry weights for 28 day old wheat plants grown in the Minnipa soil, after addition of no nutrients (Nil) , nutrient solutions (NH_4, NO_3, PO_4) and addition of sucrose at 1% soil dry weight with nutrient solutions (NH_4 and NO_3). All treatments were inoculated with the pathogen *Rhizoctonia solani* AG8. Different letters indicate significant differences for the lsd of treatment means at P=0.05.

(a)



(b)

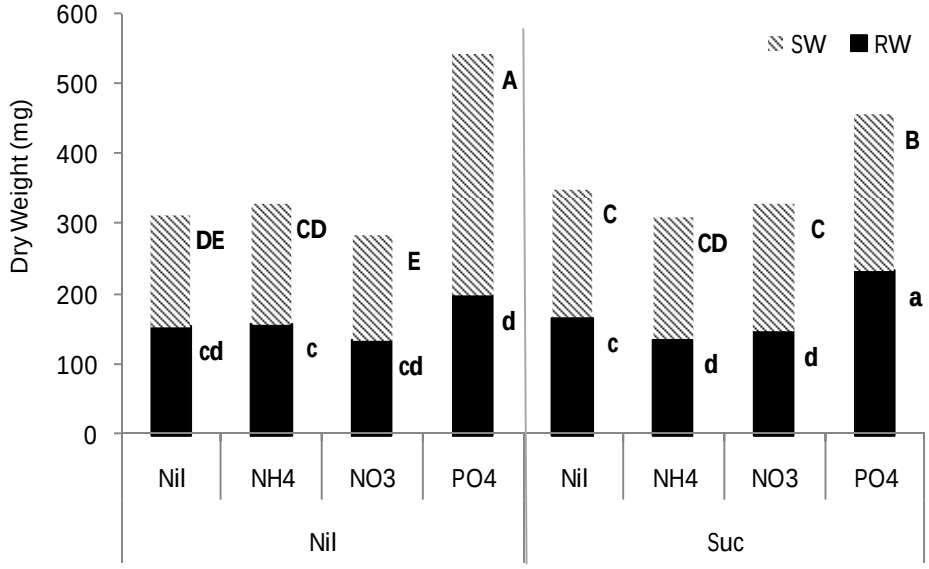


Figure 6.3: (a) Percent root infection and Root Score *Rhizoctonia solani* AG8 (b) root (RW) and shoot (SW) dry weights for 28 day old wheat plants grown in the Streaky Bay 2 soil, after addition no nutrients (Nil), nutrient solutions (NH_4, NO_3, PO_4) and addition of sucrose at 1% dry weight to soil with no nutrients (Nil) and nutrient solutions (NH_4, NO_3, PO_4). All treatments were inoculated with the pathogen *Rhizoctonia solani* AG8. Different letters indicate significant differences for the lsd of treatment means at P=0.05.

6.4 Discussion

Overall, it appears that the addition of NH_4, NO_3 and PO_4 to alleviate abiotic nutrient constraints in these two EP soils did not necessarily reduce percent root infection of Rhizoctonia root rot or result in increased plant growth. Furthermore, observed effects of nutrients were altered in the presence of a biologically available carbon source. Thus, the addition of NH_4-N either did not affect or decreased disease severity in these soils in the absence of added biologically available carbon, whereas addition of $N0_3$-N in the absence of added carbon increased disease, irrespective of soil type. These effects of added NH_4-N and NO_3-N were reversed where biologically available carbon was added. Phosphorus addition in the highly calcareous soil increased disease severity in the absence of carbon, and decreased disease severity with the addition of carbon.

The first part of this discussion will consider plant-soil-nutrient interactions in the absence of any added pathogen inoculum in order to focus initially on whether abiotic constraints to plant growth are in part alleviated by addition of the single rate of N or P used in this experiment. It is acknowledged that an experiment with increasing rates of mineral nutrients would be required to adequately evaluate whether the abiotic constraints had been totally alleviated in these soils, especially considering the different starting levels of both N and P of the original soils and that this discussion will consider the very high single rate used in this experiment to be adequate. Nevertheless due to the hostile nature of the soils on the EP the underlying assumption was that there would be nutrient constraints (especially P) to plant growth in this experiment (Holloway et al. 2001). The response of plant growth to the addition of N and P in this experiment varied and will be discussed in detail in the following paragraphs. The addition of NH_4-N increased shoot dry weight in the healthy control treatment relative to the NO_3-N and nil treatments for both soils. This increase may be due to the preferential uptake of NH_4-N rather than NO_3-N by the wheat plants. A similar suggestion was made for tea plants, where the authors attributed the greater leaf dry matter production under NH_4-N in part to greater NH_4-N use efficiency (Ruan et al. 2000). They proposed that the higher shoot N concentration under NH_4-N indicated a higher NH_4-N use efficiency compared to NO_3-N (Ruan et al. 2000). However, a more likely cause for the increase in shoot dry weight observed in this study may be that the application of NH_4-N, resulted in an increase in acidity of the rhizosphere soil. It is known that when plant roots absorb NH_4-N, there is a release of H+

ions, which causes localised acidity (Agrios 1988). Research on maize (Rodriguez et al. 2008) and tea (Zoysa et al. 1998), showed that application of NH_4-N resulted in a notable decrease in the pH of the rhizosphere soil. Furthermore, in the rhizosphere of tea, there was a subsequent increase in the availability of P which had been added as rock phosphate, and the authors concluded that phosphate was released as the acidity of the rhizosphere increased (Zoysa et al. 1998). It is highly likely that the application of NH_4-N would have decreased the soil pH in a similar manner in the Minnipa and SB2 soils, and that this decrease in pH would have resulted in an increase in the availability of phosphorus to the plant. Such an increase in available P in the rhizosphere is likely to have increased phosphorus uptake and growth, thus increasing the rate of plant growth. Due to the large buffering capacity of the carbonate ions in the highly calcareous SB2 soil it is probable that any decrease in rhizosphere pH would have been smaller than that of the less calcareous Minnipa soil and would have lead to less available P and lower plant growth when compared to the Minnipa soil.

Plant growth markedly increased with addition of phosphorus in the highly calcareous SB2 soil, highlighting the existence of an abiotic nutrient constraint to plant growth in this soil despite the higher initial P concentration than in the Minnipa soil. Initial Colwell P results suggest that Minnipa soil is likely to have more P limitations than the SB2 soil with only 60% of the concentration of P/kg soil compared to initial P concentration of the SB2 soil. These results are misleading as in the highly calcareous soils the Colwell P test has been shown to be unreliable at accurately detecting the available P (Mason et al. 2010) thus addition of the 50mgP/kg soil in this experiment was considered to be luxurious. Since highly calcareous soils are known to fix plant available phosphorus by forming insoluble complexes between the calcium carbonate and phosphorus from soil solution or the exchangeable pool (Bertrand et al. 2003). It is highly probable that long term phosphorus fixation and subsequent reduced availability of phosphorus inherent in a highly calcareous soil is the reason that plants grown in the SB2 soil generally were smaller than those grown in the Minnipa soil, with the exception of the phosphorus treatments. Field research studies in other highly calcareous soils on the EP report long term effects of fluid fertilizer phosphorus applications on increased crop root and shoot growth (Holloway et al. 2001). Indeed phosphorus applied as fluid has been reported to remain more readily and more rapidly available than granular phosphorus fertilizer in

calcareous soils (Lombi et al. 2005). Thus the increases in shoot and root growth recorded in this study with fluid phosphorus applied to SB2 soil are not surprising and clearly the addition of 50mg/kg phosphorus was sufficient to provide available phosphorus. This phosphorus rate is higher than those generally applied in EP farming systems (5-10mgP/kg soil) and follow-up work reported in the next chapter utilises a wider range of phosphorus rates to include those considered agronomically more relevant.

The next part of the discussion will focus on the consequences for suppression of Rhizoctonia root rot from the addition of the nutrients (nitrogen and phosphorus) to alleviate the abiotic constraints in these soils, and will refer to data from the inoculated pots without sucrose added. It was surprising that plants with greater disease severity (higher plant root infection percentage) were obtained in the unamended Minnipa soils rather than the unamended SB2 soil, since the Minnipa soil as mentioned in the introduction had been reported as suppressive, although this suppressive classification was adding from the bioassay assessment using carbon amended soils (Cook et al. 2007). The results discussion here serves to highlight the importance of contextual interpretation of any assessment for disease suppression.

The effect of nitrogen including the effects of specific form of nitrogen on disease severity is unclear and in some results increases in mineral nitrogen are linked to an increase in disease severity via loss of disease suppression (Gupta et al. 2006) and in other results an increase in nitrogen decreased the severity of Rhizoctonia root rot (MacNish 1985a). In this work increased disease suppression as indicated by a decrease in measured disease severity, for both soils, appears to be greater in the NH_4-N treatment compared to the NO_3-N treatment. It is likely that there are a variety of factors contributing to this decrease in disease severity, all of which are independent of soil type. One possibility is that NH_4-N had a direct influence on the growth of the pathogen *Rhizoctonia solani*. NH_4-N has been shown to result in lower *Rhizoctonia solani* mycelium growth than NO_3-N (Ghini et al.). If this holds true, under the NH_4-N treatments pots would have had lower soil colonisation by *Rhizoctonia solani* and less inoculum available to cause root rot disease. Alternatively the NH_4-N effect on soil pH may have decreased the disease severity via increasing the availability of previously fixed nutrients. As discussed previously, a decrease in the rhizosphere pH due to H^+ release on NH_4 uptake, would result in an increase in the availability of nutrients like P, boron, copper, zinc, iron and

manganese. The subsequent availability of these essential nutrients to the plant would result in healthier and stronger plants, which would be less susceptible to disease (Zoysa et al. 1998). It is also probable that the NH_4-N treatment may have reduced disease severity by changing the soil microbial community structure. Studies on the severity of take-all under different nitrogen forms found that NH_4 increased the populations of *Pseudomonads* which were antagonistic to take-all, while NO3 increased populations of those that were deleterious to plant growth (Sarniguet et al. 1992). Along with changes in the populations of *Pseudomonads*, different soil pH conditions influenced the production of antibiotics by *Pseudomonads* (Ownley et al. 2003). Inhibition of *Gaeumannomyces graminis* var. *tritici* colonies by the antibiotic phenazine-1-carboxylic acid (PCA) decreased as the pH was increased (Ownley et al. 2003). Addition of NH_4-N is likely to have lowered the rhizosphere pH (increasing the effectiveness of PCA) as well as shift the *Pseudomonads* populations towards a more suppressive community. Therefore, in combination the increased antibiotic effectiveness and increased populations of antibiotic producing organisms probably contributed to decreasing the incidence of disease in the NH_4-N treatments compared to the NO_3-N treatments in this work.

Although there is no specific research on the effect of P on disease suppression of Rhizoctonia root rot in EP soils. It was hypothesised that due to nutrient tie up in these EP soils (Bertrand et al. 2003) where P is limiting for plant growth (Holloway et al. 2001) P may also be limiting for the suppressive soil microorganisms preventing the expression of disease suppression. In this work addition of phosphorus has clearly had a large impact on plant growth in the SB2 soil even in the presence of the *Rhizoctonia* pathogen. Despite the increased disease severity in the PO_4 nutrient treatment, plant dry weight was still greater than any of the other nutrient or control treatments. As referred to earlier, the increase in plant growth supports evidence that these highly calcareous soils are P deficient (Holloway et al. 2001), and that when P supply is increased, it has the potential to increase plant growth and yields in these constrained soils (Holloway et al. 2001). Furthermore, it is likely, that the increase in plant growth despite the presence of the pathogen and root infection is partly a synergistic effect where a less nutrient limited plant is healthier or more robust. Plants that experience greater stress such as nutrient deficiencies (Dubuis et al. 2005), lack of moisture or sub-optimal soil temperature (Duveiller et al. 2007) are reported to be more susceptible to disease. A sulphur

deficiency in oilseed rape was linked to an increase in disease susceptibility to the pathogens *Leptosphaeria maculans, Botrytis cinerea* and *Phytopthora brassicae* (Dubuis et al. 2005). These authors suggested that the decrease in disease resistance was due to the combination of the specific nature of sulphur containing defence compounds as well as the general reduction in fitness and weakening of the plant which increases susceptibility to stress (Dubuis et al. 2005). It is likely that without additional nutrients, the high rate of P fixation in these calcareous soils results in a more disease susceptible plant due to P deficiency. Since P is not linked directly to defence compounds as is sulphur, an increase in available P does not necessarily result in decreased disease severity. Rather, the effect of additional P is likely to render plants more healthy overall, allowing them to withstand the greater disease severity and maintain or increase growth whilst not necessarily decreasing the actual rate of infection.

In combination with the suggested effects of P on the plant, P may also have had an impact on the pathogen. Indeed, there is a study reporting increased microbial respiration but no change in microbial biomass following phosphorus addition to a forest soil (Allen et al. 2004). It is quite probable that the addition of P in this study resulted in an increase in activity of the soil microorganisms, including the pathogen. If this is the case, an increase in activity of *Rhizoctonia solani* is likely to lead to an increase in virulence (that is the ability for a given quantity of pathogen to cause greater disease severity). Research on the effects of exogenous nutrition on the virulence of *Rhizoctonia solani* found that the addition of asparagine increased the virulence of *Rhizoctonia solani* (Weinhold et al. 1972). Since asparagine has a PO_4 group, it is possible that this PO_4 had some impact on the *Rhizoctonia solani* which may be comparable to the effect of PO_4 in this experiment. Alternatively, PO_4 addition may have altered the general microbial community structure towards a community that is less suppressive to *Rhizoctonia solani*. Although studies examining the direct effects of phosphorus application on disease suppression are limited, there have been some studies regarding the effects of phosphorus on the microbial community structure. Long term addition of phosphorus and potassium fertilizer in Northern China altered the microbial community structure towards a greater bacterial species richness (Ge et al. 2008). If these bacteria were non-suppressive, they may have allowed an increase in disease severity, as was seen in the results reported in this chapter.

As mentioned earlier in the literature review (Chapter 1) and Materials and Methods (Chapter 2), incubation with an added biologically available carbon source has been suggested as an assay to stimulate the soil micro-organisms involved in disease suppression and give a measure of the potential of a soil to biologically suppress disease (Roget et al. 1999), via increasing populations and activities of soil microorganisms. This next part of the discussion concerns shoot and root dry matter and root infection data from the nutrient treatments inoculated with *Rhizoctonia solani* AG8 and with added sucrose. The abiotic-biotic interactions in the plant-soil-pathogen continuum are considered and some hypotheses developed concerning likely biotic influences from these treatments.

There was a decrease in plant growth in the Minnipa soil for those treatments with the addition of sucrose compared to the treatments without sucrose which is considered probably due to immobilisation of the added available nitrogen. Similar immobilisation was demonstrated in a study using papermill processing residue, where with addition of the residues (a source of available carbon) to soil, nitrogen was immobilized to 0mg NO_3-N per kg of soil 14 days after the residue addition (Croteau et al. 1998). Thus, it seems likely that with the addition of sucrose, the populations of micro-organisms in the soil increased. As the populations of micro-organisms increased, they would require and use available nutrients (e.g. nitrogen) from the soil matrix. This would then decrease the amount of available nutrients for the plant, resulting in lower plant growth. Although the study using papermill residues indicated that addition of papermill residues did not alter the amount of nutrient uptake, the biomass of plants was lower with residue addition than those treatments without residue addition (Croteau et al. 1998). The decrease in biomass is similar to the observed decrease seen in plant dry weights for treatments in the Minnipa soil with added sucrose.

