A comparison of endomycorrhizal fungus colonisation, soil-organism biodiversity, and soil properties among agricultural, revegetated, and remnant vegetation sites at Port Wakefield, South Australia

Thesis submitted in partial fulfilment of the requirements for the Bachelor of Applied Science (Honours) (Biodiversity, Environmental and Park Management)

28 November 2008

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Glossary

**ADF**: Australian Defence Force.

**ANOVA**: Analysis of variance.

**Arbuscule**: Endomycorrhizal fungal structure formed in the inner cortex of roots. Arbuscules are key sites for nutrient exchange between the fungi and plant roots (Mukerji *et al.* 2000).

**Endomycorrhizae**: Fungi that form symbiotic relationships with plant roots, also known as vesicular-arbuscular mycorrhizae, and consisting of hyphae, spores and auxiliary bodies produced in the soil, as well as hyphae, arbuscules and vesicles in the roots (Brundrett *et al.* 1996).

**Hyphae**: Fungal structures that penetrate the root cortex, and extend out into the surrounding soil. Hyphae facilitate nutrient uptake in plants by accessing soil nutrients surrounding the plant roots (Marschner & Dell 1994).

**Macro-nutrient**: A nutrient needed in relatively large quantities for plant growth. The six macro-nutrients and their forms when available to plants are: nitrogen (NH$_4^+$, NO$_3^-$), phosphorus (PO$_4^{3-}$), potassium (K$^+$), calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), and sulphur (SO$_4^{2-}$) (Jones 1998).

**Micro-nutrient**: A nutrient needed in relatively small quantities for plant growth. The seven main micro-nutrients and forms when available to plants are: manganese (Mn$^{2+}$), copper (Cu$^{2+}$), zinc (Zn$^{2+}$), iron (Fe$^{2+}$), molybdenum (MoO$^-$), boron (BO$_3^{3-}$), and chlorine (Cl$^-$) (Jones 1998).

**Rhizosphere**: The region in the soil surrounding plant roots (Shepley 1973).

**Simpson’s Index of Diversity**: A measure of diversity, $1-D = 1-\sum \frac{n(n-1)}{N(N-1)}$, where $D$ is Simpson’s index, $n$ is the number of individuals of a species and $N$ is the total
number of individuals for all species (Krebs 1999). The values for Simpson’s Index of Diversity range from 0 – 1, with high numbers indicating a high species diversity.

**Species richness**: The number of species in a community (Krebs 1999).

**Trypan blue**: A stain used to selectively colour tissues or cells blue, found to be effective for the staining of endomycorrhizal structures (Phillips & Hayman 1970).

**Vesicle**: Endomycorrhizal fungal structure formed in the root cortex. Vesicles may be spherical, oval or lobed, and serve as endophytic storage organs that are rich in lipids (Mukerji *et al.* 2000).

**References**


Abstract

Agricultural land use has many impacts on the soil, which include decreases in essential plant nutrients, increased soil compaction, and changes in soil structure. The living soil biota can also be affected by agriculture. Agriculture can have negative impacts on invertebrate biodiversity and abundance, and endomycorrhizal colonisation. The major roles of soil organisms include aggregation of soil, fixation of nitrogen, and decomposition of organic matter. Mycorrhizae have an importance in facilitating nutrient uptake in plants. It may take many years before an ecosystem is restored to its original state, depending on the severity of soil disturbance.

The aim of this study was to compare physical, chemical, and biotic soil properties among an agricultural site with a 77-year history of grazing and cropping, 8-10 year old revegetated sites, and remnant vegetation sites. All sites were located in the semi-arid Port Wakefield region of South Australia. Each site type had soil samples collected and analysed for plant nutrients, pH levels, salinity, soil texture, and soil structure. Mycorrhizal colonisation and micro-organism abundance and biodiversity were also compared among site types. Soil properties of remnant vegetation sites were used as indicators of desired soil properties.

Overall findings of the study indicate that most essential plant nutrients were not significantly depleted at the agricultural site, which had relatively high levels of nitrogen, sulphur, magnesium, and potassium, likely to have been a result of the addition of fertilizer and animal excreta to the soil. Salinity was found to be very high at the agricultural site, probably because of the clearing of native vegetation and replacement with crop plants. Mycorrhizal colonisation appeared to be affected by agricultural land use, with very low colonisation in the grazed paddock. Micro-organism abundance was highest in the grazed paddock, indicating that agricultural land use may not have had a significant impact on soil organisms. Soil texture was the main variable associated with organism abundance, sandy soils having relatively low organism abundance.

Revegetation appears to have improved some components of the soil ecosystem. Very similar mycorrhizal colonisation among the remnant and revegetated sites suggests that eight years following revegetation may be sufficient for mycorrhizal recovery. Salinity was also reduced at revegetated sites, giving further evidence that revegetation may be a key to reversing the effects of salinity.
some soil nutrients including iron and manganese appear to be taking longer than eight years to return to pre-agricultural levels.
Declaration

I declare that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge it does not contain any materials previously published or written by another person, except where due reference is made in the text. I give permission for this thesis to be published online by the University of South Australia, and, in accordance with the Copyright Act, permission has been granted for the use of any third-party images contained herein.

Signature________________________

Rina Aleman

Date____________________________
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CHAPTER 1: General Introduction

Introduction

Background
Endomycorrhizal fungi (vesicular-arbuscular mycorrhizal fungi) participate in a mutualistic relationship with plant roots, whereby plant roots have improved mineral and nutrient uptake from the soil, and the mycorrhizal fungi gain direct access to carbohydrates such as glucose and sucrose produced by the plant (Brundrett et al. 1996). Endomycorrhizae are the most common type of mycorrhizae, with 90% of all land plants having a mutualistic association with endomycorrhizal fungi (Mukerji et al. 2000). These fungi are incapable of growth without host plants (Brundrett 2002). The land plants associated with endomycorrhizae include angiosperms, gymnosperms and pteridophytes, which all have true roots, as well as the gametophytes, some mosses, lycopsods and psilotales, which do not have true roots (Smith & Read 1997). Endomycorrhizae are characterised by structures formed within root cells called vesicles and arbuscules (Fig. 1.1). Arbuscules, which are highly branched structures formed in the inner cortex of roots, are key sites for nutrient exchange between plants and mycorrhizal fungi (Mukerji et al. 2000). Vesicles are spherical, oval or lobed structures formed in the root cortex, and serve as storage organs containing large quantities of lipids (Mukerji et al. 2000). Arbuscules and vesicles are formed when fungal hyphae, which extend into the surrounding soil for up to 10 cm, penetrate the walls of root cells (Coleman 1996). The hyphae have an important role in facilitating nutrient uptake in plants, whereby the hyphae access soil nutrients surrounding the roots, and also produce ectoenzymes, which provide host plants with the potential to access organic N and P forms that are not usually available to non-mycorrhizal plant roots (Marschner & Dell 1994).

Other soil micro-organisms of importance are bacteria, various soil fungi, and algae. Bacteria are defined as unicellular organisms with a prokaryotic structure. Bacterial cells are close to 1 µm in size, in the shape of rods or spheres, some able to move by means of flagellae. The nitrifying bacteria, which fix atmospheric nitrogen in the soil, are particularly beneficial to plant growth (Gobat et al. 2004). Microscopic soil algae, which can be unicellular or in filamentous colonies, are often abundant at the soil surface or in large cracks because of their photosynthetic activity
Fig. 1.1. Endomycorrhizal fungal structures formed within root cells: vesicles, arbuscules, and hyphae. The hyphae extend out into the soil, facilitating nutrient uptake in plants (Brundrett et al. 1996).
Algae have an importance in aggregating solid particles and strengthening their cohesion via the production of extracellular polysaccharides (Gobat et al. 2004). The various soil fungi are characterised by their vegetative structures, known as mycelia, which consist of a multi-nucleate mass of cytoplasm enclosed within a branched system of tubes extending into the soil (Stainer 1986). The mycelium affects the structure of the soil by entangling soil particles into water-stable aggregates (Waksman 1963). Bacteria and fungi found in the soil can also be important in the biochemical decomposition of organic matter, bioremediation, and plant growth (Sharma 2005).

As well as the living soil biota, the non-living physical and chemical components of soil have an important role in plant growth and in the functioning of ecosystems. The chemical components of soil that influence plant growth and the forms in which they are available to plants, consist of six macro-nutrients: nitrogen (NH$_4^+$, NO$_3^-$), phosphorus (PO$_4^{3-}$), potassium (K$^+$), calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), and sulfur (SO$_4^{2-}$); and seven micro-nutrients: manganese (Mn$^{2+}$), copper (Cu$^{2+}$), zinc (Zn$^{2+}$), iron (Fe$^{2+}$), molybdenum (MoO$_4^{2-}$), boron (BO$_3^{3-}$), and chlorine (Cl$^-$) (Jones 1998). Other chemical properties of soil that have varying impacts on different plant species are the soil pH and the level of salinity in the soil. These properties can influence the chemical composition of essential plant nutrients, and their availability to plants.

The main physical properties of soil include soil type, texture, porosity, and bulk density. Soil type and texture are related to the relative proportions of gravel, sand, silt and clay in a soil, and have an important role in controlling the exchange, retention and uptake of water, nutrients, and oxygen (Ashman & Puri 2002). Bulk density, which is the mass of soil particles per unit volume, and soil porosity, which is the amount of pore space in a soil, are important properties related to the measurement of soil compaction. Soil compaction, associated with animal trampling and the use of machinery, has negative impacts on nutrient cycling, water availability, water and wind erosion, and surface and ground water quality (Bronick & Lal 2005).

The focus of this study was on endomycorrhizal colonisation success, micro-organism abundance, and soil properties, because they are all interlinked components of soil that could affect revegetation success. I compared soil biota, and chemical and physical soil properties among recently grazed and cropped land, revegetated land, and patches of remnant vegetation at Port Wakefield in South Australia. The remnant
vegetation sites were used as an indicator of the desired chemical, physical and biological soil properties at revegetated and agricultural sites. The study has importance in providing knowledge on the effects that agriculture (grazing and cropping in rotation) has had on the whole soil ecosystem. Apart from determining the impacts of agriculture on the soil, this research has importance in determining whether revegetation success is associated with increased mycorrhizal infection levels in plants, which would lead to more effective revegetation methods. Because the presence of endomycorrhizae is likely to be essential for restoration of complex communities formerly present at disturbed sites (Pattinson et al. 2004), one possible solution is inoculation of plants with endomycorrhizal fungi during the revegetation process. This research has specific importance in land restoration at Port Wakefield in South Australia, but knowledge gained from this research, such as endomycorrhizal colonisation success on revegetated land, can be extended to other semi-arid locations with similar vegetation communities. There have been no known previous studies carried out on mycorrhizal colonisation and invertebrate biodiversity in the region of this study.

Aims and objectives
The overall aim of this project was to determine the effect that land disturbance, specifically agriculture, has had on the whole soil ecosystem, and to determine whether revegetation success is associated with endomycorrhizal colonisation levels, invertebrate biodiversity, and nutrient concentrations in the soil. The specific aims and objectives of the project were:

1) **Aim** - To determine the effect agricultural land disturbance has had on physical and chemical soil properties, and to determine whether revegetated land has improved soil properties.
   **Objective** - To compare soil structure and texture, and classify soil based on the international soil textural triangle among grazed, revegetated, and remnant sites by determining soil grain size, bulk density and porosity.
   **Objective** - To compare pH, salinity and plant nutrient concentrations in soils among grazed, revegetated, and remnant vegetation sites by laboratory analysis of soil samples for salinity (electrical conductivity), pH, organic carbon, sodium, aluminium and the exchangeable nutrients: nitrogen,
phosphorus, potassium, calcium, magnesium, sulphur, manganese, copper, zinc, iron, molybdenum, boron, and chlorine.

2) **Aim** - To determine the effect agricultural land disturbance has had on soil micro-organism abundance and biodiversity, and to determine whether specific soil variables influence micro-organism abundance and biodiversity. **Objective** - To identify micro-organisms present (to phylum) at different soil depths and compare their abundance and Simpson’s Index of Diversity among agricultural, revegetated and remnant vegetation sites by taking soil samples at 5-cm intervals down the soil profile until bedrock is reached, and examining the samples using a microscope. **Objective** - To determine any significant relationships between micro-organism abundance and soil variables (nutrients, pH, salinity, organic carbon, soil texture, soil porosity, and bulk density) by statistical methods known as generalised linear models (GLM).

3) **Aim** - To determine the effect that agricultural land disturbance and specific soil properties have on endomycorrhizal colonisation of plant roots, and to determine whether revegetation success is associated with endomycorrhizal colonisation. **Objective** - to compare endomycorrhizal infection levels in roots of dominant plant species among grazed, revegetated, and remnant (control) sites, using the remnant vegetation sites as indicators of desired mycorrhizal colonisation levels. **Objective** - to determine any significant relationships between micro-organism abundance and soil variables (nutrients, pH, salinity, organic carbon, soil texture, soil porosity, and bulk density) by statistical methods known as generalised linear models (GLM).

**Thesis structure**
The research presented herein is set out in six chapters. The chapters are: general introduction (this Chapter, 1), study site (Chapter 2), physical and chemical soil properties (Chapter 3), micro-organism comparisons (Chapter 4), mycorrhizal comparisons (Chapter 5), and general discussion and conclusions (Chapter 6).
Chapter 1 provides an overview of the project, and aims of the research. Chapter 2 provides a description of the study site. Chapters 3, 4, and 5 are separate topics, intended to be presented in the format of scientific articles, so some repetition may occur among chapters. Chapter 6 is a general discussion and conclusions relating to the project results.
References


CHAPTER 2: Study site

Location

The study was located at the Australian Defence Force’s (ADF) firing range in the Port Wakefield district of South Australia (Fig. 2.1). The site is located 100 km north of Adelaide, and 3 km south of the town of Port Wakefield. Physical, chemical and biological soil comparisons were made between an historically grazed and cropped site recently purchased by ADF as an easement to the firing range, revegetated sites on ADF land, and remnant vegetation sites on ADF land and nearby roadsides. (See Appendices 1.1, 1.2 and 1.3 for photographs of study sites). The landform is characterised by a complex of tidal flats, dunes, shell grit deposits, saline depressions and sandy beaches, backed by flat to gently undulating calcarenite, sand and alluvial plains which extend inland until merging with the outlying hills and footslopes of the Mount Lofty Ranges (Department of Water, Land and Biodiversity Conservation unpubl. data).

Climate and vegetation structure

The Port Wakefield region has a winter-dominant rainfall, with dry summers. The precipitation records from 1874 to 2008 at Port Wakefield indicate a mean annual rainfall of 331.4 mm (Bureau of Meteorology 2008). Temperatures are mild throughout the winter months, and warm during the summer months. Temperature records for the nearby town of Price indicate a summer mean maximum temperature of 28°C, and a winter mean maximum temperature of 16°C (Bureau of Meteorology 2008).

The natural vegetation structure of the study location is a chenopod shrubland, characterised by salt tolerant xeromorphic shrubs. The dominant chenopod species present at both revegetated and remnant vegetation sites are Enchylaena tomentosa, Maireana brevifolia, Sclerolaena obliquicuspis, and Atriplex vesicaria. Native grass species present are Stipa nitida and Danthonia caespitosa. The dominant tree and shrub species throughout the study location are Eucalyptus gracilis, Melaleuca lanceolata, Myoporum platycarpum, and Pittosporum angustifolium. The vegetation structure of the paddock has been altered by land clearance. The paddock is a
Fig. 2.1. Study site located at the Australian Defence Force’s firing range, 100 km north of Adelaide in the Port Wakefield district of South Australia.
grassland dominated by *Stipa nitida*, and the introduced species *Medicago minima*, with no tree species present. However, chenopod species including *Enchylaena tomentosa*, *Maireana brevifolia*, and *Sclerolaena obliquicuspis* are sparsely distributed across the paddock. (Plant nomenclature follows Jessop & Toelken 1986).

**Site history**

The agricultural site has a history of cropping and grazing, which ceased in 2007. The land was purchased by ADF as a buffer zone, to be revegetated during 2008. This site has a 77 year-old history of cropping and grazing, first being used for cultivation of barley for export, then cultivation of oats for livestock feed, and most recently sheep grazing (P. White pers. comm. 2008).

The land within the firing range was used for livestock grazing up until the ADF acquired the land 10-15 years ago. Approximately 80% of this land was cleared prior to Defence acquisition. However, the exclusion of stock and the occurrence of three average to better than average years of rainfall over 1999 to 2001 have resulted in an improvement in the condition and diversity of the area (Department of Water, Land and Biodiversity Conservation *unpubl. data*). Many areas within the firing range were revegetated with native species approximately 8-15 years ago.
References

CHAPTER 3: A comparison of physical and chemical soil properties among agricultural, revegetated, and remnant vegetation sites

Abstract
The effects of agriculture on physical and chemical soil properties include depletion of essential plant nutrients and increased surface soil compaction. In South Australia, the problem of increasing salinity has also been a significant result of agriculture. After the cessation of agriculture, some of these problems may take many years to be reversed. However, revegetation may be of help in reversing some of the soil damage caused by agriculture.

A comparison study of the effects of agriculture on physical and chemical soil properties was carried out in the Port Wakefield region of South Australia. Soil type, bulk density, porosity, salinity, pH, organic carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, manganese, copper, zinc, molybdenum, chlorine, boron and iron were compared among agricultural, remnant vegetation, and 8-10 year-old revegetated sites. The most noticeable result of this study was the high salinity level at the agricultural site compared with remnant vegetation and revegetated sites. Most essential nutrients were not significantly depleted at the agricultural site, which had relatively high levels of nitrogen, sulphur, magnesium, and potassium, likely to have been a result of the addition of fertilizer and animal excreta to the soil. No significant soil structural damage was evident in the grazed paddock, which had highly porous soil, indicating that little soil compaction had occurred.

Revegetation may be a key to reversing the effects that soil disturbance has had on salinity levels. Even after only eight years following revegetation with native species, soils showed lower salinity levels than at the agricultural site. However, the return of essential nutrients such as zinc, iron and manganese may be a much slower process, requiring more than 8-10 years of revegetation and no soil disturbance. The fact that the agricultural site was not continually grazed, but was grazed and cropped in rotation, could lend itself to the lack of soil compaction.
Introduction

Physical and chemical soil properties, including texture, structure, pH, salinity, and nutrients available to plants, were compared among agricultural land (sheep-grazed and cropped in rotation), revegetated sites, and remnant vegetation sites. Agriculture has been associated with many soil impacts, including reduced litter cover, loss of surface soil microtopography, increased erosion, soil compaction, and changes in the concentrations of soil nutrients (Yates et al. 2000). Hiernaux et al. (1999) observed minor decreases in pH, and organic C, N and P contents in the topsoil after four years of grazing, and further decreases in pH and P after nine years of high grazing pressure. Similarly, Su et al. (2004) observed decreases in soil organic C, N, and P after short-term cultivation. These effects can have important consequences for plant growth, including poor root growth, yellowing of leaves, and delayed flowering and fruiting (Gardiner 2001). Ultimately, nutrient deficiencies can lead to the restoration of ecosystems being a very slow process. It has been suggested that 50 years may be needed for recovery of active soil organic matter and nutrients, but recovery of total soil organic matter pools is a slower process (Burke et al. 1995). A summary of the importance of the major soil chemistry properties is outlined in Table 3.1.

In relation to salinity, agriculture has had major impacts in South Australia. The replacement of deep-rooted perennial native vegetation with shallow-rooted annual crops has resulted in a rising water table, and the development of salinity problems (Barrett-Lennard 2002). Resulting problems of salinity may include increasingly alkaline soils and deficiencies of essential plant nutrients such, as boron and manganese (Gardiner 2001).

Soil texture, a physical soil property measured by the relative proportions of gravel, sand, silt and clay, is fundamental in controlling the exchange, retention and uptake of water, nutrients, and oxygen, but changes little through time (Ashman & Puri 2002). Soil structure, the way that sand, silt and clay particles bond together in units called aggregates, is also an important physical property, more readily changed by soil disturbance (Schoenholtz et al. 2000). Changes in soil structure, notably increased soil compaction caused by agriculture, can have negative impacts on nutrient recycling, water availability, and water and wind erosion (Bronick & Lal 2005). Soil compaction has been associated with animal trampling (Basher & Lynn 1996) and the use of heavy machinery (Entry et al. 2002). Bulk density, which is the
Table 3.1. The important plant nutrients and other major chemical elements of the soil, and their significant role in the soil (Gardiner 2001).

<table>
<thead>
<tr>
<th>Element name</th>
<th>Element’s significance in soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen**</td>
<td>Necessary for proteins and amino acids in plants.</td>
</tr>
<tr>
<td>Phosphorus**</td>
<td>Stimulates root growth and hastens plant maturity.</td>
</tr>
<tr>
<td>Potassium**</td>
<td>Important in regulation of osmosis in, various enzyme actions, and formation of carbohydrates.</td>
</tr>
<tr>
<td>Calcium**</td>
<td>Most prevalent in non-acidic soils. Essential for the normal functioning of plant cells.</td>
</tr>
<tr>
<td>Magnesium **</td>
<td>Most prevalent in non-acidic soils. Used in the chlorophyll molecules of plants, and is essential to stabilise ribosome structures.</td>
</tr>
<tr>
<td>Sulphur**</td>
<td>Forms sulfide or toxic hydrogen sulfide gas in poorly aerated soil. An essential part of proteins.</td>
</tr>
<tr>
<td>Manganese*</td>
<td>Involved in enzyme systems and in electron transport.</td>
</tr>
<tr>
<td>Copper*</td>
<td>May be present in poorly aerated soils. Involved in enzyme systems and in electron transport.</td>
</tr>
<tr>
<td>Zinc*</td>
<td>Often deficient in calcareous and eroded soils. Important for numerous enzyme systems.</td>
</tr>
<tr>
<td>Iron*</td>
<td>Low solubility in most soils, and important in energy-providing reactions in plants.</td>
</tr>
<tr>
<td>Molybdenum*</td>
<td>Required by plants in very small amounts. Required by some plants in the nitrogen-fixing enzyme nitrogenase.</td>
</tr>
<tr>
<td>Boron*</td>
<td>Water-soluble plant nutrient required in small concentrations, essential for protein and cell wall formation, sugar translocation, and pollination.</td>
</tr>
<tr>
<td>Chloride*</td>
<td>Occurs in small amounts, except in saline soil. Role in plants is osmotic, and in balancing cell cationic charges.</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>Influences physical structure of soil, and the soil’s water holding capacity.</td>
</tr>
<tr>
<td>pH</td>
<td>Strong acidity can cause soluble aluminium and manganese to reach toxic levels, and microbial activity is reduced.</td>
</tr>
<tr>
<td>Salinity</td>
<td>High salinity can hinder plant growth.</td>
</tr>
</tbody>
</table>

*Micro-nutrient essential to plant growth  
**Macro-nutrient essential to plant growth
mass of soil particles per unit volume, is used to monitor the degree of soil compaction (Schoenholtz et al. 2000). Even after the cessation of agriculture on the land, soil may be permanently affected. Soil structural damage caused by sheep trampling, in particular increased surface soil hardness, was visually evident even after 16 years following sheep removal (Braunack & Walker 1985).