Addition of sucrose and no nutrients to the SB2 soil, markedly decreased the severity of Rhizoctonia disease. A recent review on suppression of soilborne fungal diseases with organic amendments found that in 45% of the studies using organic amendments, disease suppression was increased (Bonanomi et al. 2007). Additionally, and in contrast, they reported that studies specifically on *Rhizoctonia solani*, effective control of the disease was only reported in 26% of cases (Bonanomi et al. 2007). There are a limited number of studies on the effects of adding a simple carbon source (such as sucrose) on disease

suppression. Roget et al. (1999) reported that the addition of sucrose "accentuated the suppressive characteristics" of the Avon soil. Their results indicated that sucrose addition increase microbial biomass, activity and populations of active protozoa (Roget et al. 1999). Thus it is likely that the decrease in disease severity seen in the SB2 soil with addition of sucrose is due to an increase in disease suppression via increases in microbial biomass, activity and perhaps active protozoa (Roget et al. 1999). One mechanism driving these observed increases in disease suppression could be increased production of secondary metabolites. Disease suppression of *Rhizoctonia* using compost amendments in Impatiens was linked to increased microbial biomass carbon and nitrogen (Diab et al. 2003). In this work they proposed that the growth of micro-organisms lead to an enhanced production of secondary metabolites e.g. antibiotics which directly impacted the *Rhizoctonia* fungus (Diab et al. 2003). Alternatively, experiments using olive oil mill waste water, suggested that with the addition of a carbon source, the microbial community shifted towards fast growing bacteria (Kotsou et al. 2004). This shift in community structure was linked to *Rhizoctonia solani* suppression, via antagonism for nutrient and energy supplies (Kotsou et al. 2004). It is likely that sucrose addition altered the microbial community structure in the SB2 soil towards a community with greater disease suppression against Rhizoctonia root rot.

The effect of added biologically available carbon on suppressing disease severity over-rides the effect of added phosphorus on increasing disease severity. This effect is probably due to the increase in populations and activities of the soil microorganisms, including those involved in disease suppression (as discussed previously). Despite the decrease in disease severity with added available carbon, shoot dry weights did not increase. It is likely that the increase in microbial populations immobilized available nitrogen in a similar manner to experiments described earlier using paper mill residues as a carbon source (Croteau et al. 1998). The authors reported restricted plant growth similar to the lower shoot dry weights as seen in this C and P treatment for SB2 soil (Croteau et al. 1998). It is worth noting that decrease in disease severity with the addition of sucrose occurred despite the SB2 soil having more organic carbon than the Minnipa soil. The most likely explanation is that a large proportion of the native organic carbon in SB2 soil is recalcitrant and thus less available to the soil micro-organisms (Baldock 2002).

Therefore, the addition of sucrose as, a readily available carbon source, the microorganisms in the SB2 access to an energy source.

In the SB2 soil, the substantial decrease in disease severity after addition of a labile carbon source is negated when available nitrogen is added at the same time, and the effect of nitrogen form was also lost. Again, the most likely possibility for this is immobilization of nitrogen which would also result in less available nitrogen within the soil matrix to exert direct effects on the populations of soil organisms as described previously. For example, in the case of NH_4-N alone, disease severity decreased possibly due to direct effects of NH_4-N on the balance of suppressive and deleterious microbial populations (Sarniguet et al. 1992; Ownley et al. 2003). When NH_4-N was added in combination with an available carbon source, the rapid utilization of both carbon and nitrogen by increasing microbial populations would have resulted in no available NH_4-N to exert direct effects on the specific suppressive or deleterious microbial populations. In a similar manner, rapid utilization of NO_3-N and carbon would have decreased the negative impact that NO_3-N had on the suppressive organisms. Overall, the impact of nitrogen form with added sucrose on the severity of disease seems to be similar irrespective of soil type. In both soils, the addition of sucrose increases the disease severity for the NH_4-N treatment and decreases the disease severity for the NO_3-N treatment.

The work described in this chapter assessed whether nitrogen and phosphorus were a limitation to plant growth in these 2 EP soils and further how these added nutrients affected infection by Rhizoctonia root rot. Overall, plant growth was less constrained in the weakly calcareous Minnipa soil than in the highly calcareous SB2 soil. There were no plant responses to added orthophosphate or nitrate-nitrogen, only to ammonium-nitrogen in the Minnipa soil whereas in the SB2 soil there was a small but significant response to ammonium-nitrogen and a large response to orthophosphate applied at a relatively high rate. Addition of ammonium increased shoot growth in the absence of the pathogen in both soils; and shoot and root growth in the presence of the pathogen in Minnipa soil. Furthermore, in the Minnipa soil ammonium-nitrogen addition reduced *Rhizoctonia* infection, although this latter effect lessened when an available carbon source was also added. The major constraint in SB2 soil was available phosphorus, hence addition of this nutrient increased growth of shoots and roots in all treatments and also increased *Rhizoctonia* infection without compromising effects on plant growth. Available

carbon was identified as a potential constraint in SB2 soil since substantial reductions in Rhizoctonia root rot infection were observed in the nil and phosphorus nutrition treatments with added sucrose. These abiotic-biotic integrations in this agriculturally important SB2 soil warrant further investigation to test some of the hypotheses discussed regarding the influence of nutrients on the pathogen and other organisms associated with disease suppression.

The next two chapters in this thesis will focus on this highly calcareous soil. Chapter 7 will investigate the interactions between added orthophosphate at agronomic rates and the pathogen, as well as the effects of this orthophosphate on the soil microbial communities and how these manifest as disease suppression. The final research chapter (Chapter 8) will investigate interactions between phosphorus fertilizer additions and wheat stubble, the common organic matter and labile carbon input in these farming systems.

Chapter 7

Effects of added inorganic phosphorus on virulence of *Rhizoctonia solani* and population dynamics of soil microbial communities in a highly calcareous soil from the Eyre Peninsula

7. Effects of added inorganic phosphorus on virulence of *Rhizoctonia solani* and population dynamics of soil microbial communities in a highly calcareous soil from the Eyre Peninsula

7.1 Introduction

Increased disease severity after addition of inorganic phosphorus to the highly calcareous soil (SB2) was observed in the study reported in the previous chapter (Chapter 6) is pertinent in agricultural farming systems. On the Eyre Peninsula fertiliser P application is common practice in grain cropping systems. If these P applications are potentially increasing Rhizoctonia root rot severity, it may help explain why changes in farming systems that might be expected to reduce incidence of the disease, have not decreased the prevalence of Rhizoctonia root rot.

One hypothesis that arose from discussion of the work in the last chapter was that in the SB2 soil adding available P removed an abiotic limitation to *Rhizoctonia solani*, although it was not possible to distinguish whether it was the growth of the pathogen or the virulence with which it was able to infect plants that was affected. Virulence has been used to describe severity of disease caused by different isolates of the same pathogen, where inoculation of the specific pathogen isolates remains constant between treatments (Erper et al. 2006; Taheri et al. 2012). Virulence has also been used to describe differences in disease severity caused by different isolates of the same pathogen compared to an un-inoculated control (Schroeder et al. 2011). In this thesis work, virulence is used to describe disease severity caused by the same pathogen isolate for each quantified unit of pathogen in the soil.

It was also hypothesised that the effects of additional available P on Rhizoctonia root rot may be linked to shifts in microbial community structure associated with a decline in suppressive organisms. Addition of nutrients to soil has been linked to changes in microbial biomass C and N which are linked to microbial community structure (Wardle 1992). Specific addition of P in old-forest soils has been reported to shift the microbial community structure, while in the mixed forest there was no change in the microbial community structure (Liu et al. 2012). The work in this experiment aimed to evaluate

changes in to microbial community structure using carbon source utilization profiles after application of P.

The work in this chapter will explore these aspects for a range of P rates more applicable to the farming systems on the EP where the normal rates of P addition are between 5-10mg P/kg soil.

The specific aims of the work in this data chapter were to investigate;

(1) population dynamics of *Rhizoctonia solani* with increasing rates of P application using DNA technology,

(2) whether increasing rates of P application altered the virulence of *Rhizoctonia solani*,

(3) population dynamics of potentially suppressive organisms with increasing rates of P application using DNA technology, and

(4) whether or not disease severity could be linked to changes in the overall microbial community structure using catabolic diversity profiles.

7.2 Materials and methods

The bioassay experiment was carried out using procedures described in the materials and methods chapter (Chapter 2). Air-dried soils were moistened with their respective phosphorus nutrient solution treatment (details below) and mixed well. This moist soil was then placed into bioassay pots and kept moist at 75% WHC with regular application of sterilised reverse osmosis water, for the growth period. At the start of the experiment *Rhizoctonia solani* AG8 inoculum (Chapter 2, Section 2.3) was added at two plugs per pot (5cm deep in a 10cm high pot) and each treatment was replicated six times.

The P nutrient solution treatments were 5mg P / kg soil, 25 mg P / kg soil and 50mg P / kg soil as Disodium Hydrogen Phosphate (Na_2HPO_4) dissolved in sterile reverse osmosis water. The healthy and disease control treatments had no nutrient solution added.

Additional bioassay pots allocated for soil sampling to allow DNA analysis of specific organisms were prepared (described in Materials and Methods Section 2.4). Six replicate soil samples from each of the following nine different treatments were sent for DNA

extraction and analysis (as described in Materials and Methods Section 2.6.2). These were:

(1) soil from the initial pot with pathogen load added as two agar plugs (without added P),

(2) soil from pots with added *Rhizoctonia solani* inoculum with the following P treatments after 2 weeks incubation (prior to plants being grown):

a. 0 mg P / kg soil,
b. 5 mg P / kg soil,
c. 25 mg P / kg soil, and
d. 50 mg P / kg soil,

(3) soil from pots incubated for 2 weeks as above with added *Rhizoctonia solani* inoculum with the following P treatments plus another 4 weeks for plant growth:

a. 0 mg P / kg soil,
b. 5 mg P / kg soil,
c. 25 mg P / kg soil, and
d. 50 mg P / kg soil).

Furthermore, from each pot allocated for DNA analysis after incubation with *Rhizoctonia solani* at 2 weeks and for similar pots without *Rhizoctonia solani* (not for DNA analysis), a sub-sample of soil was removed for microbial community analysis using carbon source utilization profiles (Materials and Methods Section 2.6.3). The sampling regime is summarised in Figure 1.

Disease severity was measured as percent root infection and root score (Described in detail in Materials and Methods Section 2.4.4). Plant growth was estimated using root and shoot dry weights which were measured as described in detail in Materials and Methods Section 2.4.4.

Pathogen virulence, a measure of disease severity per unit of pathogen was estimated as

$$\frac{\text{Percent Root Infection}}{\log_{(10)} \text{pg } \textit{Rhizoctonia} \text{ DNA}_{(2\text{weeks})}}$$

The effect of the disease severity on plant growth was estimated as

$$\frac{\text{(un-inoculated plant dry weight – inoculated plant dry weight)}}{\text{PRI}}$$

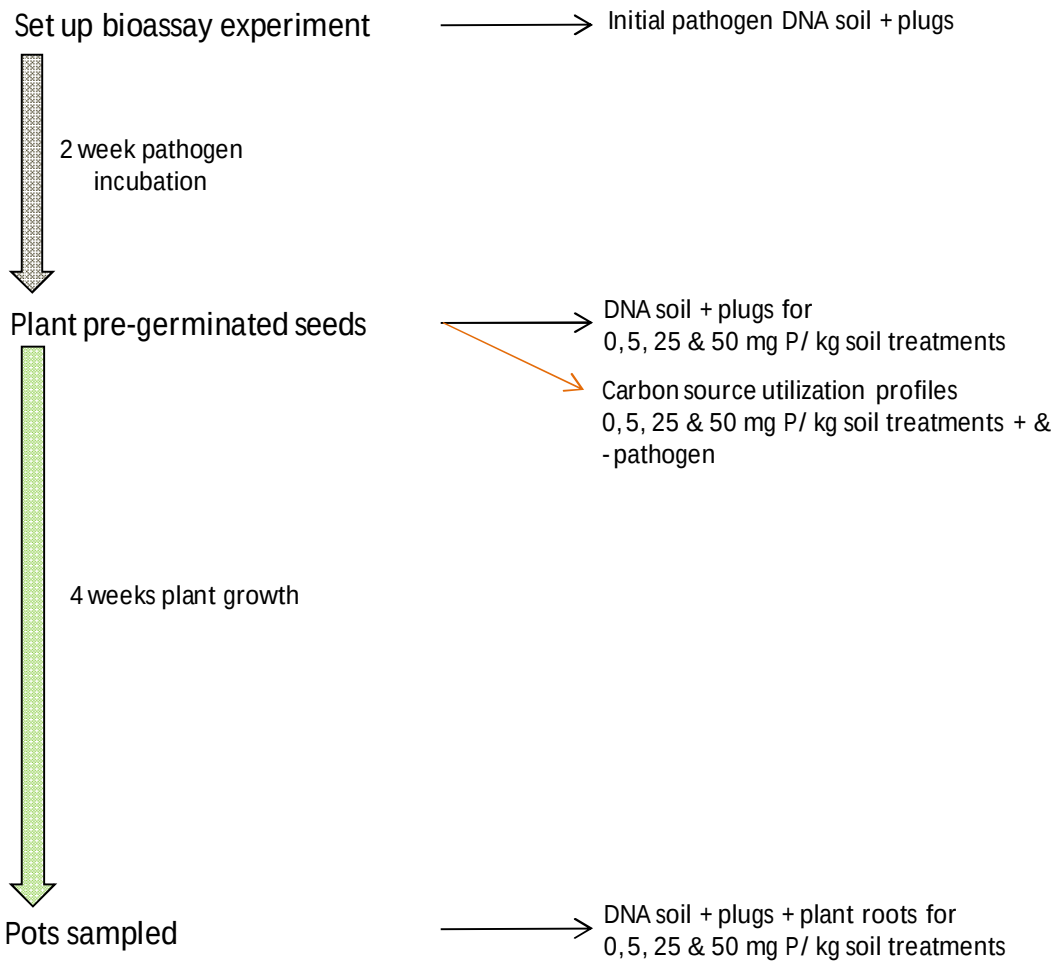


Figure 7.1: Schematic diagram showing when DNA analysis and carbon source utilization profiles were undertaken during this bioassay.

7.3 Results

7.3.1 Effect of increasing P application rates on disease severity and plant growth

Disease severity in the healthy control plants, i.e. those without *Rhizoctonia solani* inoculum, was relatively low (<11%) and was not significantly influenced by added inorganic P (Table 7.1). Plant dry weight data shows that both shoot and root growth are P limited in this soil. Compared to the control treatment, root dry weight was 11% greater in the 25mg P treatment, and 24% greater in the 50mg P treatment, additionally, the 50mg P treatment was significantly greater than the 25mg P treatment (Figure 7.2). Shoot dry weight data trends were similar to root dry weight data trends, with larger differences

between P treatments and the control treatment of 18% in 25mg P and 30% in the 50mg P treatment (Figure 7.2).

Table 7.1: Percent root infection and root scores (0-5 scale, where 0 = no disease and 5 = maximum disease) for Rhizoctonia root rot measured on plant roots after 4 weeks growth in un-inoculated bioassay pots, and addition of 0, 5, 25 or 50 mg P/kg soil (healthy controls).