Physical soil properties can influence soil microbial populations (Chapter 4) and mycorrhizal colonisation (Chapter 5), which is why the investigation of soil properties is an important part of this study. Soil type, including structure, influences the abundance of mycorrhizae, with studies indicating that mycorrhizal colonisation increases most rapidly in silty soils, but total infection levels are highest in clay soils (Gaur & Adholeya 2000). The micro-organism community structure can also be affected by soil particle size, with higher diversity of microbes found in soils with small size fractions compared with coarse size fractions (Sessitsch et al. 2001). However, physical soil properties such as bulk density and the degree of soil compaction may have little to no effect on microbial populations (Shestak & Busse 2005). The effects of soil structure and chemistry on mycorrhizal colonisation and soil micro-organisms are further discussed in Chapters 4 and 5.

This study examined the impacts of agriculture on physical and chemical soil properties by comparing soil quality on agricultural land with previously revegetated land and remnant vegetation sites. This knowledge will be important in determining the success of soil recovery after the cessation of agricultural practices, which would be a determinant of revegetation success. The overall aims of the study were to determine the effect agricultural land disturbance has had on physical and chemical soil properties, and to determine whether revegetated land has improved soil properties. (Note that the determination of the impacts of physical and chemical soil properties on soil biota will be focused on in Chapters 4 and 5). The objectives of this study were:

1) To compare soil structure and texture, and classify soil based on the international soil textural triangle, among grazed, revegetated, and remnant sites by determining soil grain size, bulk density and porosity.

2) To compare pH, salinity and plant nutrient concentrations in soils among grazed, revegetated, and remnant vegetation sites by laboratory analysis of soil samples for salinity (electrical conductivity), pH, organic carbon, sodium, aluminium and the
exchangeable nutrients: nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, manganese, copper, zinc, iron, molybdenum, boron, and chlorine.

Methods

Survey design
A total of 24 plots (eight at the agricultural site, one at each of eight revegetated sites, and one at each of eight remnant vegetation sites) was surveyed for soil chemistry, soil texture and soil structure. The agricultural site was sampled by a grid format of eight 100 m x 100 m plots spaced out as evenly as possible. A soil sample was collected from the centre of each plot. Since there was only one grazed paddock in the study location, interspersion of study plots to avoid pseudoreplication (Hurlbert 1984) was not possible; therefore, plots had to be spaced out as widely as possible to lessen the impacts of any background effects that might be concentrated in one area. The eight remnant vegetation (control) sites, and eight revegetated sites had a 100 m x 100 m plot established at each site, and a soil sample collected from the centre of each plot for analysis of soil properties. The selection of critical limits for soil properties, which is the desired range of values for a soil property, was based on the soil properties of remnant vegetation sites within the same ecological region as the agricultural and revegetated sites to eliminate the influence of climate and geomorphology (Arshad & Martin 2002).

Chemical soil properties
From the centre of each plot, a 25-cm deep soil core (or to the depth of bed-rock, if shallower), consisting of 200 g of soil was taken. Soil samples were mixed thoroughly, air dried, and stored in airtight containers. Analysis of pH, organic carbon, salinity (electrical conductivity), sodium, aluminium, chlorine, and exchangeable nutrients being: nitrogen compounds (ammonia (NH$_3$) and nitrate (NO$_3^-$)), phosphorus (Olsen and Colwell methods), potassium, calcium, magnesium, sulphur, manganese, copper, zinc, iron, molybdenum, and boron was carried out by Australian Perry Agricultural Laboratory in South Australia.
**Physical soil properties**

A sedimentation test was carried out to determine soil texture, which is the proportion of different sizes of inorganic particles that make up the soil. Soil particles are classified according to their diameter as: gravel (greater than 2 mm); sand (2.0 – 0.02 mm); silt (0.02 – 0.002 mm); and clay (less than 0.002 mm) (Andrews 1973). The sedimentation test is based on the rate at which different sized soil particles settle in water. Following the sedimentation test described by Plaster (2003), half a cup of soil, 875 ml of water, and five tablespoons of Calgon® laundry powder (a dispersing agent to help separate soil particles), were placed in a jar which was then shaken for five minutes. After shaking, the soil solution was poured into a 1000-ml measuring cylinder (Fig. 3.1). After one minute the depth of settled soil was measured and recorded as sand, then after one hour the depth of soil was measured and sand depth subtracted to obtain silt depth. After 24 hours most of the soil particles had settled, and the total soil depth was measured, and sand and silt depth subtracted to obtain clay depth. The percentage of each soil fraction was then calculated for the purpose of comparing the proportions of sand, silt and clay that made up the soil of each site. In addition, the percentages of sand, silt and clay were used to determine the textural class of the soil based on the international soil textural triangle (Fig. 3.2).

Soil structure was determined by measuring the soil’s bulk density, a measurement of the mass of particles in a given volume, and by measuring the soil’s total porosity, which is the percentage of pore space that makes up a soil. Following the methods of Ashman and Puri (2002), bulk density was determined by hammering a steel cylinder with a volume of 502.4 cm$^3$ into the soil so that its top was level with the soil surface. The soil contents were then removed from the cylinder, dried in an oven for ten hours at 105°C, and weighed to determine soil mass. Bulk density (g / cm$^3$) was calculated by: $rac{\text{soil mass}}{\text{volume of cylinder}}$. The percentage soil porosity was then determined by: $[1 - \frac{\text{bulk density}}{2.7 \text{ (average density of soil particles)}}] \times 100$.

**Statistical analysis**

One-way analysis of variance (ANOVA) was used to determine if soil properties (soil texture, porosity, bulk density, pH, salinity, and all chemistry variables) differed among at least one site type (agricultural, revegetated and remnant vegetation). Generalised linear models (GLM) (Hastie & Tibshirani 1990) were then performed to investigate which soil properties were significantly different across each site type.
Fig. 3.1. Soil sedimentation test, after being left for 24 hours, to determine the fraction of sand, silt and clay in a soil. The dark colour of the water is caused by a reaction with the Calgon® laundry powder.
Fig. 3.2. International soil textural triangle, used to plot the percentage of sand, silt and clay in a soil, to determine the textural class of the soil (Charman & Murphy 2000).
The analysis was performed using the software program R (R Development Core Team 2007), with a significance level of $\alpha = 0.05$.

In subsequent GLM (Wood 2006; Dobson & Barnett 2008) analyses of soil chemistry, pH and salinity were omitted as predictors, because preliminary statistics indicated that these soil properties were highly correlated with other soil chemistry variables. Generalised linear model output included a correlation matrix (Appendix 2). Note that nitrogen, chlorine, boron and molybdenum were not included in the GLM because the analysis of these nutrients was not returned from Australian Perry Agricultural Laboratory in time for statistical analysis.

Finally, a principal component analysis (PCA) (Shaw 2003) of the 20 soil variables (nutrients, pH, salinity, texture, porosity and bulk density) was performed to establish whether a reduced number of soil chemistry principal component (PC) constructs could be used to differentiate overall soil chemistry variability among sites, rather than by using all 20 soil variables. The two most significant combinations of soil variables that can explain variability among sites (PC1 and PC2) were determined. Similarly to the GLM analysis, nitrogen, chlorine, boron and molybdenum were not included in the PCA because the analysis of these nutrients was not available until later.

**Results**

*Soil porosity and bulk density*

Soil porosity ranged from 50% to 60% at all sites (Fig. 3.3). This result means that the amount of pore space in soils is quite high, indicative of soils that have not been severely compacted: the normal pore space in soils generally varies between 30% and 60% (Charman & Murphy 2000). ANOVA indicates that soil porosity and bulk density were significantly different among sites ($P<0.05$) (Table 3.2). GLM indicates that the sites with lowest soil porosity, and highest bulk density, were the revegetated sites, and that the grazed paddock and remnant vegetation sites were not significantly different (Fig. 3.4 [a, b]).

*Soil texture*

Soil type across the paddock was consistent, being a silty loam consisting of approximately 38% sand, 59% silt, and 3% clay (Fig. 3.3). Three of the revegetated
Fig. 3.3. Actual mean of each soil variable plotted by site type. The scales shown are proportional. Where indicated by 1/10, 1/100, or 1/1000 the true values are divided by 10, 100 or 1000 to fit on the scale. Values for each soil property range from: 0-1.98 for bulk density; 0-67.41 for porosity; 0-85 for sand; 0-62 for silt; 0-5 for clay; 0-9 for pH; 0-28 for phosphorus (Olsen); 0-27 for phosphorus (Colwell); 0-1.9 for organic carbon; 0-137 for sulphur; 0-4.4 for salinity; 0-4922 for calcium, 0-927 for magnesium; 0-920 for potassium; 0-3690 for sodium; 0-1 for aluminium; 0-19 for zinc; 0-1.55 for copper; 0-12 for manganese; and 0-0.1 for iron.
Table 3.2. Means and standard deviations (s.d.) of all soil variables across the three site types, and corresponding *P* value for the site effect for each variable. The asterisks indicate increasing levels of significance of a site effect (0 '***', 0.001 '***', 0.01 '*'). NS indicates no significant difference.

<table>
<thead>
<tr>
<th>variable</th>
<th>Grazed Paddock</th>
<th>Remnant</th>
<th>Revegetated</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
</tr>
<tr>
<td>Soil bulk density (g / cm$^3$)</td>
<td>1.17</td>
<td>0.04</td>
<td>1.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>56.67</td>
<td>1.66</td>
<td>62.22</td>
<td>3.36</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>38.25</td>
<td>3.28</td>
<td>68.63</td>
<td>10.01</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>59.38</td>
<td>2.50</td>
<td>28.13</td>
<td>9.66</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>2.38</td>
<td>1.92</td>
<td>3.25</td>
<td>1.49</td>
</tr>
<tr>
<td>pH (H2O)</td>
<td>8.94</td>
<td>0.19</td>
<td>7.98</td>
<td>0.92</td>
</tr>
<tr>
<td>Nitrogen (NO$_3$) (ppm)</td>
<td>50.00</td>
<td>9.67</td>
<td>14.30</td>
<td>13.33</td>
</tr>
<tr>
<td>Nitrogen (NH$_3$) (ppm)</td>
<td>2.88</td>
<td>0.62</td>
<td>4.15</td>
<td>3.33</td>
</tr>
<tr>
<td>Chlorine (ppm)</td>
<td>1279.05</td>
<td>791.00</td>
<td>253.30</td>
<td>263.60</td>
</tr>
<tr>
<td>Boron (ppm)</td>
<td>20.09</td>
<td>15.81</td>
<td>2.21</td>
<td>1.51</td>
</tr>
<tr>
<td>Molybdenum (ppm)</td>
<td>0.65</td>
<td>0.32</td>
<td>0.43</td>
<td>0.15</td>
</tr>
<tr>
<td>Phosphorus (Olsen) (ppm)</td>
<td>4.48</td>
<td>1.04</td>
<td>5.84</td>
<td>1.96</td>
</tr>
<tr>
<td>Phosphorus (Colwell) (ppm)</td>
<td>13.43</td>
<td>3.11</td>
<td>17.51</td>
<td>5.89</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>1.09</td>
<td>0.23</td>
<td>1.54</td>
<td>0.19</td>
</tr>
<tr>
<td>Sulphur (ppm)</td>
<td>84.63</td>
<td>45.83</td>
<td>46.75</td>
<td>47.89</td>
</tr>
<tr>
<td>Salinity (dS/m)</td>
<td>1.71</td>
<td>1.18</td>
<td>0.39</td>
<td>0.29</td>
</tr>
<tr>
<td>Calcium (ppm)</td>
<td>4520.59</td>
<td>261.87</td>
<td>3900.84</td>
<td>944.79</td>
</tr>
<tr>
<td>Magnesium (ppm)</td>
<td>680.39</td>
<td>165.82</td>
<td>428.75</td>
<td>167.95</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>675.31</td>
<td>129.59</td>
<td>401.54</td>
<td>131.68</td>
</tr>
<tr>
<td>Sodium (ppm)</td>
<td>1811.26</td>
<td>1111.02</td>
<td>318.41</td>
<td>289.02</td>
</tr>
<tr>
<td>Aluminum (ppm)</td>
<td>0.63</td>
<td>0.52</td>
<td>0.88</td>
<td>0.35</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>5.05</td>
<td>1.94</td>
<td>7.89</td>
<td>5.18</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>1.21</td>
<td>0.23</td>
<td>0.95</td>
<td>0.24</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>3.42</td>
<td>2.19</td>
<td>7.02</td>
<td>3.12</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>0.16</td>
<td>0.06</td>
<td>0.69</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Fig. 3.4. GLM fitted means and 95% confidence intervals for soil textural and structural variables plotted by site type. Actual values range from: 0-1.98 for bulk density; 0-67.41 for porosity; 0-85 for sand; 0-62 for silt; and 0-5 for clay.
sites had loamy sand soil types, consisting of approximately 76\% sand, 23\% silt, and 1\% clay. The other five revegetated sites had silty loam soil types, consisting of approximately 61\% sand, 37\% silt, and 2\% clay. All remnant vegetation sites located in the firing range had a silty loam soil type with high sand content, consisting of approximately 63\% sand, 33\% silt, and 4\% clay. One remnant vegetation roadside site also had a silty loam soil type consisting of 62\% sand, 37\% silt, and 1\% clay. The remaining three remnant vegetation roadside sites had a loamy sand soil type, consisting of approximately 79\% sand, 19\% silt, and 2\% clay. ANOVA (Table 3.2) indicates that sand and silt content were significantly different among the site types (P<0.001), but clay was not significantly different across the three site types. GLM (Fig. 3.4 [c, d]) indicates that the grazed paddock had the lowest sand content, and the remnant vegetation and revegetated sites were not significantly different.

Soil chemistry
ANOVA (Table 3.2) indicates that the only soil nutrients and minerals that did not differ among site types were ammonia (NH₃), phosphorus, aluminium, zinc, molybdenum and calcium, which were all found in low levels (Table 3.2). All other soil nutrients and minerals (organic carbon, sulphur, magnesium, potassium, sodium, copper, manganese, nitrogen (NO₃⁻), and iron) differed significantly among site types (P<0.05). Figure 3.5 (GLM comparisons), and Figure 3.6 (comparisons of means), give an indication of where the differences among site types for each soil chemistry variable lie, and Figure 3.3 gives the actual mean of the soil variables by site type. It is noted that the grazed paddock had the lowest mean levels of manganese and iron (but not statistically different to revegetated sites) (Fig. 3.5 [n, o]), but had the highest mean levels of nitrogen (NO₃⁻), chlorine, boron, sulphur, magnesium, potassium, copper, sodium, salinity and pH (Figs. 3.5 and 3.6). In contrast, the remnant sites had the highest mean levels of organic carbon, manganese, and iron (Fig. 3.5 [d, n, o]). The revegetated sites had the lowest mean levels of sulphur, potassium and copper, although not significantly lower than the remnant vegetation sites (Fig. 3.5 [e, i, m]).

Soil pH was significantly highest in the paddock (P<0.01) (Fig. 3.5a), having a mean pH of 8.94 indicating that the soil was strongly alkaline. Revegetated sites were also strongly alkaline (mean pH=8.8), and remnant sites were moderately alkaline (mean pH=7.98). Salinity and sodium levels were closely related, the paddock having
Fig. 3.5. GLM fitted means and 95% confidence intervals for soil chemistry variables plotted by site type. Actual values for each soil property range from: 0-9 for pH; 0-28 for phosphorus (Olsen); 0-27 for phosphorus (Colwell); 0-1.9 for organic carbon; 0-137 for sulphur; 0-4.4 for salinity; 0-4922 for calcium, 0-927 for magnesium; 0-920 for potassium; 0-3690 for sodium; 0-1 for aluminium; 0-19 for zinc; 0-1.55 for copper; 0-12 for manganese; and 0-0.1 for iron.
Nitrogen (NH$_3$)

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>1.2</th>
<th>2.4</th>
<th>3.6</th>
<th>4.8</th>
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</thead>
<tbody>
<tr>
<td>P</td>
<td>8</td>
<td>2.888</td>
<td>0.620</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>R</td>
<td>8</td>
<td>4.150</td>
<td>3.331</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>2.525</td>
<td>0.896</td>
<td></td>
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Nitrogen (NO$_3^-$)

<table>
<thead>
<tr>
<th>Site</th>
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<th>Mean</th>
<th>StDev</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
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<tbody>
<tr>
<td>P</td>
<td>8</td>
<td>50.00</td>
<td>9.67</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>R</td>
<td>8</td>
<td>14.30</td>
<td>13.33</td>
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<tr>
<td>V</td>
<td>8</td>
<td>19.14</td>
<td>11.05</td>
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Chlorine

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<tbody>
<tr>
<td>P</td>
<td>8</td>
<td>1279.5</td>
<td>791.0</td>
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<td>8</td>
<td>253.3</td>
<td>263.6</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>266.3</td>
<td>400.3</td>
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Boron

<table>
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<tr>
<th>Site</th>
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<th>0.45</th>
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<tr>
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<td>20.095</td>
<td>15.817</td>
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<td></td>
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<tr>
<td>R</td>
<td>8</td>
<td>2.214</td>
<td>1.506</td>
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</tr>
<tr>
<td>V</td>
<td>8</td>
<td>3.656</td>
<td>4.744</td>
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Molybdenum

<table>
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<tr>
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<th>N</th>
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<th>StDev</th>
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<th>0.45</th>
<th>0.60</th>
<th>0.75</th>
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<tbody>
<tr>
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<td>0.6550</td>
<td>0.3164</td>
<td></td>
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<tr>
<td>R</td>
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<td>0.4350</td>
<td>0.1548</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
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<td>0.4400</td>
<td>0.0882</td>
<td></td>
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</tbody>
</table>

Fig. 3.6. Means of soil nutrients, and 95% confidence intervals for mean based on pooled standard deviation.  P indicates paddock, R indicates remnant vegetation sites, and V indicates revegetated sites.  Note that GLM analysis was not performed on these soil nutrients because of soil analysis reports for these nutrients not being available from Perry Agricultural Laboratory until later.
four times higher salinity and sodium levels than both the revegetated and remnant vegetation sites (Table 3.2 and Fig. 3.5 [f, j]). A Pearson correlation table is given in Appendix 2, giving an indication of how closely related soil variables are to each other.

**Principal component analysis (PCA) of soil variables**

Table 3.3 and Figure 3.7 show that the 20 soil characteristics can be reduced to two principal components, PC1 and PC2, which jointly account for 97.8% of the variation in the soil characteristic data. PC1 alone accounts for 68% of the variation in the soil data, and is effectively a linear combination of primarily sodium, then potassium, calcium, magnesium and sulphur. PC2 is a contrast between calcium (and magnesium and potassium) versus sodium, as evidenced by the weights for the principal components in Table 3.3. Effectively, five of the 20 soil characteristics are used in the principal component analysis (PCA), and there are two principal components that summarise the data well.

Figure 3.7 gives a scatter plot of PC2 versus PC1 with the three site types labelled. The grazed paddock is clearly lower on PC1 (+sodium + sulphur + magnesium + potassium + calcium). Both high and low values on PC2 are evident for the remnant sites. Remnant site PC1 values tend to be above 0.00. PC2 values for the revegetated site tend to be below 0.00. PC1 separates the paddock from the remnant and revegetated sites (but may be influenced by the “outlier” site high in sodium). PC2 separates the remnant and revegetated sites (but is strongly influenced by three of the revegetated sites, low in calcium, magnesium, and potassium).

**Discussion**

Soil texture was similar among site types, all soils being a silty loam or loamy sand. However, the agricultural soil had significantly less sand content than all of the remnant and revegetated soils. This result is not unusual, as sandy soils may not be of preference for agricultural purposes because the soil dries rapidly and easily loses plant nutrients, which are drained away in percolating water (Gardiner 2001). Soil texture changes little over time, so had minor importance as an indicator of land disturbance impacts. Soil structure, changing more readily as a result of soil disturbance, was different among site types. Porosity, an important structural
Table 3.3. Principal weights and proportion of variance for soil variables. Variables that were significant in the principal component analysis for PC1 and PC2 are marked in bold. Note that all 20 soil variables are not included in the table, because the ones not included are very similar in explaining site variability to the variables already listed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus (Olsen)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Phosphorus (Colwell)</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Sulphur</td>
<td>-0.033</td>
<td>0.033</td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.142</td>
<td>-0.982</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-0.097</td>
<td>-0.106</td>
</tr>
<tr>
<td>Potassium</td>
<td>-0.157</td>
<td>-0.012</td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.972</td>
<td>0.155</td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.000</td>
<td>0.003</td>
</tr>
<tr>
<td>Copper</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Iron</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1021.030</td>
<td>677.013</td>
</tr>
<tr>
<td>Proportion of Variance</td>
<td>0.680</td>
<td>0.299</td>
</tr>
<tr>
<td>Cumulative Proportion</td>
<td>0.680</td>
<td>0.978</td>
</tr>
</tbody>
</table>
Fig. 3.7. Principal component analysis (PCA): scatter plot of PC1 versus PC2. PC1 is essentially a combination of negative values of sulphur, calcium, magnesium, potassium and sodium; and PC2 is a combination of negative values of calcium, magnesium, and potassium, and positive values of sodium.
component related to soil compaction, was highest at the grazed paddock and lowest at the revegetated sites. However, it is interesting to note that soil porosity was greater than 50% at all sites, indicating that all sites had relatively high soil pore space. This result suggests that sheep grazing has not significantly compacted the soil. As sheep grazing at the paddock site was not continuous, but was rotated with cropping, this practice may have helped to avoid severe soil compaction.