P Addition (mgP/kg soil)	0	5	25	50	P Value
Percent Root Infection	10.7	1.7	5.2	6.8	0.345 (ns)
Root Score	0.40	0.07	0.22	0.20	0.354 (ns)

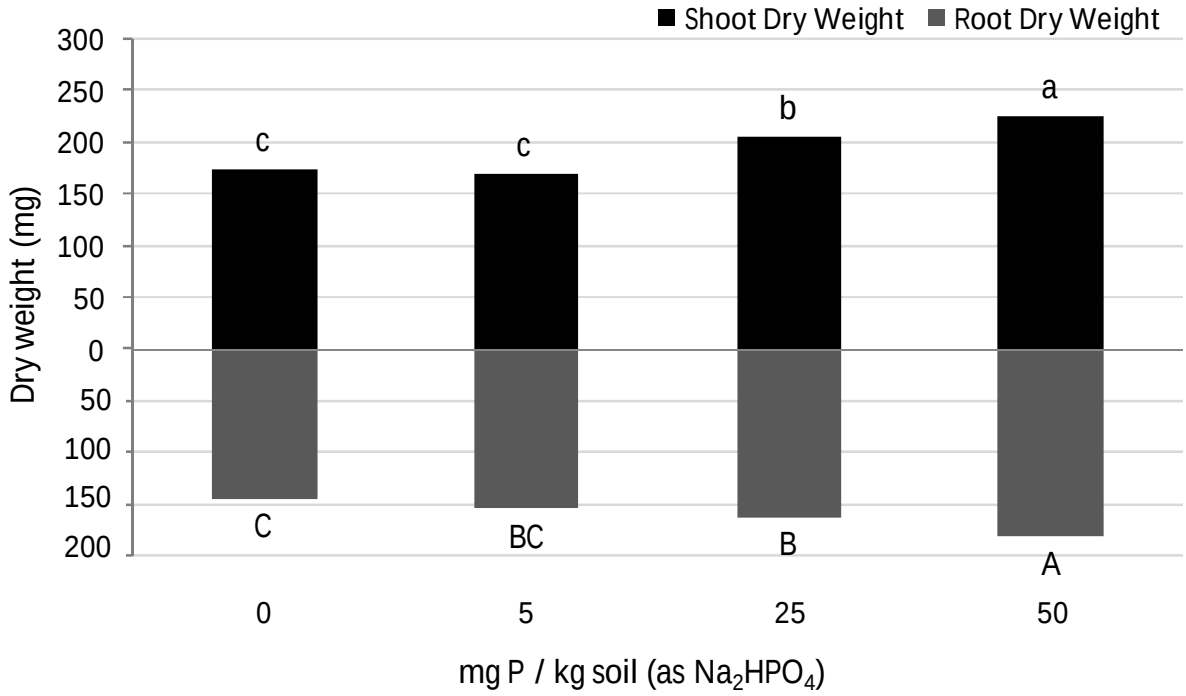


Figure 7.2: Root and shoot dry weight for plants grown in a highly calcareous soil (Streaky Bay 2) with different amounts of P added as orthophosphate (0, 5, 25 & 50 mg P / kg soil). Different letters show significant differences for the treatment means at $lsd_{(0.05)}$.

Disease severity measured on roots of the plants grown with *Rhizoctonia solani* inoculum was much greater (PRI range 58-80%) than in the healthy control treatments (no

pathogen addition). All treatments with added P had greater measured percent root infection than the disease control which did not have P addition (Figure 7.3). Root dry weight was not significantly affected by the severity of disease and all treatments with P addition as well as the disease control did not show differences in root dry weight (Figure 7.4). Shoot dry weight was responsive to P despite the severity of root disease. The disease control and 5 mg P treatments had similar shoot dry weights, which were also similar to the healthy control shoot dry weights for the same treatments (Figure 7.4). The 25mg P treatment had significantly greater shoot dry weight (40%) than the disease control, and the 50mg P treatment a 95% increase (Figure 7.4).

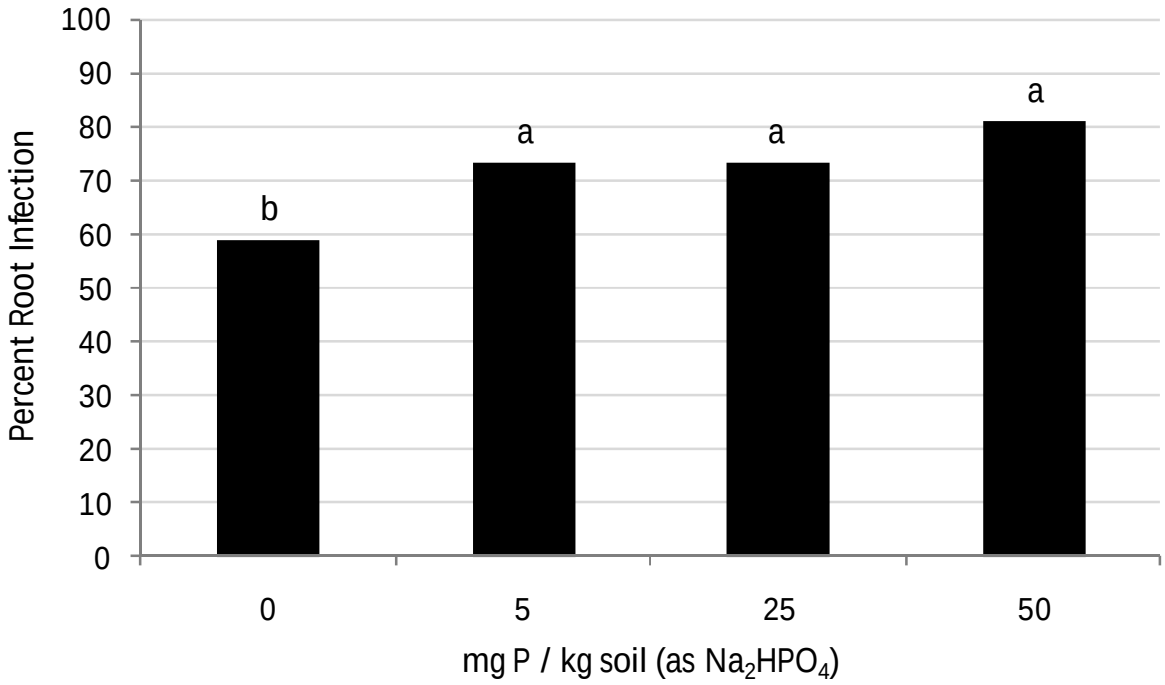


Figure 7.3: Percent root infection measured on roots of plants grown in Streaky Bay 2 soil after inoculation with 2 agar plugs of *Rhizoctonia solani* AG8 and addition of P (as orthophosphate) at different rates (0, 5, 25 and 50 mgP / kg soil). Different letters show significant differences between treatment means at l.s.d. = 0.05.

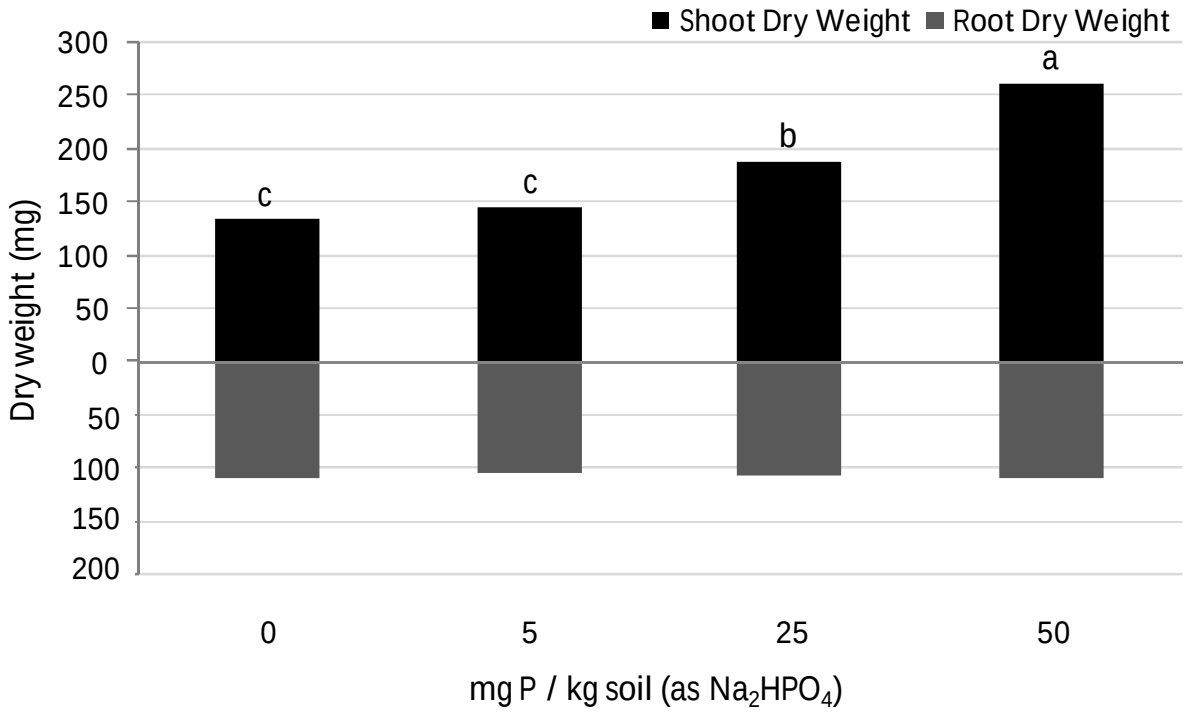


Figure 7.4: Root and shoot dry weight for plants grown for 28 days in the Streaky Bay 2 soil. All pots were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 and P (as orthophosphate) added at different rates (0, 5, 25 and 50 mg P / kg soil). Different letters show significant differences for treatment means at l.s.d.= 0.05.

7.3.2 Effect of P application rate on DNA of Rhizoctonia solani

After incubating soil inoculated with *Rhizoctonia solani* inoculum and amended with P for 2 weeks, the amount of DNA for *Rhizoctonia solani* AG8 decreased in the unamended control soil compared to the soil at initial inoculation with agar plugs (Figure 7.5). The treatments amended with P did not have *Rhizoctonia solani* DNA amounts different from that of the initial pot and plug, although there was a distinct trend with DNA amount in the 5 and 25mg P/ kg soil treatments lower than that in the 50mg P/ kg soil treatment (Figure 7.5).

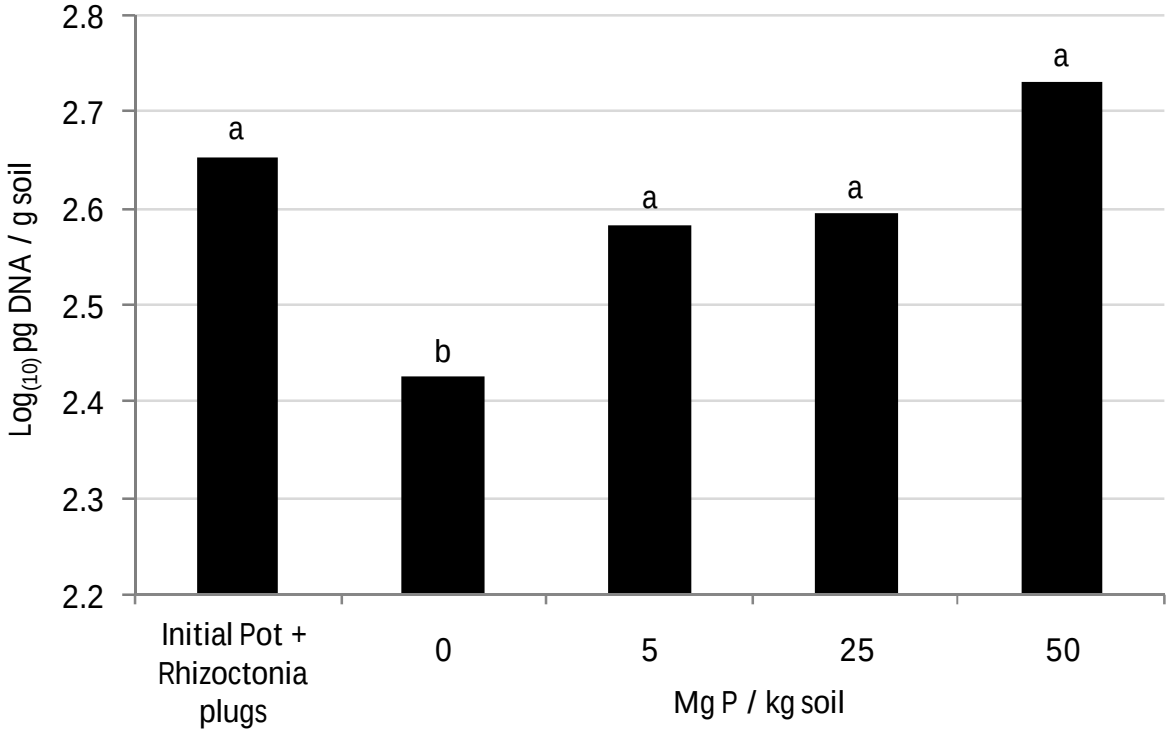


Figure 7.5: Amount of *Rhizoctonia solani* AG8 DNA (Log $_{(10)}$ pg DNA per g soil) from an inoculated pot + 2 agar plugs and from pots with different rates of phosphorus addition as orthophosphate (0, 5, 25 and 50 mgP / kg soil) after incubation with 2 agar plugs of *Rhizoctonia solani* AG8 for two weeks in a controlled environment room. Different letters show significant differences for the treatment means at an l.s.d = 0.05.

7.3.3 Effects of increasing rates of P application on virulence of Rhizoctonia solani

The virulence of *Rhizoctonia solani* in this grey calcareous soil did not change significantly with addition of P (Table 7.2). That is, the severity of disease caused by each $\log_{(10)}$pg of *Rhizoctonia solani* DNA was no different for each mg of P applied per kg soil.

Disease severity data from this experiment suggests that increasing P application reduces the impact that each unit of Rhizoctonia root rot has in terms of plant growth depression (Figure 7.6).

Table 7.2: Virulence (percent root infection / $\log_{(10)}$ pg *Rhizoctonia solani* AG8 DNA g soil $_{(2weeks)}$) and reduction in plant dry weight (mg) for plants grown in bioassay pots inoculated with 2 agar plugs of *Rhizoctonia solani* AG8. Different letters show significant differences between treatment means at P= 0.05.

mg P added per kg soil	0	5	25	50	l.s.d.$_{(0.05)}$
Virulence	24.79	28.72	28.82	30.43	ns
Reduction in Plant Dry Weight (mg)	-23.4 a	-21.6 ab	-19.8 bc	-8.1 c	12.68

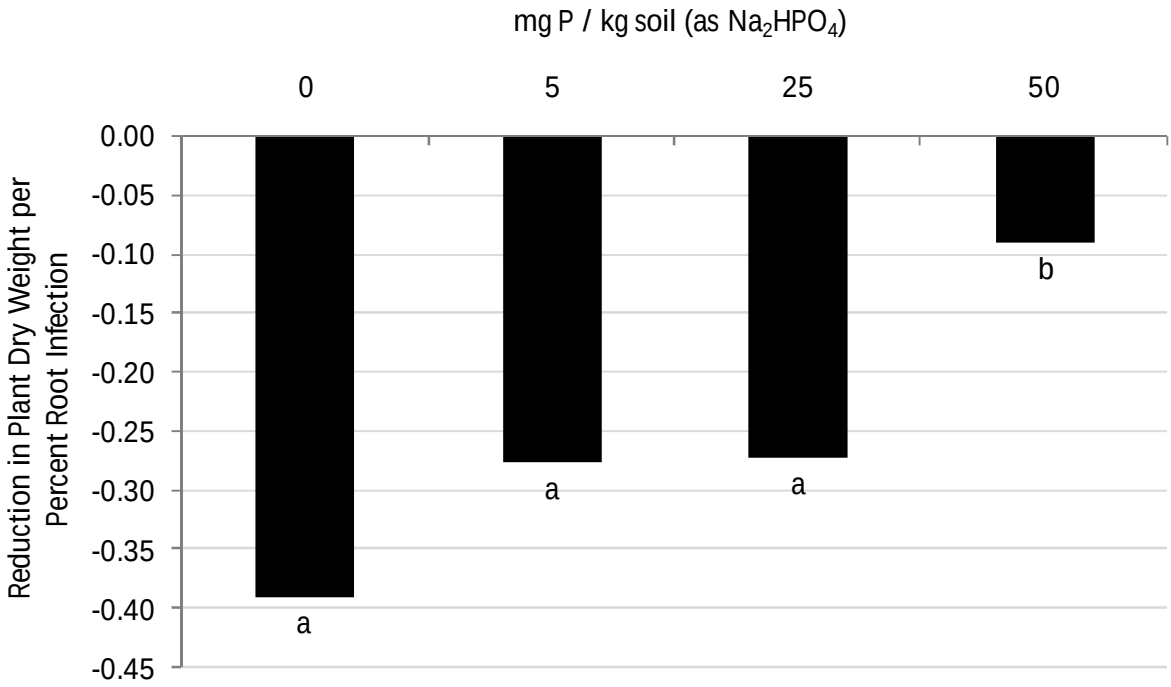


Figure 7.6: Reduction in plant dry weight (mg) per percent root infection for plants grown in Streaky Bay 2 soil with addition of P as orthophosphate at different rates (0, 5, 25 and 50 mg P/ kg soil). This was calculated as ((plant dry weight from *Rhizoctonia solani* AG8 inoculated pots) minus (plant dry weight from healthy controls which were not inoculated with *Rhizoctonia solani* AG8) per percent root infection for pots inoculated with *Rhizoctonia solani* AG8). Different letters indicate significant differences at l.s.d.=0.05.