The soil nutrients that did not differ among site types were nitrogen (NH$_3$), phosphorus, aluminium, zinc, molybdenum and calcium, all occurring in low concentrations. Phosphorus, an important macro-nutrient required for stimulation of root growth and hastening plant maturity, is commonly deficient in soils (Gardiner 2001), so this result was not unusual. Interestingly, manganese and iron were the only nutrients to be lowest in the paddock when compared to remnant and revegetated sites, possibly because of absorption by crop plants. However, manganese and iron levels in the paddock were not significantly lower than at the revegetated sites. Even though remnant vegetation sites had significantly highest levels of iron and manganese, their levels were still considered very low compared with a normal Australian soil. Iron and manganese deficiencies are most common in arid soils and sands (Gardiner 2001), which may explain the very low iron and manganese levels at all sites. The nutrients found in highest concentrations in the paddock when compared with remnant and revegetated sites were nitrates (NO$_3$), sulphur, magnesium, potassium, chlorine, boron and copper. Sulphur, nitrogen, magnesium, and potassium, all macro-nutrients, had medium to high concentrations in all of the paddock plots. With the common additions of sulphur, nitrogen, potassium, and magnesium to fertilizers, it was not unusual that the agricultural site had significantly high concentrations of these nutrients. In addition, animal excreta could have contributed to the high levels of nitrogen, magnesium and potassium in the sheep-grazed paddock (Smith & Wheeler 1979).

The recovery of organic carbon, zinc, iron, and manganese in the soil appears to be slow, as revegetated sites had similar nutrient levels to the grazed paddock, whereas remnant sites had significantly higher levels of these nutrients. This result suggests that eight years (the number of years since revegetation occurred) is not long enough to allow for the recovery of some nutrients and organic carbon. It is possible that 50 years or more may be required before sufficient nutrient recovery is evident (Burke et al. 1995).
Salinity at the agricultural site was very high. Both salinity (conductivity) and sodium levels were at least four times higher in the paddock than at both the remnant vegetation and revegetated sites. A common cause of excess salt in agricultural soils is the removal of large amounts of water from the soil but very small amounts of salt by crop plants (Wolf & Snyder 2003). Excessive soil salts can also be introduced and built up by various fertilizers applied to the soil. High levels of salinity can have consequences for revegetation, in that water uptake by plants may be impeded, affecting the growth and establishment of plants (Wolf & Snyder 2003).

All sites had alkaline soils, with the paddock being strongly alkaline. One of the main hazards of strongly alkaline soils is a deficiency of manganese (Gardiner 2001), which was evident in this study, all sites having very low manganese levels. Alkaline soils with a pH of above 8.3 are usually caused by sodium (Gardiner 2001), which explains the very high pH levels in the paddock.

The most important findings of the principal component analysis were that sodium, followed by potassium, calcium, magnesium and sulphur were the main soil chemistries influencing variability among soils. In particular, high sodium was influencing soils in the grazed paddock. Remnant and revegetated sites tended to be lowest in the sulphur, calcium, magnesium, potassium and sodium combination.

Overall, salinity at the agricultural site was the most significant soil chemistry property to be concerned about. The most apparent results of this study were the high sodium and salinity levels at the agricultural site, which has more than likely been the cause of strongly alkaline soils, which has then led to deficiencies of manganese. It is possible that revegetation with native species can help to reverse salinisation by lowering the water table (Schofield 1992). Significantly lower levels of salinity at all revegetated sites than the agricultural site in this study give evidence that revegetation may help to reverse the salinity problem. Essential plant nutrients and soil structure in the paddock did not appear to be significantly degraded. The addition of animal excreta and fertilizer to the soil probably increased sulphur, nitrogen, potassium, and magnesium levels in the paddock.
References


R Development Core Team. (2007) R Version 2.6.0. NZ.


CHAPTER 4: A comparison of soil micro-organism abundance and biodiversity among agricultural, revegetated, and remnant vegetation sites

Abstract
The three major micro-organism groups in soil are the bacteria, fungi, and microscopic algae, all playing an important part in promoting nutrient cycling, organic matter decomposition, and aggregation of soil particles, leading to improved plant growth. Previous literature on the effects of agriculture on microbial populations has shown varying outcomes, from significantly reduced microbial numbers to no changes in microbial populations. This study aimed to determine the impact of a 77-year history of sheep grazing and cropping in rotation on micro-organism abundance and biodiversity. Micro-organism abundance and biodiversity at an agricultural site were compared with organisms at revegetated and remnant vegetation (control) sites in the Port Wakefield region of South Australia.

Micro-organisms identified in this study were Proteobacteria, Cyanobacteria, Chlorophyta and Ascomycota, all occurring at the three site types. The grazed paddock had the highest abundance of Proteobacteria and Cyanobacteria, but there was no difference in abundance of Chlorophyta and Ascomycota among site types. Soil texture was the main variable affecting micro-organism abundance, with sandy soils having significantly lower numbers of organisms than silty soils. Biodiversity was lowest in the grazed paddock and in the upper layers of the soil profile, because of a dominance of Proteobacteria at these locations.

The findings of this study agree with previous literature that external factors such as agricultural land use, fertilizer, or sheep grazing only have a small effect on determining microbial abundance compared with factors such as soil particle size. It has been suggested that finer sized soil particles (silt and clay) provide a protective habitat for micro-organisms through pore-size exclusion of protozoan predators (Sessitsch et al. 2001). However, it can not be concluded that agriculture has little or no impact on soil micro-organisms, as higher-intensity agriculture may have a greater impact on the soil biota.
Introduction

In ecosystems that have been used for agriculture, the effects of soil disturbance on the vegetation have been widely studied; however, the effects of soil disturbance on microscopic invertebrate communities have been less widely studied. Microorganisms are an important part of the soil ecosystem, returning organic matter to the mineral state so that nutrients become available to plants, and maintaining soil structure and aeration (Davet 2004). I chose to study the impacts of land disturbance on soil micro-organisms as well as on mycorrhizae (as outlined in Chapter 5), because they both occur in the soil surrounding plant roots, known as the rhizosphere (Shepley 1973), and have an important interaction with each other. The four major groups of micro-organisms recognised in soils are bacteria, fungi, microscopic algae and protozoa. However, protozoa are conventionally included in the micro-fauna (Gobat et al. 2004).

Microscopic soil organisms greatly affect soil structure, nutrient cycling, organic matter decomposition and plant growth. The specific micro-organisms present in a soil vary, depending on soil type, moisture content, light penetration and pH (Waksman 1963). The depth at which organisms are sampled from in the soil will also be a determinant in organism numbers and types, with bacteria, fungi and algae most abundant in the first few centimetres of soil where light penetration is greatest (Waksman 1963). In particular, algal production is correlated with light intensity, because algae are photosynthetic and therefore need light for growth and reproduction (Shimmel & Darley 1985). The extent of light penetration through soil is affected by factors such as soil moisture content, and particle size and colour (Tester & Morris 1987).

Bacteria, which include spore-forming rods, cocci, vibrios and spirilla, are the most abundant of all soil organisms, with numbers in thousands per gram estimated to be as high as 7340 in some soils (Waksman 1963). Bacterial community structure and abundance are thought to be affected to a greater extent by soil type, for example particle size fraction of the soil, than by external factors such as the kind of fertilizer applied to the soil (Sessitsch et al. 2001). Bacterial abundance is positively correlated with high nitrogen and moisture levels in the soil (Aguilera et al. 1999). The three major functions of bacteria in soils are nutrient mobilisation in the soil, fixation of nitrogen, and protection of plants against pathogens (Johansson et al. 2004; Frey-Klett
et al. 2007). Many bacterial groups, including Proteobacteria, Firmicutes, and Actinomycetes are also known as ‘mycorrhiza helper bacteria’ because they promote establishment and growth of mycorrhizae by stimulating mycorrhizal spore germination, increasing the growth rate of hyphae, and reducing soil-mediated stresses (Frey-Klett et al. 2007).

The various soil fungi and algae positively influence soil structure. For example, aggregation of soils by filamentous organisms such as fungal mycelium and algae can improve the structure of fine textured soils (Bond & Harris 1964). The mycelium of fungi, which is a multi-nucleate mass of cytoplasm enclosed within a branched system of tubes, affects the structure of soil by entangling soil particles into water-stable aggregates (Waksman 1963). The aggregation of soil is essential to plant growth, because aggregation promotes gas exchange, penetration of water, and resistance to erosion (Gray & Williams 1971). The soil-aggregating mycelial network may be considerably large in fertile soil, sometimes attaining a total length of 10,000 km in just one square metre of soil (Gobat et al. 2004). Algae also aggregate solid soil particles and strengthen their cohesion, but in a different way to fungi. The main function of algae is the production of extracellular polysaccharides that assist with soil cohesion (Gobat et al. 2004).

Soil disturbance by agricultural activities can affect the production and abundance of microbial populations. Tillage has been found to affect soil fungi and bacteria, both organisms having greater biomass in soils that have not undergone tillage (Kladivko 2001). This greater microbial biomass in no-tillage soils is likely to be due to cooler, wetter conditions, and less fluctuation in temperature and moisture (Kladivko 2001). However, some agricultural impacts such as soil compaction caused by sheep grazing and machinery may have little impact on microbial populations. Shestak and Busse (2005) found that increased soil compaction was unrelated to microbial biomass including bacteria abundance and fungi abundance.

This study used data collected on invertebrate biodiversity, abundance, and species richness to assess biological soil quality at agricultural, revegetated, and remnant vegetation sites. Organism phyla, rather than individual species (because of the difficulty in identifying organisms to finer taxa), were used to determine the structure of microbial populations. This study has importance in providing knowledge on the impacts of soil disturbance on micro-organism communities, and
also in the conservation of invertebrate biodiversity, which is necessary for maintaining soil health.

The aims of this study were to determine the effect that agricultural land disturbance has had on soil micro-organism abundance and biodiversity, and to determine whether specific soil variables influence micro-organism abundance and biodiversity. The objectives of the study were:

1) To identify micro-organisms present (to phylum) at different soil depths, and compare their abundance and Simpson’s Index of Diversity, among revegetated, agricultural and remnant vegetation sites, by taking soil samples at 5-cm intervals down the soil profile to a depth of 30 cm or until bedrock was reached, and examining the samples using a microscope.