7.3.4 "Beneficial organisms" and microbial community structure determined by carbon source utilization profiles

After the two week incubation period in the soil not inoculated with *Rhizoctonia solani* the DNA amounts for *Pantoea agglomerans* were not affected significantly by addition of P (Table 7.3). The populations of Microbacterium spp. decreased in the treatment with 50mgP compared to 0mgP (Table 7.3). *Trichoderma* spp. were absent in all treatments (Table 7.3).

Table 7.3: Amounts of DNA ($log_{(10)}$ pg DNA per g soil) for 'beneficial organisms' (*Pantoea agglomerans*, *Microbacterium* spp. and *Trichoderma* group A and B) isolated from the initial bioassay soil after 2 weeks incubation with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum and varying rates of P application (0, 5, 25 and 50 mg P / kg soil). Different letters show significant differences between treatment means at P= 0.05

mg P added per kg soil		0	5	25	50	$l.s.d_{(0.05)}$
Pantoea agglomerans	0.83	0.97	1.00	1.21	0.92	ns
Microbacterium spp.	4.06 a	4.08 a	4.10 a	4.01 ab	3.95 b	0.07
Trichoderma group A	0	0	0	0	0	
Trichoderma group B	0.75	0	0	0	0	

Using CO_2 evolution as an estimate of microbial activity, microbial activity increased with increased application of P in the water only treatment. For all types of carbon source added, the main effect of *Rhizoctonia solani* AG8 inoculum was to increase CO_2 production suggesting an increase in utilization of the carbon sources by the *Rhizoctonia solani* AG8 inoculum (Table 7.4). Overall, CO_2 evolution for all amino acid groups (Secondary, Neutral, Basic, Acidic and Sulphur) increased with increasing P application. The CO_2 evolution significantly ($p<0.05$) increased with increasing P application for 3 types of carbon source; neutral amino acids, basic amino acids and sulphur amino acids. This may indicate a specific preference for type of carbon source by the *Rhizoctonia solani* AG8 inoculum (Table 7.4).

Table 7.4: Average carbon dioxide evolution for carbon sources grouped into chemical types measured by carbon source utilization profiles from soil taken after two weeks incubation with (+) and without (-) 2 agar plugs of *Rhizoctonia solani* AG8 inoculation and different rates of P application (0, 5, 25 and 50 mg P / kg soil). Numbers in bold show lsd values for main effects of *Rhizoctonia* inoculation (Rs) & phosphorus application (P) for those chemical types which had significant differences at P<0.05.

Rhizoctonia plugs	+	+	+	+	-	-	-	-	$lsd_{(0.05)}$	
P (mg/kg soil)	0	5	25	50	0	5	25	50	P	Rs
Monosaccharide	0.864	0.762	0.848	0.836	0.398	0.366	0.423	0.380		**0.087**
Oligo-saccharide	0.740	0.751	0.744	0.773	0.392	0.359	0.382	0.333		**0.080**
Secondary aa	0.305	0.316	0.352	0.397	0.113	0.109	0.123	0.113		**0.045**
Neutral aa	0.379	0.428	0.472	0.526	0.172	0.167	0.188	0.155	**0.058**	**0.042**
Basic aa	0.164	0.177	0.214	0.236	0.092	0.091	0.098	0.097	**0.031**	**0.049**
Acidic aa	0.654	0.686	0.660	0.828	0.120	0.149	0.165	0.169		**0.095**
Sulphur aa	0.250	0.266	0.312	0.358	0.094	0.087	0.104	0.099	**0.042**	**0.031**
Carboxylic acid	0.710	0.751	0.761	0.822	0.341	0.371	0.453	0.386		**0.057**

Multidimensional scaling analysis based on Bray-Curtis similarity discriminated between + and – *Rhizoctonia solani* inoculation (MDS stress value 0.14), but not between P application rates for carbon source utilization profiles after 2 weeks incubation in bioassay pots (Figure 7.7). There was no interaction between P and *Rhizoctonia solani* inoculation, nor was there a significant effect of different rates of P addition. Cluster analysis (Figure 7.8) indicates that + and –*Rhizoctonia solani* inoculum treatments were 90% similar. There does not appear to be any clear effect of P either with or without *Rhizoctonia* inoculation, suggesting that there is little effect of P on the microbial community structure as measured by carbon source utilization profiles.

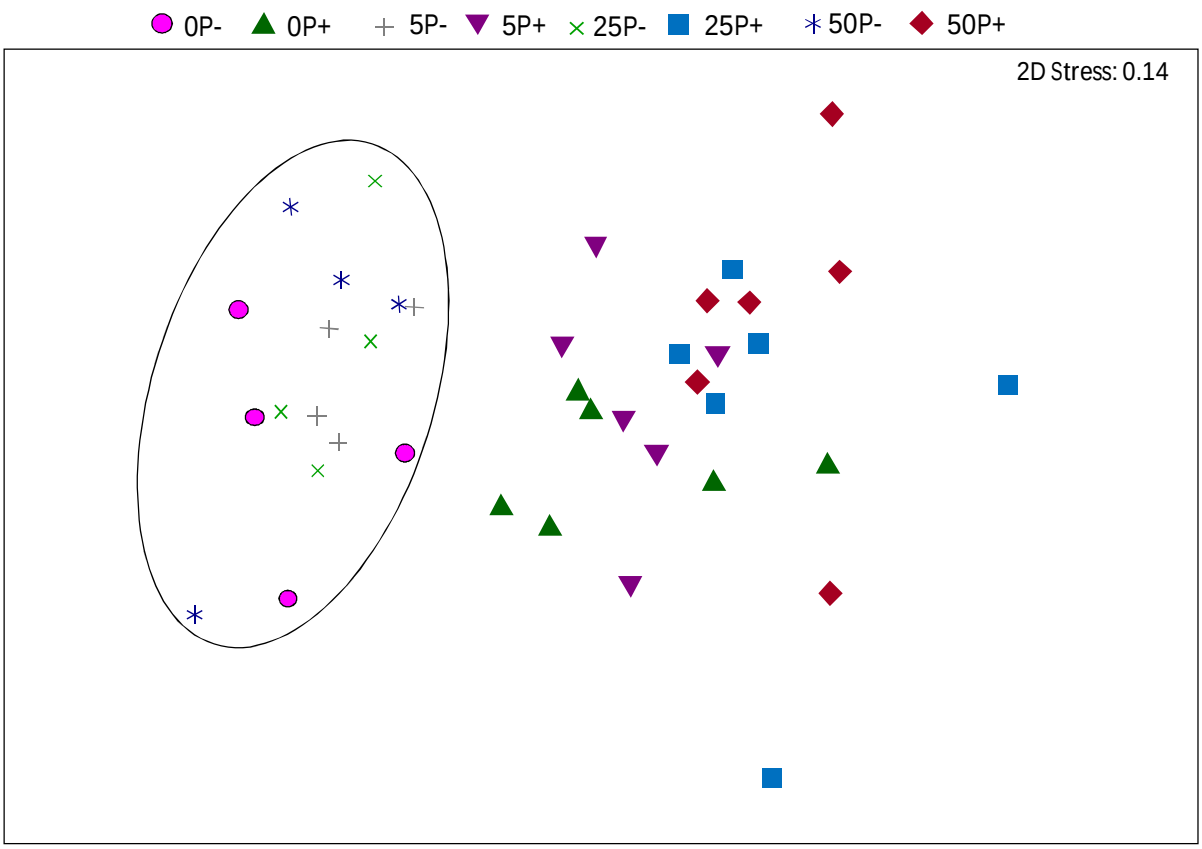


Figure 7.7: Multidimensional scaling plot based on Bray Curtis similarities for log(x+1) transformed carbon source utilization data from soils after 2 weeks incubation with (+) and without (-) 2 agar plugs of *Rhizoctonia solani* AG8 inoculum and different rates of phosphorus addition (0P, 5P, 25P and 50P mg P/ kg soi). There was a significant difference (indicated by oval grouping) between *Rhizoctonia solani* AG8 inoculated and un-inoculated treatments (P (permanova) 0.001). Neither phosphorus nor the interaction between phosphorus and *Rhizoctonia solani* AG8 inoculum were significant.

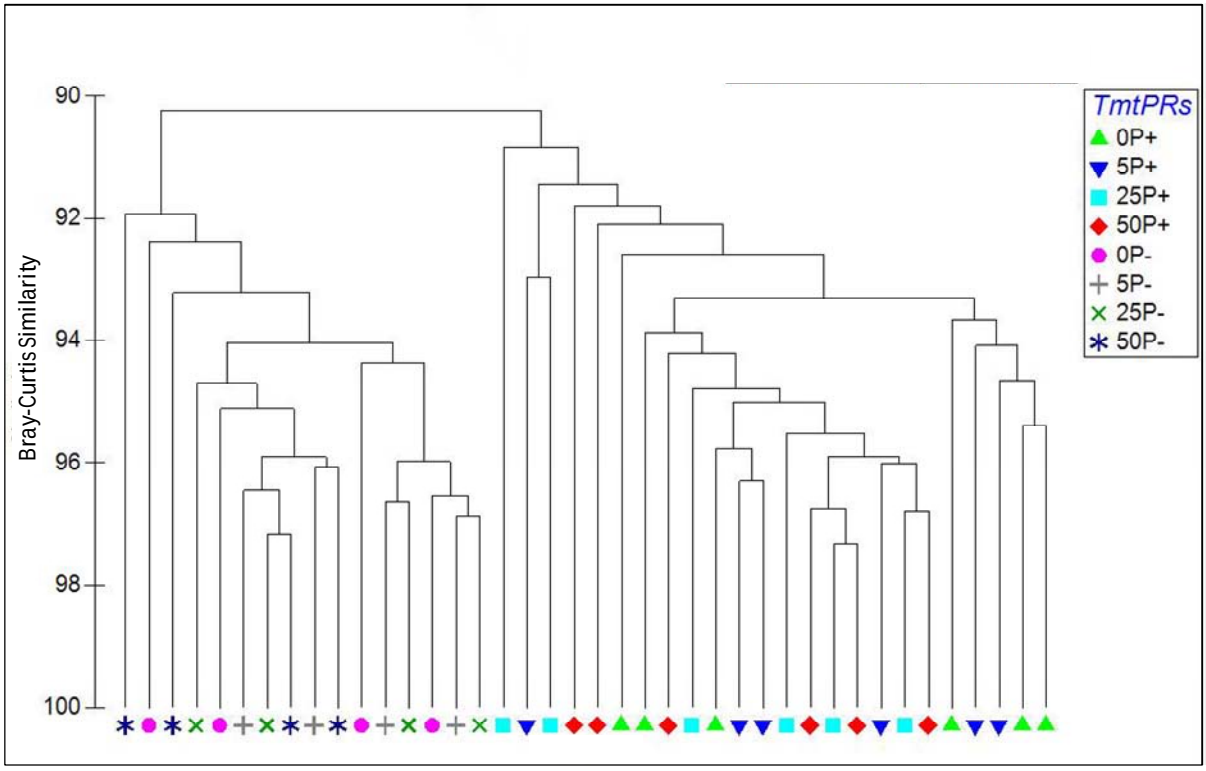


Figure 7.8: Cluster analysis showing Bray-Curtis similarity between carbon source utilization profiles for *Rhizoctonia solani* AG8 inoculated (+) and un-inoculated pots (-) with varying rates of phosphorus application (0P, 5P, 25P & 50P mg / kg soil) after 2 weeks incubation in bioassay pots.

7.3.5 Interactions between plant, pathogen & beneficial organisms in the bioassay

The amount of DNA of *Microbacterium* spp. increased during the four weeks of the bioassay (ie when plants were grown) from 4.24 $\log_{(10)}$ pg DNA/g soil to 4.63 $\log_{(10)}$ pg DNA/g soil with increasing P application (Table 7.5). There were no changes in the amount of *Pantoea agglomerans* DNA nor was there a notable presence of *Trichoderma* spp. DNA (Table 7.5). *Rhizoctonia solani* DNA (*Rhizoctonia* DNA $_{(4\ weeks)}$) increased with increasing addition of P (Table 7.5). The amount of *Rhizoctonia solani* DNA in the 25mg P treatment was greater than in the disease control treatment, while the *Rhizoctonia solani* DNA in the 50mg P treatment was even greater than the amount of *Rhizoctonia solani* DNA in the disease control treatment (Table 7.5).

Table 7.5: Amounts of DNA ($log_{(10)}$ pg DNA per g soil) for specific beneficial organisms (*Pantoea agglomerans*, Microbacterium spp. and *Trichoderma* group A and B) and *Rhizoctonia solani* AG8 in soil isolated from bioassay pots after 4 weeks growth of wheat plants. Soils inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 and with varying rates of P application (0, 5, 25 and 50 mg P / kg soil). Different letters show significant differences between treatment means at P= 0.05

mg P added per kg soil	0	5	25	50	l.s.d.$_{(0.05)}$
Pantoea agglomerans	0.80	0.76	0.95	0.80	ns
Microbacterium spp.	4.24 d	4.34 c	4.50 b	4.63 a	0.07
Trichoderma group A	0.69	0	0.36	0.72	
Trichoderma group B	0	0	0	0	
Rhizoctonia solani AG8$_{(4weeks)}$	3.6 c	3.7 c	3.9 b	4.1 a	0.09

7.4 Discussion

Overall, the results from this experiment show that the addition of orthophosphate P in a highly calcareous soil increased the amount of pathogen (detected in the soil as *Rhizoctonia solani* DNA) and severity of Rhizoctonia root rot, as assessed from root symptoms (PRI/root score) , but did not affect the virulence of the pathogen. Shoot dry weight also increased with increasing P application, despite the increase in disease severity. Although, the addition of P increased carbon dioxide evolution (microbial activity) in the presence and absence of *Rhizoctonia solani* inoculum, the increase could not be linked to shifts in the microbial community structure determined from carbon source utilization profiles. Effects of P addition on a selection of specifically isolated beneficial organisms were inconclusive with only Microbacteria showing any significant differences, decreasing in uninoculated soils and increasing in the bioassay in response to P.

Root disease symptoms and root and shoot dry weights for healthy control and inoculated treatments were consistent with the results of the previous chapter (Section 6.3). That is, increasing P application increased the disease severity as well as shoot dry weight. Since the previous chapter (Chapter 6) discussed in detail the effects of added P

on shoot growth in highly calcareous soil, this discussion will focus on possible mechanisms driving the increase in disease severity.

The hypothesis that P fixation due to high calcium carbonate content in SB2 soil was likely to be limiting P availability for the soil micro-organisms specifically *Rhizoctonia solani* appears to be supported by the results from the work in this chapter. In the previous chapter (Chapter 6) the results indicated that P was limiting for *Rhizoctonia solani* AG8 but it was unclear whether P was altering *Rhizoctonia solani* AG8 growth or virulence. Whereas data from this work suggests that P is indeed limiting for *Rhizoctonia solani* growth as measured by DNA isolation. The data also suggests that microbial activity (determined by CO_2 evolution) is also limited by P availability which is alluded to in other work done in P limited soils from moist tropical forests in Costa Rica (Cleveland et al. 2002) and tropical pastures in Mexico (Galicia et al. 2004). In the forest soils, addition of P increased microbial activity over a 30 day period (Cleveland et al. 2002), while in the pasture soil, addition of P increased both microbial activity (CO_2 evolution) and C-immobilization in the microbial biomass after 20 days (Galicia et al. 2004). Although neither of the soils in these studies were highly calcareous, it is probable that a similar phenomenon is occurring in the highly calcareous SB2 soil in this work due to P fixation. Joergensen et al. (1999) stated that it was presumable soil micro-organisms were P limited in a calcareous soil due to the formation of Ca-phosphates. The data here agrees with these studies and suggests that P is limiting microbial activity (CO_2 evolution) and microbial biomass (populations) for some specific species e.g. *Rhizoctonia solani* AG8 in the SB2 soil.

The second aim of this study was to investigate if virulence of *Rhizoctonia solani* was altered by P application. The data suggests that virulence (an integration of symptoms and plant growth) was not altered by increasing P application to soil (Table 7.2) but that the increase in disease severity was a direct result of an increase in pathogen inoculum with increasing P application. Results from studies specifically looking at disease severity and inoculum density of *Rhizoctonia solani* AG8 in American soils and *Rhizoctonia solani* AG4 in Cuba, found that increasing inoculum density increased the severity of disease (Schroeder et al. 2008; Nerey et al. 2010). Although not a direct study on inoculum density and disease, Smith et al. (2003) used a green bridge to increase inoculum density in field soils. In their trial, the plots which had higher inoculum density had consistently

higher disease severity (Smith et al. 2003). Interestingly, research done on *Rhizoctonia* in radish suggests that both conducive and suppressive soils had an increase in disease severity with increasing inoculum density (Wijetunga et al. 1979).Therefore it is not surprising that in the SB2 soil, an increase in inoculum density increased the disease severity. This data also supports results from Chapter 3 the preliminary bioassay experiments, where increasing the number of *Rhizoctonia solani* plugs per pot increased the severity of disease on wheat roots.

The third aim of this study was to investigate the response of populations of specific potentially suppressive soil organisms to additions of P, and in contrast to the *Rhizoctonia* DNA, DNA data for these potentially suppressive organisms suggests that populations of the specific organisms isolated did not increase in response to addition of P after incubation for two weeks. In fact, there was a decrease in Microbacteria DNA with increasing P application. Cleveland et al. (2002) suggested in less fertile oxisol forest soils microbial respiration was more limited by available C than by available P. Since *Rhizoctonia solani* is known to be highly saprophytic (Bonanomi et al. 2007; Bonanomi et al. 2010), it is likely that in SB2 soil, Rhizoctonia can access the more recalcitrant carbon sources and thus is P limited rather than C limited. It is hypothesised that available carbon is more limiting than available P for the specific potentially suppressive organisms quantified (e.g. Microbacteria and *Pantoea*) in the hostile SB2 soil. This hypothesis will be further explored in the work described in the next chapter (Chapter 8) which will examine the interaction of C added in the form of stubble plus fluid fertilizer P on Rhizoctonia root rot severity and the associated populations of organisms in soil.

A notable result from the DNA analysis of these beneficial organisms is the absence of *Trichoderma* species in this highly calcareous soil. Trichoderma species are well documented antagonistic fungi, to a variety of pathogens including *Rhizoctonia solani* (Reviewed by Grosch et al. 2006; Verma et al. 2007; Vinale et al. 2008), as well as being plant growth promoting micro-organisms (Duffy et al. 1997; Avis et al. 2008; Vinale et al. 2008). Studies on *Rhizoctonia solani* AG2-2 in sugar beet found that populations of antagonistic *Trichoderma* species were higher in disease patches (Anees et al. 2010). The authors suggest that these disease patches became less conducive to disease in the following seasons due to the increase in antagonistic *Trichoderma* populations (Anees et al. 2010). This lack of *Trichoderma* in the SB2 soil studied here may help explain why

Rhizoctonia root rot continues to be such a persistent problem in farming systems on this soil type in particular on the Eyre Peninsula. Another contributing factor to the absence of *Trichoderma* DNA in this soil may be inherent abiotic characteristics of the soil. Although relating to Take-all rather than *Rhizoctonia solani*, *Trichoderma koningii* was reported to be less effective at suppressing Take-all disease under conditions which are inherent in SB2 soil, namely high pH and a sandy textured soil (Duffy et al. 1997).

The measured microbial community structure was not shifted by P application, but rather by inoculation with *Rhizoctonia solani*. These results are comparable to a study on field soils in France which were infested with *Rhizoctonia solani* AG2-2 (Anees et al. 2010). DNA analysis on soil from the diseased patch had high inoculum densities and would be comparable to +*Rhizoctonia* treatments, while the healthy areas had low inoculum and thus comparable to – *Rhizoctonia* treatments (Anees et al. 2010). Community structures determined using Biolog EcoPlates clearly distinguished between diseased and healthy areas (Anees et al. 2010), which could be considered comparable to the + and – *Rhizoctonia solani* inoculum treatments in the bioassay pots in this present study.

Microbial community structure data from the – *Rhizoctonia* treatments, indicates that with addition of a variety of carbon sources, CO_2 evolution did not increase with increasing P application. Galicia et al. (2004) found that limitations in microbial activity and microbial biomass due to C, N and P in combination or alone differed depending on the specific soil environment. In their work, they found that nitrogen application alone and the combination of nitrogen, P and carbon was limiting for C mineralisation in pasture soils (Galicia et al. 2004). Data from the last 3 bioassay chapters suggests that perhaps in the SB2 soil, it is the combination of nitrogen, phosphorus and carbon limiting microbial activity, rather than any single nutrient alone.

Data from treatments which had + *Rhizoctonia* inoculum suggest that *Rhizoctonia* alone or the resultant microbial community structure (+Rs as shifted from –Rs) has a greater capacity to utilize amino acids with increasing P application. *Rhizoctonia solani* as a saprophyte has the ability to produce extracellular enzymes (Sneh et al. 1996). Perhaps it is these extracellular enzymes contributing to amino acid break down and the subsequent release of available N and C, which in combination with the P added in this experiment, increased microbial activity. Research on ectomycorrhizal fungi suggested that activity of

cellobiohydrolase, laccase and chitinase were related to the nature of substrates and ecological niches (Buee et al. 2007). It may be that, in a similar manner to ectomycorrhizal fungi, addition of available P leads to *Rhizoctonia* inoculum alone or in combination with other soil organisms switching on genes to release enzymes for the breakdown of amino acids. The subsequent nitrogen and carbon products would then have been available to the soil microorganisms, resulting in the increase in CO_2 evolution seen in this data set. This may partially explain the observed increase in *Rhizoctonia solani* DNA, compared to organisms such as Microbacteria and *Pantoea*.

After 4 weeks of a growing plant, the *Rhizoctonia solani* DNA increased from initial amount of DNA after incubation for 2 weeks as well as increasing with increasing P application. Clearly in all pots, growing wheat roots are highly likely to release C rich exudates into the soil providing the *Rhizoctonia* inoculum with readily available carbon substrates to use for growth and infection. This would be equivalent to the "green bridge" alluded to in field conditions (Roget 1987) as discussed in the Literature Review (Chapter 1). In the field, grassy weeds are reported to have high rates of infection by *Rhizoctonia solani*, and are therefore sources of large quantities *Rhizoctonia solani* inoculum (Rovira 1990; Anees et al. 2010). In these pot bioassays, growing wheat roots would provide a source of carbon rich exudates and also a host for infection. Both contribute towards an increase in *Rhizoctonia solani* inoculum as seen by increases in amounts of DNA in this data set. Also, the relative differences in *Rhizoctonia solani* DNA after 4 weeks of root growth with increasing P application is likely to be a function of:

a) Higher initial amounts of DNA in higher P treatments at the start i.e. after 2 weeks incubation,

b) Larger quantities of root material with increasing P application, providing increased carbon rich root exudates as well as larger root area to host Rhizoctonia root rot infection.

Since Microbacteria are commonly associated with the rhizosphere of plants (Barnett et al. 2006) it is not surprising that their populations increased after 4 weeks of plant growth as seen in this work and seen in the young root carbon treatment in Chapter 5. What is unexpected is that the presence of roots does not appear to have significantly affected *Pantoea agglomerans* populations. This would suggest that neither carbon nor

phosphorus is limiting population growth of *Pantoea agglomerans*. It also implies that there is likely to be an additional constraint or constraints in the SB2 soil preventing the growth and activity of some soil organisms, including *Pantoea agglomerans*. If *Pantoea agglomerans* and the other soil organisms with limited growth and activity are those that contribute to disease suppression of *Rhizoctonia solani*, then these results help to explain why Rhizoctonia root rot continues to be prevalent in highly calcareous soils from the Eyre Peninsula.

In summary, the combination of stable virulence and *Pantoea agglomerans* populations, increase in microbial activity with increasing addition of P and lack of *Trichoderma* populations as indicators of a disease suppressive biotic community suggests that the biotic component of this highly calcareous SB2 soil may not be highly supportive of suppression to Rhizoctonia root rot. Despite a difference in the community structure between +*Rhizoctonia* and – *Rhizoctonia* treatments, there was no effect of P on community structure within either + or – *Rhizoctonia* treatments. This implies that addition of P does not "turn off" the suppressive communities, but that the suppressive communities may either not be functioning due to other unknown constraints (abiotic or biotic) or that the community itself is not suppressive.

Chapter 8
Effects of stubble input and P fertilizer on *Rhizoctonia solani* AG8 DNA, Rhizoctonia root rot severity, DNA of beneficial organisms and community structure

8. Effects of stubble input and P fertilizer on *Rhizoctonia solani* DNA, Rhizoctonia root rot severity, DNA of beneficial organisms and microbial community structure

8.1 Introduction

Data from the previous chapters in this thesis indicates that application of P to a highly calcareous soil increased the amount of *Rhizoctonia solani* inoculum and subsequent severity of Rhizoctonia root rot, albeit without compromising shoot dry matter (Chapter 7). Conversely, the addition of P with a biologically available carbon source (sucrose) decreased the severity of Rhizoctonia root rot (Chapter 6). As mentioned in Chapter 5 there are 2 key inputs of C that can be considered to influence the ultimate expression of disease suppression in a soil. One being C input as crop residues such as cereal stubbles and the other being C exudates from living roots. Stubble, being lignified mature plant tissue compromises a more complex C source that may not be readily available to most soil organisms, with the exception of cellulose decomposers. Whereas exudates tend to be low molecular weight compounds such as the simple sugar sucrose used in the work described in Chapter 7 and are considered to provide biologically readily available C. This chapter will investigate whether a more complex carbon source (stubble) in combination with P application will decrease the severity of Rhizoctonia root rot as the more simple sugar sucrose did (see Chapter 6). Using the techniques applied in the previous chapter, the effects of interactions between C input and P fertiliser on the amount of pathogen DNA, DNA of potentially suppressive organisms and overall microbial community structure will be assessed.

The overarching hypothesis is that disease suppression in SB2 soil is limited by available carbon as suggested from work in Chapter 5 and 6 and that C input as stubble will alleviate this constraint (as shown in Chapter 5) by increasing populations of suppressive organisms and ultimately decreasing disease severity.

Specifically, the study will assess the effect of

1) stubble carbon on the
    a. native population of *Rhizoctonia solani* AG8,

b. populations of potentially suppressive organisms *Pantoea agglomerans*, Microbacterium spp. and *Trichoderma* measured by DNA quantification, and
c. microbial community structure measured using catabolic diversity profiles.

2) stubble carbon in combination with phosphorus on
   a. population dynamics of *Rhizoctonia solani* AG8,
   b. disease severity of Rhizoctonia root rot,
   c. potentially suppressive organisms *Pantoea agglomerans*, Microbacterium spp. and *Trichoderma* measured by DNA isolation,
   d. whether these changes in suppressive organism populations be linked to disease severity,
   e. microbial community structure measured using catabolic diversity profiles, and
   f. whether shifts in microbial community structure can be linked to disease severity.

8.2 Materials and methods

Wheat stubble was finely chopped before mixing into moist SB2 soil and incubating in a controlled environment room for 6 weeks at 15°C. Containers were weighed twice a week and sterile reverse osmoses water added as required to maintain moisture at 75% WHC. Soil from these containers and relevant stubble treatments were used for the bioassay experiment carried out as described in the Materials and Methods (Chapter 2, section 2.4).

Briefly soils were moistened with their respective phosphorus nutrient solution and mixed well. This moist soil was then placed into bioassay pots and kept moist at 75% WHC, for the growth period. *Rhizoctonia solani* AG8 inoculum (Materials & Methods section 2.4) was added at two plugs per pot for inoculated treatments and each treatment was replicated six times.

The stubble treatments were finely chopped wheat shoot stubble at 0 g stubble / kg soil, 2.8 g stubble / kg soil and 5.6 g stubble / kg soil. The P nutrient solution treatments were 0 mg P / kg soil, 25 mg P / kg soil and 50mg P / kg soil as Na_2HPO_4 added to autoclaved reverse osmosis water. Assuming an average harvest index of 0.32 (Unkovich et al. 2010) and an average yield of 1.6t/ha (http://www.pir.sa.gov.au/aghistory/left_nav/dept_of_agriculture_as_an_organisation/locations/minnipa_ag), the average annual stubble production on the Upper Eye Peninsula

would be approximately 5.12 g/kg. The average P application rate for the Upper Eyre Peninsula is 5 to 10 mg/kg.

Sacrificial bioassay pots for DNA analysis of specific organisms were included at the time of setting up this experiment (described in Materials and Methods Section 2.6.2). Six replicates of each specific treatment were sent for DNA extraction and analysis. These were

(1) Pots for each stubble treatment after 6 weeks incubation
    a. 0 g stubble / kg soil (control)
    b. 2.8 g stubble / kg soil
    c. 5.6 g stubble / kg soil
(2) Pots from each stubble treatment, with Phosphorus and *Rhizoctonia solani* inoculum
    a. 0 g stubble + 0mg P, 25 mg P or 50 mg P / kg soil
    b. 2.8g stubble + 0mg P, 25 mg P or 50 mg P / kg soil
    c. 5.6g stubble + 0mg P, 25 mg P or 50 mg P / kg soil

From each pot allocated for DNA analysis, a sub-sample of soil was removed to analyse using catabolic diversity (Materials and Methods Section 2.6.3).