2) To determine any significant relationships between micro-organism abundance and soil variables (nutrients, pH, salinity, organic carbon, soil texture, soil porosity, and bulk density) by statistical methods known as generalised linear models (GLM).

**Methods**

**Survey design**

Micro-organism biodiversity, abundance, and richness were compared among agricultural, revegetated and remnant vegetation sites. Soil cores were used to determine the abundance and type of micro-organisms present at various depths in the soil profile. A total of 24 plots (eight at the agricultural site, one at each of eight revegetated sites, and one at each of eight remnant vegetation sites) were established to sample micro-organisms. The agricultural site was sampled by a grid format of eight 100 m x 100 m plots, while the eight remnant vegetation sites (controls), and eight revegetated sites were sampled by a 100 m x 100 m plot located at each site.

**Sampling procedure**

Soil organisms were sampled by taking a soil core from the centre of each plot (eight plots per site type), first taking the litter layer, and then each subsequent 1-cm interval to a depth of 30 cm, or until bed-rock was reached. Sample depths of less than 30 cm are not likely to give a good estimation of the vertical distribution of soil invertebrates (Andre et al. 2002). Following pilot work, the 1-cm deep soil samples were pooled to 5-cm samples, which were sufficient to describe the distribution of micro-organisms.
down the soil profile. Intact soil core samples were isolated in 8-cm diameter polyvinyl chloride (PVC) tubes, which were hammered into the soil. Each soil interval was then carefully taken from the core using a teaspoon and stored in sealed plastic bags until micro-organisms would be analysed in the laboratory. Micro-organisms were stored at room temperature and were analysed within five days of collection.

Data for soil properties, including salinity, pH, organic carbon, sodium, aluminium and the exchangeable nutrients: phosphorus, potassium, calcium, magnesium, sulphur, manganese, copper, zinc and iron were gathered by the methods outlined in Chapter 3. These data were then used to determine any statistically significant associations between soil variables and micro-organism abundance. Note that nitrogen, molybdenum, chlorine, and boron were not included in the statistical analysis as laboratory analysis results for these nutrients were not available until later.

Quantitative analysis of micro-organisms
In the laboratory, 2 g of each 5-cm soil sample were added to 20 ml of distilled water and shaken for 10 minutes to separate soil particles and evenly distribute organisms. Using a pipette, one drop of the soil-water solution was placed between a glass microscope slide and cover-slip. Micro-organisms were counted under a light microscope at 1000 x magnification. Using the measurement scale in the microscope eyepiece, all microorganisms that were intercepted by the 100µm measurement line were counted. Any rarely-occurring organisms were noted as occurring in the whole microscope field of view. A total of ten counts was carried out for each 1-cm soil sample. It was not possible to classify organisms to species level because of the taxonomic difficulty in accurately identifying the various bacteria and algae. For the purpose of this study, identifying organisms to phylum was sufficient to be able to make soil biodiversity comparisons among sites.

Statistical analysis
Biodiversity was calculated using Simpson’s Index of Diversity

\[ 1-D = 1 - \sum_{n=1}^{N} \frac{n(n-1)}{N(N-1)} \]

where \( D \) is Simpson's index, \( n \) is the number of individuals of a taxon and \( N \) is the total number of individuals for all taxa (Krebs 1999). The values for Simpson’s Index of Diversity range from 0 – 1, with high numbers indicating a high species diversity.
The index was calculated for each 5-cm interval down the soil profile at each of the site types (remnant vegetation, revegetated and grazed paddock). Invertebrate richness was determined by the number of different taxa recorded per site. Relative abundance for each taxon was determined by dividing the abundance of a taxon by the total abundance of all taxa.

Statistical comparisons of micro-organism abundance and biodiversity among site types, and associations between organisms and soil variables were carried out using the software program R (R Development Core Team 2007), with a significance level of \( \alpha = 0.05 \). One-way analysis of variance (ANOVA) was used to determine if there were any significant soil property influences on micro-organism abundance, and to determine any significant differences in micro-organism abundance among site types. A stepwise generalised linear model (GLM) analysis was then carried out to determine specific associations between micro-organism abundance and soil variables. Significant differences in biodiversity among site types were determined by ANOVA, and stepwise GLM analyses were carried out to establish the significance of soil depth and soil properties as covariates.

Statistical analyses were first carried out to determine differences among all three site types (paddock, remnant vegetation and revegetated), and then to determine differences between only two sites (remnant vegetation and revegetated). The reason for these separate comparisons was that there was a distinction between the three-site versus two-site data, in that soil bulk density and porosity data were only available for the remnant and revegetated sites, so could not be used as covariates when including the paddock as a comparison site. Even though soil bulk density and porosity data were taken from the paddock, samples were not taken from the exact same plots as micro-organism samples because of weed-poisoning in some plots, which may have influenced micro-organism abundance if organisms were sampled from these plots.

**Results**

*Micro-organism richness*

The phyla identified at all sites were: Proteobacteria, characterised by their brown colour and round shape, with flagellae allowing them to move rapidly; Cyanobacteria, also referred to as ‘blue-green algae’ because of their photosynthetic pigments giving them a green tinge; Chlorophyta, also known as ‘green algae’, which have green
chloroplasts surrounded by two membranes; and Ascomycota, which are fungi that grow as filamentous mycelia composed of septate hyphae (Barnes 1998). For a photographic description see Appendix 3. All sites had all four phyla present at all soil depths (richness = 4).

**Relative abundance of micro-organisms**

Mean relative abundances of each organism in the paddock were: Proteobacteria 49%, Cyanobacteria 32%, Chlorophyta 17%, and Ascomycota 1%. At revegetated sites mean relative abundances were: Proteobacteria 46%, Cyanobacteria 30%, Chlorophyta 23% and Ascomycota 1%. At remnant vegetation sites mean relative abundances were: Proteobacteria 45%, Cyanobacteria 31%, Chlorophyta 23% and Ascomycota 1%.

**Micro-organism abundance: three-site analysis (paddock, remnant, and revegetated)**

Proteobacteria were highly site dependent, with the grazed paddock having significantly higher abundance than remnant vegetation sites ($P<0.001$) and revegetated sites ($P<0.01$) (Fig. 4.1a and Table 4.1a). Soil properties may account for the differences among site types, as abundance for Proteobacteria was highly significantly and negatively correlated with soil depth ($P<0.0001$), sand ($P<0.006$), silt ($P<0.01$), phosphorus ($P<0.001$), and copper ($P<0.001$) (Fig. 4.1 [b, c, d, e, j]). However, Proteobacteria abundance was positively correlated with calcium ($P<0.05$), sodium ($P<0.001$), aluminium ($P<0.05$), zinc ($P<0.001$) and iron ($P<0.001$) (Fig. 4.1 [f, g, h, i, k]). Note that in the GLM output Proteobacteria (and Cyanobacteria) were negatively correlated with silt because the statistical analysis takes into account the whole range of soil variables. Without adjusting for other soil variables, silt is actually positively correlated with abundance, and sand negatively correlated with abundance as indicated by Figure 4.2 (a, b).

Cyanobacteria were highly site dependent with the grazed paddock having significantly highest abundance when compared to remnant ($P<0.05$) and revegetated ($P<0.05$) sites (Fig. 4.3a and Table 4.1b.) Abundance for Cyanobacteria was negatively correlated with soil depth ($P<0.0001$), sand ($P<0.05$), and silt ($P<0.05$) (Fig. 4.3 [b, c, d]). Unlike Proteobacteria, Cyanobacteria were not significantly negatively correlated with copper and phosphorus levels. Abundance of
Fig 4.1. GLM partial plots for Proteobacteria abundance, showing fitted mean and 95% confidence intervals: three-site analysis.
Table 4.1. Stepwise GLM analysis for micro-organism abundance and significantly associated soil variables: three sites with soil chemistry and texture as indicators. Note that the relative order of importance is indicated by the order in which variables are listed in the table.

(a) Proteobacteria abundance

<table>
<thead>
<tr>
<th>site</th>
<th>- depth</th>
<th>- sand</th>
<th>- silt</th>
<th>- Phosphorus (Olsen)</th>
<th>+ Ca</th>
<th>+ Sodium</th>
<th>+ Alum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddock &gt; Remnant</td>
<td>(P=0.00001)</td>
<td>(P&lt;0.00001)</td>
<td>(P=0.006)</td>
<td>(P=0.008)</td>
<td>(P=0.006)</td>
<td>(P=0.05)</td>
<td>(P=0.0003)</td>
</tr>
<tr>
<td>Paddock &gt; Revegetated</td>
<td></td>
<td>+ Zinc</td>
<td>+ Iron</td>
<td>- Copper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remnant &lt; Revegetated</td>
<td>(P=0.0004)</td>
<td>(P=0.002)</td>
<td>(P=0.0002)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Cyanobacteria abundance

<table>
<thead>
<tr>
<th>Site</th>
<th>- depth</th>
<th>- sand</th>
<th>- silt</th>
<th>- Phosphorus (Olsen)</th>
<th>+ Magnesium</th>
<th>+ Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddock &gt; Remnant</td>
<td>(P=0.0000)</td>
<td>(P&lt;0.00001)</td>
<td>(P=0.01)</td>
<td>(P=0.04)</td>
<td>(P=0.06)</td>
<td>(P=0.0008)</td>
</tr>
<tr>
<td>Paddock &gt; Revegetated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remnant &lt; Revegetated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) Chlorophyta abundance

<table>
<thead>
<tr>
<th>site</th>
<th>- depth</th>
<th>+ Magnesium</th>
<th>+ Alum</th>
<th>+ Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>(P&lt;0.00001)</td>
<td>(P=0.0006)</td>
<td>(P=0.002)</td>
<td>(P=0.0006)</td>
</tr>
</tbody>
</table>
Fig. 4.2. Scatter plot showing the association between micro-organism abundance and sand, and silt content of the soil. Note that these graphs are actual values, and not adjusted to account for other soil variables.
Fig 4.3. GLM partial plots for Cyanobacteria abundance, showing fitted mean and 95% confidence intervals: three-site analysis.
Cyanobacteria was highly significantly and positively correlated with magnesium ($P<0.001$), zinc ($P<0.001$), and iron (10% significance level) (Fig. 4.3 [f, g, i]).

Chlorophyta abundance was not significantly different across revegetated, remnant vegetation and grazed paddock sites (Table 4.1c). Abundance was negatively correlated with soil depth ($P<0.0001$) (Fig. 4.4a). The only significant associations with soil chemistry were a positive correlation with magnesium ($P<0.001$), aluminium ($P<0.01$), and zinc ($P<0.001$) (Fig. 4.4 [d, e, f]).

Ascomycota had no significant association with depth of sampling, or any of the soil chemistry variables. The only variable found to have a slight positive association ($P=0.05$) was sand. There was no difference in Ascomycota abundance among site types. (See Appendix 4 for ANOVA tables).