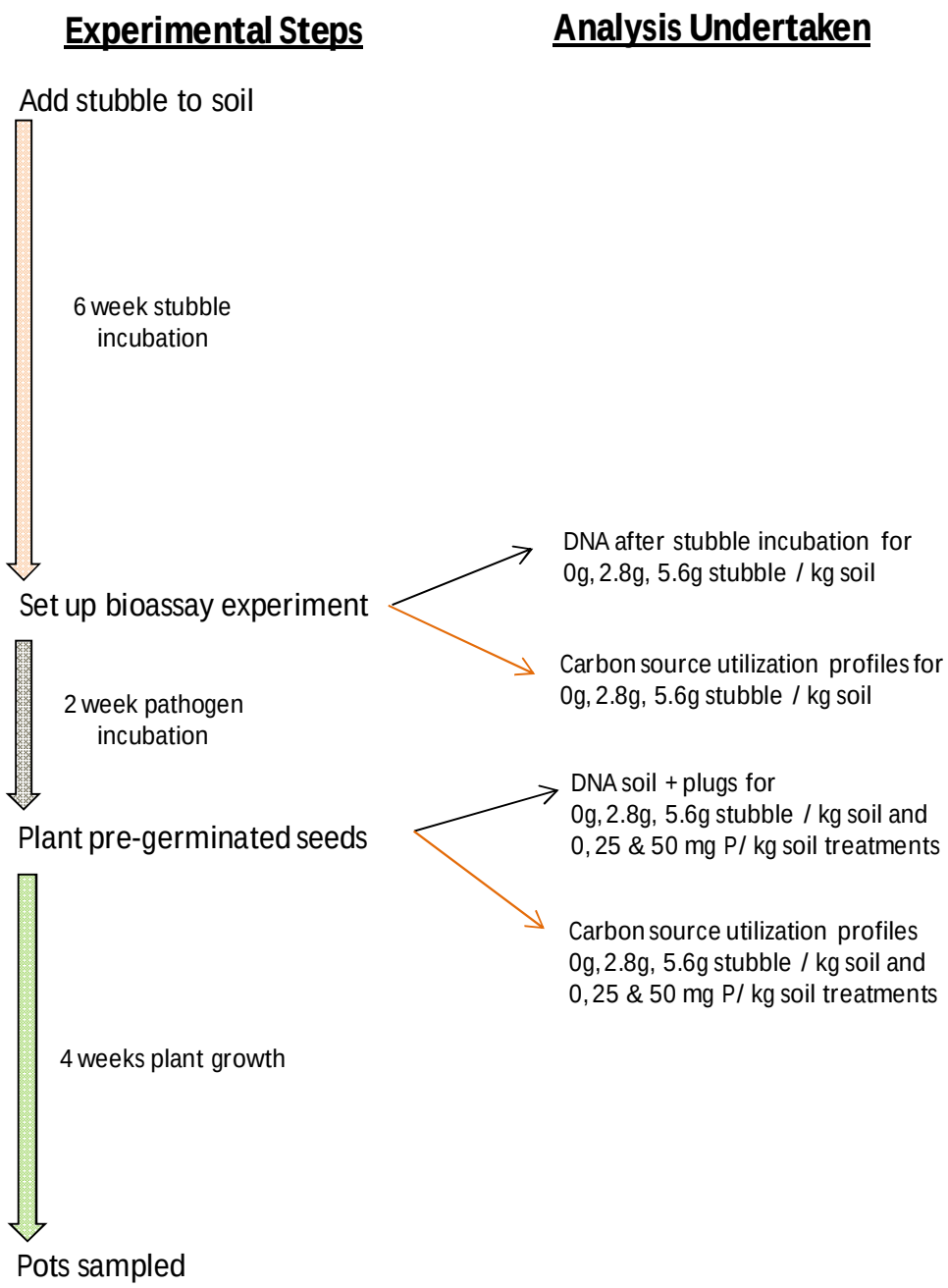


Figure 8.1: Schematic representation of the experimental steps and the microbial analysis undertaken during this experiment

8.2.1 Experimental Treatments

There were 3 stubble carbon treatments and 3 phosphorus treatments, i.e. 9 treatment combinations, as in Table 8.1.

Table 8.1: Summary of stubble carbon and phosphorus treatment combinations for bioassay pots.

Stubble (g/kg)	0	0	0	2.8	2.8	2.8	5.6	5.6	5.6
Phosphorus (mg/kg)	0	25	50	0	25	50	0	25	50

8.3 Results

8.3.1 The effect of stubble input on native populations of Rhizoctonia solani AG8, potentially suppressive organisms Pantoea, Microbacteria and Trichoderma, and microbial community structure

Addition of stubble had no effect on the native population of *Rhizoctonia solani* AG8 as measured by DNA quantification. The amount of $\log_{(10)}$ *Rhizoctonia solani* AG8 DNA for nil carbon was 1.1, for 2.8 stubble carbon was 1.3 and for 5.6 stubble carbon was 1.3 $\log_{(10)}$ pg DNA/ g soil. Amounts of $\log_{(10)}$DNA for the beneficial organisms, Microbacterium increased with increasing rate of stubble addition, while amount of DNA for *Pantoea* is greater with addition of stubble irrespective stubble rate (Table 8.2). *Trichoderma* group A increases with increasing stubble application while group B is mostly absent from these samples (Table 8.2)

Table 8.2: Amounts of DNA ($\log_{(10)}$pg DNA/ g soil) for potentially suppressive organisms *Pantoea agglomerams*, Microbacterium spp. and pg DNA/ g soil for *Trichoderma* Groups A & B measured after incubation for 6 weeks with varying rates stubble addition (0, 2.8 & 5.6g stubble / kg soil). Different letters indicate significant differences for each organism between stubble rates at P=0.05.

Stubble	*Pantoea*	Microbacterium spp.	*Trichoderma* A	*Trichoderma* B
g/kg	$\log_{(10)}$pg DNA per g soil		pg DNA per g soil	
0	1.2 b	3.9 c	0.0	0.0
2.8	2.5 a	4.2 b	5.4	0.0
5.6	2.7 a	4.4 a	16.6	0.7
lsd $_{(0.05)}$	0.3	0.1		

Stubble carbon did not cause any significant changes in the microbial community structure as analysed by PERMANOVA of catabolic diversity profiles (Figure 8.2). Analysis on each of the individual carbon sources showed no significant effects of stubble application on CO2 evolution (Data not shown).

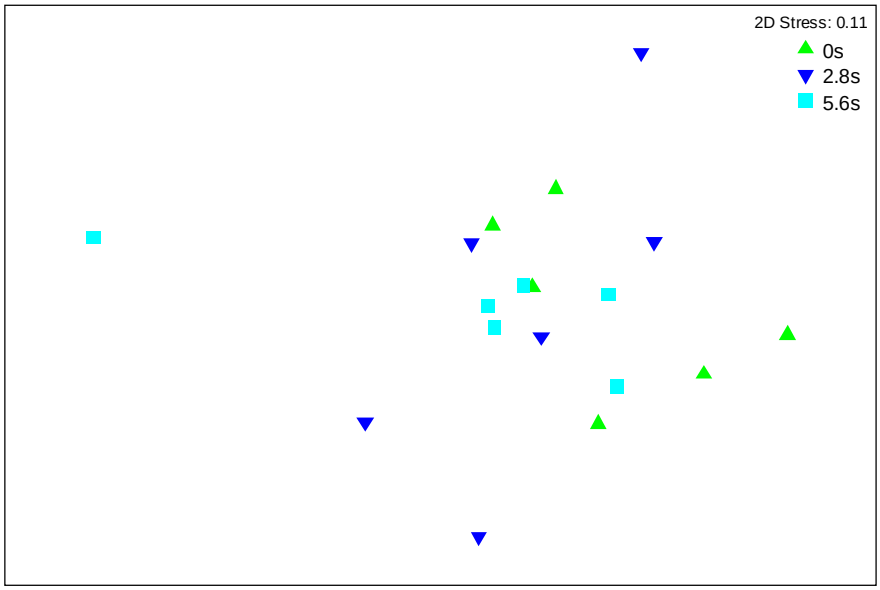


Figure 8.2: Multidimensional scaling plot based on Bray Curtis similarities for log(x+1) transformed carbon source utilization data from soils after 6 weeks incubation with ground stubble carbon at 0, 2.8 and 5.6 g stubble / kg soil. PERMANOVA analysis detected no significant differences between microbial community structures for all carbon application rates.

8.3.2 The effect of stubble plus added P on the population dynamics of Rhizoctonia solani AG8 and disease severity of Rhizoctonia root rot

Increased stubble addition was the only treatment to affect the amount of *Rhizoctonia solani* AG8 DNA, increasing rate of stubble addition decreased amounts of *Rhizoctonia solani* AG8 DNA (Table 8.3). There was no interaction between stubble and phosphorus, and addition of phosphorus did not affect the amount of *Rhizoctonia solani* AG8 DNA (Table 8.3).

Table 8.3: Amount of DNA for *Rhizoctonia solani* AG8 ($log_{(10)}$ pg DNA per g soil) from bioassay pots after 2 weeks incubation with *Rhizoctonia solani* AG8 inoculum, addition of phosphorus (0, 25 & 50mg P /kg soil) and previous 6 weeks incubation with 0, 2.8 and 5.6 g stubble / kg soil. Different letter shows significant differences for treatment means of the main effect stubble. The interaction between phosphorus and stubble was not significantly different.

Stubble g/kg	0	0	0	2.8	2.8	2.8	5.6	5.6	5.6
Phosphorus mg/kg	0	25	50	0	25	50	0	25	50
Rhizoctonia solani AG8 (log(10) pg DNA per g soil)									
	2.4	2.3	2.3	2.2	2.2	2.2	2.2	2.0	2.0
Stubble P value = <.001	2.3 a			2.2 b			2.1 c		

Only the main treatments of stubble and phosphorus had an effect on disease severity. Addition of either 2.8g or 5.6g of stubble decreased Rhizoctonia disease severity on plants grown in soil with any rate of phosphorus addition (Table 8.4). The main effect of P was also significant and all plants grown with P addition had lower disease severity than in plants from the control treatment with no P addition (Table 8.4). Addition of both stubble and P had an effect on root and shoot dry weights, with increasing application of either stubble or P, root and shoot dry weights increased from the control, which had neither stubble nor P addition (Table 8.4).

Virulence of *Rhizoctonia solani* AG8 appears to be influenced only by stubble application. Addition of stubble decreases the virulence of *Rhizoctonia solani* AG8. The nil stubble treatment had 26 percent root infection per $log_{(10)}$ pg *Rhizoctonia solani* AG8 DNA, while both 2.8 and 5.6g stubble had about half the virulence at 14 and 13 percent root infection per pg $log_{(10)}$ *Rhizoctonia solani* AG8 DNA respectively.

Table 8.4: Treatment means of the main effects for Percent Root Infection (PRI), root score (0-5 scale where 0 = no disease and 5 = maximum disease) and root and shoot dry weights (mg) of plants grown in bioassay pots after 2 weeks incubation with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum, addition of phosphorus (0, 25 & 50mg P /kg soil) and previous 6 weeks incubation with 0, 2.8 and 5.6 g stubble / kg soil.

Stubble	Phosphorus	PRI	Root Score	Root Weight	Shoot Weight
0		56 a	2.1 a	137 c	159 b
2.8		30 b	1.1 b	179 b	172 a
5.6		28 b	1.0 b	193 a	165 ab
	0	45 a	1.6	154 c	136 c
	25	34 b	1.3	168 b	163 b
	50	35 b	1.3	186 a	196 a
lsd $_{(0.05)}$		8	0.3	13	9

8.3.3 Effects of stubble carbon and phosphorus in combination on populations of the potentially suppressive organisms Pantoea, Microbacteria and Trichoderma, and the microbial community structure as a whole

There was no interaction between stubble and P for populations of the potentially suppressive organism *Pantoea agglomerans*, which only increased with increasing amounts of stubble carbon addition (Table 8.5). There was an interaction between stubble and P for Microbacterium spp., the amounts of DNA increased with increasing stubble addition and the 50mg P treatment had consistently greater amounts of DNA than the 0 and 25 mgP treatments (Table 8.5). Neither *Trichoderma* group A nor B was present in the 0 stubble treatment (Table 8.5). *Trichoderma* group A may have been incorporated with the addition of stubble as low quantities of DNA was isolated in some of the 2.8g stubble treatments and all of the 5.6g/kg stubble treatments (Table 8.5).

Table 8.5: Amounts of DNA ($log_{(10)}$ pg DNA per g soil) for specific beneficial organisms *Pantoea agglomerans*, Microbacterium spp. and pg DNA per g soil for *Trichoderma* group A & B in soil isolated from bioassay pots after 2 weeks incubation with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum, addition of phosphorus (0, 25 & 50mg P /kg soil) and previous 6 weeks incubation with 0, 2.8 and 5.6 g stubble / kg soil. Different letters show significant differences between treatment means at P= 0.05.

Stubble	Phosphorus	*Pantoea*		Microbacterium	*Trichoderma* A	*Trichoderma* B
		$log_{(10)}$pg DNA per g soil			pg DNA per g soil	
0	0	1		3.8 f	0.0	0.0
0	25	0.9	1.0 c	3.8 f	0.0	0.0
0	50	1.1		3.9 e	0.0	0.0
2.8	0	2.6		4.2 d	5.9	0.0
2.8	25	2.3	2.5 b	4.2 d	3.8	0.0
2.8	50	2.6		4.4 c	0.0	0.0
5.6	0	2.8		4.4 bc	7.1	0.0
5.6	25	2.9	2.8 a	4.5 a	12.6	0.0
5.6	50	2.9		4.5 ab	7.5	0.0
SxP Interaction		ns		0.008		
Stubble main effect		<.001		<.001		
Phosphorus main effect		ns		<.001		

The microbial community structure was shifted by carbon addition and phosphorus application in combination with carbon addition, indicated by PERMANOVA analysis (Table 8.6). The MDS figure for all treatment combinations was not a good fit as the 2D stress value was 0.23 which is slightly greater than 0.2 implying that the replicate variability is considered to be too high (Figure 8.3).

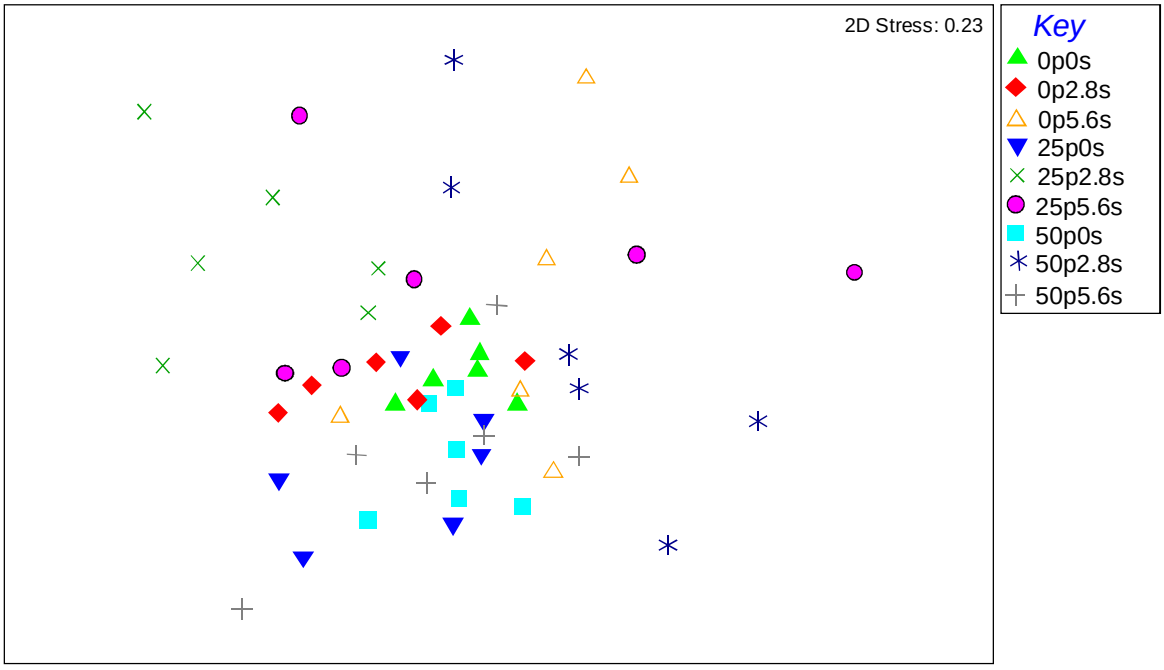


Figure 8.3: Multidimensional scaling plot based on Bray Curtis similarity for log(x+1) transformed carbon source utilization profiles after 6 weeks incubation with ground stubble (0, 2.8, 5.6 g stubble /kg) and 2 weeks incubation with 0, 25 and 50 mg P/kg soil. All treatments were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum. The high stress value indicates that this data is not a good fit for this MDS plot.

A restricted data set of 25P, 50P in combination with all stubble addition rates was analysed, the MDS plot was a better fit (Figure 8.4). In this plot lines have been drawn around some of the more separate treatment combinations to indicate where the treatment differences may lie (Figure 8.4). It is important to note that these groupings are an indication only.

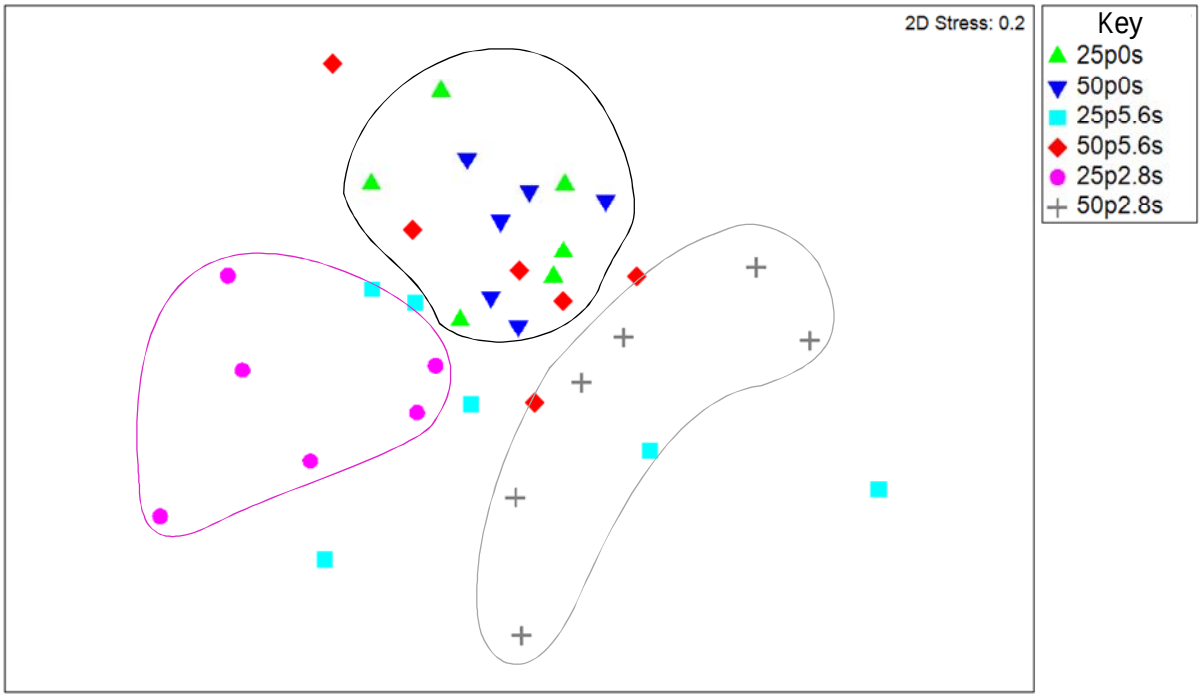


Figure 8.4: Multidimensional scaling plot based on Bray Curtis similarity for log(x+1) transformed carbon source utilization profiles after 6 weeks incubation with stubble (0, 2.8, 5.6 g/kg) and 2 weeks incubation with 25 and 50 mg P/kg soil. All treatments were inoculated with 2 agar plugs of *Rhizoctonia solani* AG8 inoculum. Lines indicate which treatment combinations lie further away from one another and which could be different from each other.

The data indicates that shifts in the community structure with phosphorus addition are more noticeable when carbon was also added. There is strong similarity between the 25P and 50P data sets for 0g stubble (indicated by the black ring in Figure 8.4). With the addition of 2.8g of stubble, the results of the two P levels both move noticeably away from the 0g stubble results and each other (indicated by the pink and grey rings in Figure 8.4).

Table 8.6: PERMANOVA & ANOSIM values for analysis of catabolic diversity profiles shown in Figures 8.3 and 8.4

Analysis	PERMANOVA	ANOSIM (Global R)
All treatment combinations	P(perm)	0.205
Phosphorus	0.002	
Stubble	0.001	
Phosphorus x stubble	0.001	
Restricted treatments (0, 2.8, 5.6 stubble & 25, 50P)		0.262
Phosphorus	0.002	
Stubble	0.001	
Phosphorus x stubble	0.003	

8.4 Discussion

Overall, the data from this experiment indicates that addition of stubble without fertilizer P in this highly calcareous soil had no effect on the native population of *Rhizoctonia solani* AG8 but it did result in an increase in the populations of specific potentially suppressive organisms *Pantoea agglomerans* and Microbacterium spp. Disease severity of Rhizoctonia root rot decreased with addition of stubble with P fertiliser. Shifts in the microbial community structure appeared to be linked to stubble input with P.

Rhizoctonia solani is known to survive on organic carbon sources in the top layers of field soil (Neate et al. 1996). Similar amounts of *Rhizoctonia solani* DNA isolated in all stubble carbon treatments suggests that pathogen suppression is occurring in these soils and that despite the stubble not being sterile addition of the stubble does not appear to have introduced additional pathogen populations into these treatments. Data from isolation of *Microbacterium* spp., *Pantoea agglomerans* and *Trichoderma* species indicates that general pathogen suppression via the general microbial community is likely to be playing a role in maintaining similar amounts of *Rhizoctonia solani* DNA in all treatments. General microbial suppression is reported to act via antibiosis and competition for nutrients and space (Baker 1968). Although no measure of overall microbial biomass was done in this work, measured DNA amounts for Microbacterium spp, *Pantoea agglomerans* and

Trichoderma species suggest that there were populations within this soil microbial community which increased with stubble addition. However, it is evident from the lack of change in DNA amounts for *Rhizoctonia solani* AG8 that some of these organisms are providing a level of general suppression by maintaining the *Rhizoctonia solani* AG8 DNA at similar levels to the nil stubble control. It is highly likely that specific pathogen suppression is also playing a role in this bioassay. Specific suppression is linked to specific organisms or groups of organisms (Cook et al. 1983). There are numerous reports on the specific antagonism between *Trichoderma* species and *Rhizoctonia* (Henis et al. 1979; Liu et al. 1980; Chet et al. 1990). Indeed; Grinyer et al. (2005) linked production of proteins N-acetyl-b-D-glucosaminidase and 42-kDa endochitinase by *Trichoderma atriviride* to degradation of *Rhizoctonia* cell walls. Therefore, it is likely that the combination of general suppression by the whole microbial community and specific suppression by *Trichoderma* species is preventing large increases in populations of *Rhizoctonia solani* AG8. It must be noted that addition of stubble in this way may not accurately represent what happens in a field situation, in some field paddocks stubble will harbor the pathogen in its resting phase (Neate 1984; Gupta et al. 2007) thus the stubble will be a source of inoculum as well as a source of carbon for the disease suppressive microbial community and the pathogen.

Stubble addition decreased *Rhizoctonia solani* AG8 populations in soils inoculated with *Rhizoctonia solani* AG8. As discussed above a combination of general and specific suppression is still likely to be reducing the amount of *Rhizoctonia solani* DNA with an addition of stubble. In contrast to the previous data chapter in this thesis, addition of P did not increase the amount of *Rhizoctonia solani* DNA. It is highly likely that the combined general and specific suppressive capacity of the soil enhanced by addition of available C in stubble was sufficient to prevent an increase in *Rhizoctonia solani* even with additional P supply. This observation is similar to that from work done on *Rhizoctonia solani* in sugar beet where soil amendment with residues increased the activity of bacteria antagonistic to *Rhizoctonia solani* (Kasuya et al. 2006). The study also noted that the activity of antagonistic bacteria was greater in a soil with a history of chemical fertilizer application (Kasuya et al. 2006). In this data set, it may be that the addition of P behaved in a similar manner to the fertilizer application and increased the activity of bacteria which were antagonistic to *Rhizoctonia solani*, therefore decreasing *Rhizoctonia*

solani inoculum compared to the nil stubble control. Another possible reason for lower amounts of *Rhizoctonia solani* DNA where stubble was added may be due to competition and immobilization of available C by the soil microbial biomass. Demoling et al. (2007) found that the most common limitation for soil microorganisms was C and in some of the soils tested there was also a N limitation. Since SB2 is highly calcareous, and P is likely to be fixed, perhaps P is more limiting than N for microorganisms in this specific soil, as was shown in plants grown in this soil (Chapter 6). Thus, the combination of carbon and P would alleviate nutrient constraints on the microorganisms resulting in greater activity and pathogen suppressive capabilities as seen by decreased *Rhizoctonia solani* DNA in these results.

It is not surprising that disease severity decreased with increasing stubble application as pathogen inoculum levels also decreased with increasing stubble application. This observation supports numerous experiments that link the amount of *Rhizoctonia solani* inoculum to subsequent disease severity (van Bruggen et al. 1986; Schroeder et al. 2008; Anees et al. 2010), and also supports previous experiments described in this thesis (Chapter 3 and Chapter 7). The effect of decreased *Rhizoctonia solani* inoculum after stubble addition does not, however, explain the decrease in virulence of *Rhizoctonia solani* with increasing stubble application. Applying the definition of disease suppression as defined by Mazzola (2007), which is "Soils suppressive to soilbone plant disease have been defined as those in which disease development is minimal even in the presence of a virulent pathogen and a susceptible plant host". In this bioassay, a susceptible plant host and virulent pathogen can be confirmed by the disease severity measured in non-amended controls. In work done on *Rhizoctonia* in radishes, disease severity was lower in the suppressive soil compared to the non-suppressive soil despite the same inoculum density (Wijetunga et al. 1979). Although they did not measure virulence, by definition in this work the calculation of virulence is disease severity per unit pathogen inoculum and would be equivalent to this data set. Therefore, the decrease in *Rhizoctonia* virulence indicates that SB2 soil does indeed have the capacity to support and develop a disease suppressive microbial community.

Since the MDS plot grouped the microbial community for nil stubble carbon treatments, compared to treatments with stubble addition it suggests that there was a shift in microbial community structure after addition of stubble and P, although stubble appears

to be the more important factor. This results support previous work done on soils from South Australia which showed that soil treatment with stubble addition shifted the soil microbial community structure (Gupta et al. 2010b). In all three soils tested addition of stubble increased the diversity of the soil fungal community and highlighted that *Pseudomonads* spp are one of the most important drivers of disease suppression (Gupta et al. 2010b). These results are also consistent with other research done on increasing organic carbon and microbial community structure with specific relation to disease suppression, for example compost amendments in different soil types (Perez-Piqueres et al. 2006), stubble retention in direct drilled farming systems (Pankhurst et al. 2002), organic amendments and yield decline in sugarcane fields (Pankhurst et al. 2005) and carbon amendments to control root diseases (Hoitink et al. 1999). These examples relate to the overall community structure, and in some cases researchers have isolated specific organisms associated with specific disease suppression. For example *Pseudomonads* suppressing *Rhizoctonia* in apple orchards (Mazzola et al. 2004), *Pseudomonads* spp and *Streptomyces* spp. antagonistic to *Rhizoctonia solani* and *Fusarium oxysporum* (Adesina et al. 2007). In this work, isolation of *Microbacteria* and *Panteoa agglomerans* indicates increased in populations with increasing stubble application. These organisms have previously been linked specifically to disease suppression of *Rhizoctonia solani* (Barnett et al. 2006). It is highly likely that in this experiment, they contributed to disease suppression of Rhizoctonia root rot. As discussed previously, *Trichoderma* species are well known antagonists of *Rhizoctonia solani* as reviewed by Vinale et al. 2008. The increase in *Trichoderma* populations with increasing stubble addition probably contributed to disease suppression. The effect of P on microbial community structure appears to be stubble dependent, which suggests that carbon is the more limiting factor and that P may become limiting for microbial activity after addition of stubble. This agrees with work done by Cleveland et al. (2002) who demonstrated that carbon was more limiting than phosphorus for microbial respiration rates, but utilization of carbon was further limited by phosphorus availability. Shifts in the microbial community structure after mineral phosphorus and potassium fertilizer application have been reported in soils from Northern China (Ge et al. 2008). In this work reported here it is likely that the combined relative limitations of C and P are affecting shifts in microbial community structure as seen in the MDS plots. Overall, it appears that the shifts in microbial community structure and increases in DNA of specific organisms isolated in this work correspond to the

observed decrease in disease severity. These results suggest that the original hypothesis holds true and that disease suppression mediated via the microbial communities in the SB2 soil is indeed limited by carbon.

Chapter 9
Final discussion and Conclusions

9. General Discussion

9.1 Introduction

Biological disease suppression of Rhizoctonia root rot has been reported at Avon in the mid-north of South Australia (Roget 1995, Wiseman et al. 1996, Gupta et al. 1999a). Despite modification to seeding practices and other changes in farming systems that increase carbon inputs and control summer weeds, the EP in South Australia has been unable to establish biological disease suppression to Rhizoctonia root rot (Chapter 1). One avenue for sustainable long term control of Rhizoctonia root rot on the EP would be to develop biological disease suppression as seen at Avon, in the mid-north of South Australia. Biological disease suppression has been defined as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." (Weller et al. 2002). Soils on the EP have been termed as hostile due to their highly calcareous nature and inherent nutrient constraints (Coventry et al. 1998). Due to these hostile characteristics and inherent abiotic constraints there has been concern that these soils may be unable to develop biological disease suppression. Thus, the overarching aim of this thesis was to assess the potential of these hostile soils from the EP in South Australia to biologically suppress Rhizoctonia root rot in wheat crops (Chapter 1).

The following sections of this discussion will highlight important findings from each of the experimental studies in this thesis in relation to current scientific literature and attempt to provide a synthesis in the context of farming system practices in semi-arid Mediterranean environments. The discussion will firstly consider results from the preliminary study investigating the effect of increasing soil moisture and amount of pathogen inoculum on Rhizoctonia root rot disease severity (Chapter 3). Highlighting the important links between pathogen survival and growth, disease development and incidence especially for semi-arid farming systems such as those on EP. The discussion will then focus on findings from the first major study which investigated whether the inherent abiotic constraints in soils from the Upper EP prevented expression of biological disease suppression to Rhizoctonia root rot (Chapter 4). Following this will be discussion of the results from the second major study where the impact of increasing carbon inputs on the

development and expression of disease suppression in some EP soils was clearly highlighted (Chapter 5). Then it will look at the final part of the experimental studies which investigated the interaction between key fertilizer inputs (nitrogen and phosphorus) and disease suppression of Rhizoctonia root rot, in the presence or absence of an additional carbon source (Chapters 6). Chapters 7 and 8 focused on the effect of phosphorus fertilizer on the pathogen *Rhizoctonia solani* and subsequent disease expression of Rhizoctonia root rot. Chapter 8 also investigated the effect of an additional carbon source with phosphorus application on the pathogen *Rhizoctonia solani* and subsequent disease expression of Rhizoctonia root rot. Finally, the key conclusions of this work and recommendations for future studies in this area will be presented.