Micro-organism abundance: two-site analysis (remnant and revegetated)
Proteobacteria were highly site dependent, with the remnant vegetation sites having lower abundance than the revegetated sites ($P<0.03$) (Fig. 4.5a). Soil properties may account for the differences among site types, as abundance of Proteobacteria was negatively correlated with depth of sampling ($P<0.00001$) (Fig. 4.5b and Table 4.2a). Proteobacteria were significantly and positively correlated with soil bulk density ($P<0.05$), soil porosity ($P<0.05$), magnesium ($P<0.002$), aluminium ($P<0.005$) and zinc ($P<0.00001$) (Fig. 4.5 [c, d, f, i, j] and Table 4.2a).

Cyanobacteria abundance was not different across revegetated and remnant vegetation sites. Abundance for Cyanobacteria was negatively correlated with soil depth ($P<0.00001$) and calcium ($P<0.001$), and positively correlated with phosphorus ($P<0.01$), sulphur ($P<0.02$) and magnesium ($P<0.00001$) (Fig. 4.6 [a, b, c, d, e] and Table 4.2b). Unlike Proteobacteria, Cyanobacteria were not significantly positively correlated with aluminium or zinc.

Chlorophyta abundance was not significantly different across revegetated and remnant vegetation sites. Abundance was negatively correlated with soil depth ($P<0.00001$) (Table 4.2c and Fig. 4.7a). The only significant associations with soil chemistry were a positive correlation with magnesium ($P<0.006$), aluminium ($P<0.05$), and zinc ($P<0.000001$) (Fig. 4.7 [b, c, d] and Table 4.2c).

Ascomycota abundance was not significantly different among remnant vegetation and revegetated sites, and had no significant association with soil depth, or
Fig 4.4. GLM partial plots for Chlorophyta abundance, showing fitted mean and 95% confidence intervals: three-site analysis. Note: silt and sulphur have no significant association with Chlorophyta abundance.
Fig. 4.5. GLM partial plots for Proteobacteria abundance, showing fitted mean and 95% confidence intervals: two-site analysis (includes soil bulk density and porosity as predictors, as well as the soil chemistries).
Table 4.2. Stepwise GLM analysis for micro-organism abundance and significantly associated soil variables: two sites (remnant vegetation and revegetated) with soil chemistry, soil density, and soil porosity as indicators. Note that the relative order of importance is indicated by the order in which variables are listed in the table.

<table>
<thead>
<tr>
<th>(a) Proteobacteria abundance</th>
<th>Site</th>
<th>Site-depth</th>
<th>density</th>
<th>porosity</th>
<th>sand</th>
<th>Magnesium</th>
<th>Aluminum</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remnant &lt; Revegetated</td>
<td>P=0.026</td>
<td>P&lt;0.00001</td>
<td>P=0.04</td>
<td>P=0.04</td>
<td>P=0.002</td>
<td>P=0.001</td>
<td>P=0.004</td>
<td>P&lt;0.00001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Cyanobacteria abundance</th>
<th>Site</th>
<th>Site-depth</th>
<th>Phosphorus (Olsen)</th>
<th>Sulphur</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>P&lt;0.00001</td>
<td>P=0.009</td>
<td>P=0.01</td>
<td>P=0.0005</td>
<td>P&lt;0.00001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(c) Chlorophyta abundance</th>
<th>Site</th>
<th>Site-depth</th>
<th>Magnesium</th>
<th>Aluminum</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>P&lt;0.00001</td>
<td>P=0.005</td>
<td>P=0.04</td>
<td>P&lt;0.00001</td>
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Fig. 4.6. GLM partial plots for Cyanobacteria abundance, showing fitted mean and 95% confidence intervals: two-site analysis.
Fig. 4.7. GLM partial plots for Chlorophyta abundance, showing fitted mean and 95% confidence intervals: two-site analysis. Note: silt and sulphur have no significant association with Chlorophyta abundance
soil bulk density, or porosity. The only soil variable found to have a slight positive impact was sand ($P<0.05$).

**Micro-organism biodiversity: three-site analysis (paddock, remnant, and revegetated)**

Simpson’s Index of Diversity indicates medium diversity for all sites. The paddock had a mean Simpson’s Index of Diversity of 0.652, the revegetated sites of 0.695, and the remnant vegetation sites of 0.689 (Fig. 4.8). The remnant and revegetated sites were not significantly different in biodiversity, but the paddock was significantly lower than the other two sites (Fig. 4.9a). The most significant soil variable affecting biodiversity was soil depth ($P<0.00001$) (Table 4.3a and Fig. 4.9b). At all sites diversity significantly increased with increasing depth of sampling.

After adjusting for the significant positive effect of soil depth, ($P<0.00001$), sand ($P<0.05$), silt ($P<0.05$) and phosphorus ($P<0.0001$) on species diversity, the grazed paddock still had significantly lower diversity than the remnant vegetation ($P<0.0001$), and the revegetated sites ($P<0.002$). Indeed these site differences remained significantly different after adjusting the significantly negative impacts of organic carbon ($P<0.05$), calcium ($P<0.05$), sodium ($P<0.05$), zinc ($P<0.0005$), and iron ($P<0.00001$).

**Micro-organism biodiversity: two-site analysis (remnant vegetation and revegetated)**

From Table 4.3b, which shows the GLM results of micro-organism biodiversity using soil chemistry as predictors (in addition to site, species, depth, soil texture and structure, and density and porosity), it is noted that no significant difference exists between remnant and revegetated sites.

Micro-organism biodiversity increased significantly with increasing soil depth ($P<0.00001$), but biodiversity decreased significantly with increasing soil bulk density ($P<0.01$) and porosity ($P<0.01$) (Fig. 4.10 [a, b, c]). After adjusting for the negative effects of bulk density and soil porosity, the effects of sand ($P<0.01$), silt ($P<0.01$), and phosphorus ($P<0.05$) were positive. (Note: negative correlations were observed for the three-site analysis with no bulk density or porosity as predictors, shown in Figure 4.10 (d, e, f). Micro-organism biodiversity was negatively correlated with organic carbon ($P<0.006$), magnesium ($P<0.001$), potassium ($P<0.05$), aluminium ($P<0.01$) and particularly with zinc ($P<0.00001$) (Fig. 4.10 [g, h, i, j, k] and Table 4.3b).
Fig. 4.8. Scatter plot comparing Simpson’s Index of Diversity among grazed, remnant vegetation and revegetated site types, at different soil depths. Overall mean Simpson’s Index of Diversity for paddock is 0.652, for revegetated sites mean index is 0.695, and for remnant vegetation sites mean index is 0.689.
Fig. 4.9. GLM partial plots for Simpson’s Index of Diversity showing fitted mean and 95% confidence intervals for the three sites (paddock, remnant vegetation and revegetated site types).
Table 4.3. Stepwise GLM analysis for micro-organism biodiversity and significantly associated soil variables: three sites, and two sites, with soil properties as predictors. Note that the relative order of importance is indicated by the order in which variables are listed in the table.

(a) Three sites with soil chemistry predictors

<table>
<thead>
<tr>
<th>site</th>
<th>+ depth (0.00001)</th>
<th>+ sand (0.02)</th>
<th>+ silt (0.04)</th>
<th>+ Phosphorus (Olsen) (0.00001)</th>
<th>- Orgcarbon (0.0015)</th>
<th>- Calcium (0.0015)</th>
<th>- Magnesium (0.001)</th>
<th>- Sodium (0.0001)</th>
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<td>(0.0015)</td>
<td>(0.0015)</td>
<td>(0.001)</td>
<td>(0.002)</td>
<td>(0.04)</td>
<td>(0.005)</td>
<td>(0.0002)</td>
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<td></td>
<td>(0.016)</td>
<td>(0.002)</td>
<td>(0.00001)</td>
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(b) Two sites with soil chemistry, texture, soil porosity and density

<table>
<thead>
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<th>Site</th>
<th>depth (0.00001)</th>
<th>- density (0.0015)</th>
<th>- porosity (0.0015)</th>
<th>- sand (0.001)</th>
<th>- silt (0.002)</th>
<th>- Phosphorus (Olsen) (0.0001)</th>
<th>- Orgcarbon (0.005)</th>
<th>- Magnesium (0.0002)</th>
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Fig 4.10. GLM partial plots for Simpson’s Index of Diversity showing fitted mean and 95% confidence intervals for the two sites (remnant vegetation and revegetated), with soil depth, soil bulk density and porosity, and soil chemistries as predictors.
Discussion

The paddock had a significantly higher abundance of Proteobacteria and Cyanobacteria than revegetated and remnant vegetation sites, but the other two micro-organisms, Chlorophyta and Ascomycota, showed no significant difference in abundance among site types. Therefore, this result suggests that agricultural activities may not be significantly detrimental to microbial populations. The most noticeable result relating to micro-organism abundance was that sand content, which was significantly high at remnant vegetation and revegetated sites (Chapter 3), was negatively correlated with Cyanobacteria and Proteobacteria abundance. From this result, it appears that soil texture influenced the abundance of micro-organisms more than external factors such as agricultural land use. This result agrees with the findings of Sessitsch et al. (2001), which show that external factors such as fertilizer have only a small effect on determining microbial abundance compared with factors such as soil particle size. It has been suggested that finer sized soil particles (silt and clay) provide a protective habitat for micro-organisms through pore size exclusion of predators such as protozoa (Sessitsch et al. 2001).

In addition to soil texture, it appears that some soil nutrients may be affecting micro-organism abundance. The most significant results, occurring in both three-site and two-site analyses, were that: Proteobacteria abundance was positively correlated with aluminium and zinc; Cyanobacteria abundance was positively correlated with magnesium; and Chlorophyta abundance had positive correlations with magnesium, aluminium and zinc. To my knowledge there have been no previous studies to indicate what the specific relationship is between these soil chemistries and micro-organism abundance.

All micro-organism abundance, apart from Ascomycota, was greatest in the first five centimetres of soil, and gradually decreased as soil depth increased. Light intensity was likely to have been the cause of high micro-organism abundance in the top layers of soil (Shimmel & Darley 1985), especially since both Cyanobacteria and Chlorophyta depend on light for photosynthesis. Ascomycota, fungi that grow as filamentous mycelia, did not have any association with soil depth or any of the soil chemistry and structural variables. Because Ascomycota had very low abundance and were rarely counted during microscope analysis, it may have been difficult to accurately determine their significance in the soil microbial community.
Biodiversity was medium at all sites according to Simpson’s Index of Diversity. However, organism diversity was lowest in the grazed paddock, and also lowest in the upper levels of the soil profile. The cause of lower organism diversity was an increase in the number of Proteobacteria, which were the dominating species in the paddock and in the top layers of soil. The dominance of Proteobacteria can be seen by the high relative abundance of this species (45-49%) at all sites, which was expected according to previous literature (Waksman 1963).

The overall findings of this study indicate that agricultural land use in the paddock has not had a negative impact on micro-organism abundance. It is possible that the input of certain nutrients into the soil has caused an increase in organism abundance. However, micro-organism abundance appears to be more significantly correlated with soil texture, sandy soils having a lower abundance of organisms. Remnant vegetation and revegetated sites were not significantly different in organism abundance, and had similar soil types, indicating that revegetated sites have the desired natural level of micro-organism abundance.
References


R Development Core Team. (2007) R Version 2.6.0. NZ.