9.2 The effect of soil moisture and pathogen inoculum on Rhizoctonia root rot

Results from preliminary experiments (Chapter 3) highlighted the important negative relationship between increasing soil moisture and development of Rhizoctonia root rot disease. In the Minnipa soil, a 40% increase in soil moisture from 55% WHC to 95%WHC decreased measured disease from 95 percent root infection (PRI) to 80 PRI, in the Streaky Bay soil this effect was more pronounced and measured disease decreased from 96 to 60 PRI (Table 3.1). These results agree with previous work done on *Rhizoctonia solani* in Western Australia, where an increase in moisture content decreased the development of disease in a controlled environment (Gill et al. 2001a; Gill et al. 2001b) and on *Pellicularia lamentosa* (Pat.) Rogers and *Sclerotinia homeacarpa* Bennett (Kerr 1955). These results also agree with results from recent field investigations into the effects of summer rainfall on the amount of pathogen DNA in paddock soil in the South Australian Mallee region which showed that, with regular rainfall, the amount of *Rhizoctonia solani* DNA decreases over time (Gupta et al. 2010a). Conversely, if there is no summer rainfall, the amount of *Rhizoctonia solani* DNA in the soil increases over the dry summer period (Gupta et al. 2010a). In the absence of a host crop *Rhizoctonia solani* AG8 colonizes and survives in semi-decomposed particulate organic matter (Neate 1984). It is known that in the presence of adequate soil moisture populations and activities of soil microorganisms would increase (Gill et al. 2001b) resulting in competition for carbon and nutrients with the pathogen (Kotsou et al. 2004). It appears that under increased moisture conditions *Rhizoctonia solani* the pathogen is not a good competitor and increased soil microbial activity leads to a decrease in the population of *Rhizoctonia solani*. Soils from EP present a

number of constraints to biological activity due to their physico-chemical properties (high pH, calcareous nature, lower levels of biologically available carbon, low P availability) and thus favour opportunistic pathogens such as *R. solani*. Other preliminary experiments showed that, with increasing levels of pathogen inoculum, there was a subsequent increase in measured disease (Chapter 3.2). Increasing the amount of inoculum from 1 plug to 3 plugs increased percent root infection in the Minnipa soil from 54 to 80 PRI, while in the Streaky Bay soil it increased from 76 to 83 PRI. Since amount of pathogen inoculum in the soil is related to Rhizoctonia root rot disease incidence and severity (Chapters 3.2 and 7; Smith et al. 2003; Schroeder et al. 2008; Nerey et al. 2010), it is likely that in soils from the EP the semi-arid Mediterranean type climatic characteristics of this region contribute to high pathogen populations and thus a predisposition to greater disease incidence and severity. That is, the characteristic dry summer conditions possibly lead to an increase in *Rhizoctonia solani* populations over summer and the characteristic low growing season rainfall resulting in low soil moisture contents and higher Rhizoctonia root rot disease incidence and severity. This combination of the effects of moisture on the pathogen inoculum, and the pathogen inoculum relationship with disease severity help to explain why Rhizoctonia disease at a paddock scale continues to be a large constraint to farming systems on the EP.

9.3 Complexity of abiotic-biotic interactions influences disease suppression of Rhizoctonia root rot

The overarching hypothesis in this thesis was that the hostile nature of soils from the EP has a direct effect on the ability of these soils to biologically suppress Rhizoctonia root rot (Chapter 1). Results from Chapter 4 suggest that the interactions between biotic and abiotic characteristics of the soil that result in disease suppression of Rhizoctonia root rot are complex. In some soils the biotic characteristics appeared to dominate, in others it was the abiotic characteristics, whilst in other soil, biological suppression of Rhizoctonia root rot appeared to be determined by a combination of both the biotic and abiotic characteristics. The Avon soil was consistently biologically suppressive in this work, confirming previous investigations of biological disease suppression in the Avon soil (Wiseman et al. 1996). Conversely, Minnipa soil did not appear suppressive, which contradicts other bioassay studies (Cook et al. 2007). PLFA profile results suggest that biological disease suppression may be working through different organisms and pathways

in each individual soil tested as some of the PLFA profiles were different yet the soils still exhibited the same biological disease suppression to Rhizoctonia root rot. Although these PLFA results were not directly correlated to observed patterns of disease suppression in these soils, the differences in microbial communities responsible for suppression may be at a finer scale than can be determined by PLFA. Thus these PLFA results may not represent the actual microbial communities since no links were found. Within the broader groups described by PLFA a variety of phenotypic groups of microorganisms would have been responsible for disease suppression and all of them can contribute towards the suppressive function, i.e. functional redundancy. Functional redundancy, that is a lack of direct linkage between PLFA community analysis and other soil process measures, e.g. enzyme activity is not new (Marschner et al. 2003). These results also support the hypothesis that disease suppression may involve multiple organisms, that is a complex process involving many different organisms (Gupta et al. 1999a; Garbeva et al. 2004).The occurrence of functional redundancy highlights the complexity associated with biological systems, including that of biological suppression to soil-borne diseases. Results from this experiment suggested that although the interactions are soil specific and complex, the abiotic-biotic properties of soils from the EP can indeed support biological disease suppression to Rhizoctonia root rot. Therefore, the lack of notable expression of biological disease suppression under field conditions is likely to be due to highly specific abiotic or biotic characteristics of each different soil (for example, low biologically available carbon), or due to the climatic characteristics of the region as discussed previously.

9.4 Biologically available carbon positively influences organisms linked to disease suppression but rainfall may limit both parameters in semi-arid farming systems

Biological disease suppression is believed to be mediated by the effects of biologically available carbon on the soil microorganisms. It was hypothesised that an increase in carbon inputs led to the development of biological disease suppression at Avon in the mid-north of South Australia (Roget et al. 1999, Gupta et al. 1999a). The effect of increasing carbon inputs through crop residues in hostile soils from the EP had not been previously investigated. The work in this thesis investigated the effect of adding the two main types of carbon inputs at a field scale (young root carbon and cut stubble carbon) on

biologically mediated disease suppression of Rhizoctonia root rot in three soils from the EP (Chapter 5). Addition of both types of carbon source decreased disease severity, addition of young root carbon decreased root infection by 10%, 20% and 30% in Kimba, Mount Damper and Port Kenny soils, respectively. While addition of cut stubble carbon decreased root infect by 24%, 27% and 18% in Kimba, Mount Damper and Port Kenny soils, respectively. This result is new and supports previous work where carbon addition as a simple carbon substrate (sucrose) increased expression of biological disease suppression (Roget et al. 1999). It also supports previous studies on the addition of a variety of carbon substrates and their effects on disease suppression towards pathogens such as *Verticillium*, *Thielaviopsis*, *Fusarium*, *Phytophthora* and *Rhizoctonia solani* which suggested that addition of carbon enhanced disease suppression, and compost was the most effective carbon source (Bonanomi et al. 2007). The effect of addition of young root carbon and cut stubble carbon in EP soils on the specific organisms, *Microbacterium* spp. and *Pantoea agglomerans* linked to biological disease suppression in the Avon soil (Barnett et al. 2006) also had not been demonstrated previously. Addition of young root carbon increased DNA amounts of *Microbacterium spp.* by on average 1 $\log_{(10)}$ pgDNA/g soil compared to the unamended control in all soils and *P. agglomerans* by between 1 and 2.5 $\log_{(10)}$ pgDNA/g soil compared to the unamended control in all soils. Addition of stubble carbon increased DNA amounts of *Trichoderma* spp by on average 0.5 $\log_{(10)}$ pgDNA/g soil compared to the unamended control for all three soils used. These results suggest that these EP soils have biologically available carbon limitations for soil micro-organisms which in other studies have specifically been shown to be associated with disease suppression of Rhizoctonia root rot. Since biologically available carbon appears limiting for the specific organisms quantified by DNA analysis, it is likely that it is also limiting for other soil microorganisms. This observation is supported by previous studies where, for most soils tested, carbon was limiting for bacterial growth (Demoling et al. 2007). Results from this experiment suggest that development of biological disease suppression is possible in soils from the EP despite the hostile nature of these soils. Since both moisture and amount of available carbon are essential to the development of biological disease suppression, the characteristic low rainfall experienced in this region is likely to limit both the moisture required to develop biological disease suppression, as well as limit crop growth during the growing season therefore the carbon input into the soil. One possible option to increase carbon inputs at a field scale would be to introduce

high biomass crops to the current farming system rotations that are adapted to semi-arid climates, such as rye.

9.5 Addition of phosphorus increases the amount of *Rhizoctonia solani* AG8 DNA, Rhizoctonia root rot disease and plant growth; while addition of carbon positively influences disease suppression of Rhizoctonia root rot

A previously unreported effect was observed when application of 50mg P/kg soil (in controlled conditions) increased Rhizoctonia root rot on plants in the highly calcareous Streaky Bay soil by more than 25% (Chapter 6). Further investigation (Chapter 7) concluded that this increase in disease was related to an increase in pathogen DNA in those treatments with added P, addition of 50mgP/kg soil increased the amount of *Rhizoctonia solani* DNA by 0.3 $\log_{(10)}$ pg DNA/g soil (Chapter 7). The increase in pathogen DNA corresponded to an increase in measured disease severity, but the virulence of the pathogen remained constant at 29% infection per $\log_{(10)}$ pg DNA/g soil (Chapter 7). It has been suggested that P fixation as calcium phosphates in highly calcareous soils could result in P limitations for microorganisms in calcareous soils (Joergensen et al. 1990). There appears to be no previous literature on the effects of added P on disease suppression in highly calcareous soils. Nevertheless, in non-calcareous pasture and forest soils application of P increased microbial activity (Cleveland et al. 2002; Galicia et al. 2004). Since microbial respiration and *Rhizoctonia solani* DNA increased with added P in this work, it is reasonable to assume that these hostile soils from the EP are indeed also P limited for the soil microorganisms. Despite this P limitation, observations after addition of available carbon and P indicate that carbon is likely to be more limiting than P in these highly calcareous soils (Chapter 6 and 8). In both experiments, the addition of an available carbon source decreased disease severity compared to the unamended control and the treatments with added P (greater disease severity). In combination these results add to the understanding of why Rhizoctonia root rot is still such a problem in EP farming systems. The abiotic constraints of inherently low biologically available carbon in these soils and the regular application of P as part of the farming system are likely to be contributing to increases in pathogen DNA in the field leading to increases in disease severity as seen in the current cropping systems. One possible means of addressing these production constraints may be the development and adoption of management systems which increase carbon inputs to improve biological activity in these soils. Alternatively,

changes to P fertilizer type or application method could be made such that P application no longer increases the amount of pathogen DNA in the soil, but is used by the crop to alleviate P limitations to crop growth.

It is difficult to reach any firm conclusions regarding the effect of fertiliser N addition from the work described in this thesis. Addition of ammonium-N decreased root infection by 30% in Minnipa soil and 20% in Streaky bay soil compared to nitrate-N which had greater percent root infection. Previous studies on the effects of nitrogen on Rhizoctonia disease are also inconclusive and in some cases addition of nitrogen decreased disease (MacNish 1985a) while in other cases there was no consistent response to increasing rates of nitrogen application (Wall et al. 1994). The impact of N form on expression of disease for pathogens other than *Rhizoctonia solani* has been investigated in some detail. For example, NH_4-N increased *Pseudomonads* populations antagonistic to take-all and subsequently decreased take-all disease (Sarniguet et al. 1992) and perhaps a similar biotic response was occurring in the experiment reported in this thesis. Nevertheless, the mechanisms driving this obviously complex set of interactions, in terms of nitrogen, between the abiotic-biotic-pathogen components is not clear and requires further investigation.

9.6 Conclusion

In conclusion, the studies reported by this thesis found that stimulating biological disease suppression of Rhizoctonia root rot is certainly possible in hostile soils from the semi-arid Eyre Peninsula. It appears that the main limitation for development of biological disease suppression in the soils of this region is the availability of carbon for the soil microorganisms. As discussed previously, carbon availability in soil is reported to be limiting in a range of soil types worldwide. Therefore, these results have implications for dry land cropping in semi-arid farming systems worldwide. Although the specific edaphic and climatic characteristics of the EP region may preclude the development of disease suppression via lack of moisture for the soil microorganisms and for plant growth, therefore stubble carbon input back into the soil. If there is adequate moisture and increasing carbon inputs is viable in these farming systems, it is highly likely that biological disease suppression will develop.

Despite the complexity of interactions between the abiotic-biotic soil characteristics, the work in this thesis on the effects of commonly applied fertilizers, especially P, contributes to an understanding of why Rhizoctonia root rot continues to be such an issue on the EP. As P is applied regularly in these farming systems, with any improvement to general microbial activity it would lead to higher *Rhizoctonia solani* populations in the soil and result in greater Rhizoctonia root rot in crops, as is seen regularly in these farming systems. This data also further supports the conclusion that available carbon is limiting in these soils, as this effect of P would not be observed if there was no carbon limitation as adequate carbon overrides the effect of P on *Rhizoctonia solani* populations as seen in the results from this thesis.

9.7 Future work

While there is merit in extrapolating results and conclusions from controlled conditions to field conditions, it is essential for the effects of P on *Rhizoctonia solani* DNA and disease severity to be confirmed in field studies. If, in the field, the combination of low inherent available organic carbon in EP soils and regular application of P increases *Rhizoctonia solani* DNA and subsequent Rhizoctonia root rot there is potential to adapt farming systems to account for these effects.

Carbon limitation in these EP soils needs to be investigated further under controlled conditions as well as under field conditions. Varying rates of carbon addition should to be investigated to confirm that available carbon is indeed limiting for disease suppression in these soils and to establish a base line amount of available carbon required to develop disease suppression in these hostile soils.

As discussed previously, the effects of nitrogen and nitrogen form on disease expression and suppression are complex. More work on understanding these interactions in the plant-biotic-pathogen continuum is needed. Nitrogen application plays an integral part of farming systems in South Australia and around the world, if the application of nitrogen is contributing to the incidence and severity of Rhizoctonia root rot there would be potential to manage Rhizoctonia root rot with nitrogen fertilizer management.

Another aspect that needs to be investigated is the length of time required to develop disease suppression in these hostile soils in the field. The controlled environment

conditions set up an ideal situation in which moisture, temperature and light are not limiting plant production. Since field conditions are highly variable and likely to have moisture and temperature constraints it will be important for growers in this region to know how long it will take for disease suppression to develop in their region and farming system.

Once the climatic and edaphic parameters associated with biological disease suppression under field conditions are established, the microbial communities or specific microorganisms involved in disease suppression under these conditions will also need to be investigated. Results from this work suggest that PLFA may only offer a limited degree of resolution and that other technology such as DNA isolation would be more appropriate for establishing which organisms or groups of organisms are contributing to biological disease suppression under field conditions. While the link between *Pantoea agglomerans*, Microbacteria and *Trichoderma* spp. has been described for studies in controlled conditions, the effect that these organisms have on disease suppression needs to be further investigated under field conditions.

Since edaphic and climatic characteristics of the EP region may be limiting the quantity of biomass production required to develop biological disease suppression in current farming systems, the use of alternative adapted high biomass producing crops in the farming system rotations is worth investigating. If alternative crops which enhance biomass production also promote development of biological disease suppression, changes in farming system practices may offer a sustainable long term solution to control Rhizoctonia root rot.

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Appendices

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