CHAPTER 5: A comparison of endomycorrhizal infection levels in plants among agricultural, revegetated, and remnant vegetation sites

Abstract

Endomycorrhizal fungi, which are plant root symbionts that assist with nutrient uptake by plant roots, play an important part in the restoration of disturbed land. After soil disturbance, and during the early stages of community succession, mycorrhizae are frequently absent and may take years to establish. Therefore, knowledge on the effects that agricultural land disturbance has had on endomycorrhizal infection levels, and whether revegetation success is associated with endomycorrhizal colonisation, can help to establish revegetation methods such as the inoculation of plant seedlings with endomycorrhizal fungi.

A comparison study on mycorrhizal infection levels in roots of semi-arid plants among agricultural, revegetated, and remnant vegetation sites at Port Wakefield, South Australia was carried out. Roots of the introduced species *Medicago minima*, and the native species *Stipa nitida, Sclerolaena obliquicuspis, Enchylaena tomentosa, Rhagodia parabolica, Pittosporum angustifolium, Acacia sclerophylla, Melaleuca lanceolata, Disphyma crassifolium* and *Atriplex stipitata* were stained with trypan blue, which stains endomycorrhizal structures dark blue. The proportion of root length colonised by endomycorrhizal structures, being arbuscules, vesicles and hyphae, was then determined.

Results show highest frequency of mycorrhizal colonisation in the introduced species, *Medicago minima*. Interestingly, all but one (*Enchylaena tomentosa*) of the normally non-mycorrhizal family, Chenopodiaceae, showed mycorrhizal colonisation. Revegetated and remnant vegetation sites had higher endomycorrhizal colonisation levels than the agricultural site, indicating that recent sheep grazing and cropping is likely to have affected mycorrhizal levels. Sites that had undergone revegetation approximately eight years ago appeared to have recovered to normal mycorrhizal colonisation levels, based on the results of similar colonisation levels as the remnant vegetation (control) sites.
**Introduction**

Mycorrhizae are symbiotic associations between plant roots and fungi. The plant roots gain improved mineral and nutrient uptake from the soil, and the mycorrhizal fungi gain direct access to carbohydrates such as glucose and sucrose produced by the plant. Endomycorrhizae (vesicular arbuscular mycorrhizae) are the most common type of mycorrhizae, found in 90% of the land plants (Mukerji *et al.* 2000). Studies have reported the importance of endomycorrhizae in facilitating plant growth, and it has been suggested that restoration of disturbed native ecosystems may be dependent on mycorrhizal colonisation (Pattinson *et al.* 2004). Most Australian native plants have an association with endomycorrhizal fungi, with the fungi absent or rare in only a few major Australian plant families, including Chenopodiaceae, Proteaceae and Cyperaceae (Brundrett *et al.* 1996). Non-mycorrhizal plants are generally most abundant in harsh habitats such as extremely wet, saline, or arid soils (Brundrett 2002). These plants tend to have fine roots with many root hairs, and advanced chemical strategies for water and nutrient uptake (Brundrett 2002).

It is important to note that not all plant species have the same growth response to mycorrhizae. For this reason, different vegetation communities will respond in different ways to the loss of mycorrhizae caused by land disturbance. O’Connor *et al.* (2002) found that native plant species diversity in a semi-arid herbland of South Australia increased when mycorrhizal colonisation was suppressed, because growth of a highly mycorrhiza-responsive introduced dominant species, *Medicago minima*, was reduced and previously subordinate plant species were released from competition.

The effects of soil disturbance on mycorrhizal colonisation can vary depending on the level of disturbance. For example, trampling which causes damage to vegetation has been found to destroy beech seedlings and their mycorrhizal fine roots, whereas mycorrhizal roots of mature trees were resilient to trampling (Waltert *et al.* 2002). In relation to agricultural tillage, soil subjected to low-input agriculture produce greater mycorrhizal colonisation than soil from conventionally farmed sites (Douds *et al.* 1995). High-impact soil disturbance, whether it be from trampling or tillage, can cause damage to the fungal propagules and the hyphal network, which results in the loss of endomycorrhizae (Miller & Lodge 2007).

Soil nutrients may have an impact on mycorrhizal colonisation. Treseder (2004) found that mycorrhizal abundance decreased by 15% under nitrogen
fertilization and by 32% under phosphorus fertilization, but increased by 47% under elevated carbon levels. It is thought that if nitrogen or phosphorus availability rises in the soil, plants are not so dependent on mycorrhizae, and do not allocate their carbohydrates to mycorrhizae; therefore, fewer mycorrhizae are formed (Treseder 2004).

After soil disturbance, and during the early stages of community succession (recovery of ecosystems), mycorrhizae are frequently absent and may take years to establish. Studies have indicated that it can take up to five years from the sowing of grass seed for mycorrhizal infection potential of the soil to increase (Miller 1987). In the case of severely disturbed soil, such as that of mine sites, effective endomycorrhizal fungi may not form at all, even after two years of no soil disturbance (Stahl et al. 1988). It has been suggested that inoculation of plants with mycorrhizae during the revegetation process, by adding mycorrhizal fungi to potting mixes, could have value as a conservation technique (Koske & Gemma 1995).

The aim of this study was to determine the effect that agricultural land disturbance and specific soil properties have on endomycorrhizal colonisation of plant roots, and to determine whether revegetation success is associated with endomycorrhizal colonisation. To achieve these aims, my objectives were: to compare endomycorrhizal infection levels in roots of common plant species among grazed, revegetated, and remnant (control) sites, using the remnant sites as indicators of desired mycorrhizal colonisation levels; and to determine any significant relationships between micro-organism abundance and soil variables (nutrients, pH, salinity, organic carbon, soil texture, soil porosity, and bulk density) by statistical methods known as generalised linear models (GLM).

This study examined mycorrhizal colonisation levels on revegetated land, an historically grazed site, and remnant vegetation (control) sites at Port Wakefield, South Australia. The study has importance in the restoration of ecosystems disturbed by agricultural land use. Knowledge gained by this study can help to establish what revegetation methods, such as inoculation of plants with endomycorrhizal fungi, may be required for revegetation success.
Methods

Survey design
A total of 24 plots (eight at the agricultural site, one at each of eight revegetated sites, and one at each of eight remnant vegetation sites) was surveyed. Colonisation of roots by endomycorrhizae was assessed on plant roots of the common native species, *Sclerolaena obliquicuspis*, *Enchylaena tomentosa* and *Stipa nitida*, occurring at all sites. *Medicago minima*, an introduced species, was also assessed for mycorrhizal infection levels at all sites to determine the mycorrhizal inoculum potential of the soil. Other species, though common in the area, not occurring at all sites: *Melaleuca lanceolata*, *Atriplex stipitata*, *Ragodia parabolica*, *Pittosporum angustifolium*, *Acacia sclerophylla*, and *Disphyma crassifolium*, were also assessed for mycorrhizal infection levels. The agricultural site was sampled by a grid format of eight 100 m x 100 m plots, in which I collected root samples for mycorrhizal frequency analysis. Since there was only one grazed paddock in the study location, interspersion of study plots to avoid pseudoreplication (Hurlbert 1984) was not possible. Instead, the eight plots at the agricultural site were spaced out as widely as possible to lessen the impacts of any background effects which may be concentrated in one area. Regular positioning of plots, with root samples selected evenly across each plot, was the most effective survey method for an even distribution of samples across each site (Brower et al. 1998). A 100 m x 100 m plot was established at each of the eight revegetated sites, and eight sites containing remnant vegetation (control sites). Three plants of each species were selected from each plot by locating the plant of each species closest to the centre of the plot, and two plants of 25 m away from the centre in the directions of north and south.

Mycorrhizal staining procedure
Root samples were stored in vials containing 50% ethanol (Smith & Dickson 1997) until the staining process was carried out. Following the procedure outlined by Phillips and Hayman (1970), trypan blue, which stains both live and dead mycorrhizal structures dark blue while leaving the root structures pale blue was carried out. (See Appendix 5 for photos of stained mycorrhizal structures). The staining process, which involved clearing, bleaching, and staining took place in a 95ºC water bath. Roots were washed and cut into 1 cm portions, which were cleared in a 10% KOH
solution. After clearing, the root segments were washed with water and rinsed in a 0.1 N HCl solution to bleach the roots. Finally, roots were stained in a solution containing lactic acid, glycerol, water (1:1:1), and 0.05% trypan blue. The optimal durations for clearing and staining can vary among plant species, so preliminary trials were carried out to determine the best staining process as follows: preliminary tests of clearing in KOH were done for each plant species at 30, 60, 120, 180, 240, 300, and 480 minutes, followed by brief rinsing in HCl to bleach the roots. The root samples were then each stained for 20 and 60 minutes, giving 14 alternative procedures. The procedure that produced the best staining result for each plant species was used in the final analysis. Stained roots were stored in vials containing lactic acid, glycerol, and water (1:2:1) (Smith & Dickson 1997).

**Quantitative analysis of mycorrhizal frequency**

Visual assessment of the proportion of root length colonised by mycorrhizal structures followed the ‘magnified intersections method’ of McGonigle et al. (1990). Samples of 1-cm root segments were mounted in glycerin on microscope slides. Between two and four slides were used for each sample, which contained 20 randomly selected root segments. Each root segment was observed under a light microscope at magnification 100 x. The vertical crosshair of the microscope was used to intersect each root segment by moving the stage graticule to record ten evenly spaced intersects along each root segment. A total of 200 intersects was recorded for each sample. Each intersection was recorded as ‘negative’ (no mycorrhizal structures), ‘arbuscules’, ‘vesicles’, or ‘hyphae only’. If the vertical crosshair intersected one or more arbuscules or vesicles at the same intersect, the appropriate category was increased by one. The category for ‘hyphae only’ was only increased if the intersection contained hyphae, and no arbuscules or vesicles, because hyphae were assumed to be present with arbuscules and vesicles. Colonisation by mycorrhizal structures in plant roots was calculated as a frequency. Total mycorrhizal colonisation was calculated as the frequency of non-negative intersections, arbuscular colonisation was calculated by dividing the count for arbuscules by the total number of intersections examined, and similarly vesicular colonisation was calculated by dividing the count for vesicles by the total number of intersections examined. The extent of total mycorrhizal colonisation was categorised as non-mycorrhizal (0%), low (<10%), medium (10-30%), and high (>30%) (O'Connor et al. 2001).
**Statistical analysis**

Statistical comparisons of endomycorrhizal colonisation among sites, and among plant species were carried out with stepwise generalised linear models (GLM). Stepwise GLMs were also carried out to determine whether vesicular, arbuscular and total mycorrhizal colonisation are associated with any chemical or physical soil properties. A significance level of $\alpha = 0.05$ was used. The analysis was performed using R, a statistical software program (R Development Core Team 2007).

Statistical analyses were first carried out to determine differences among all three site types (paddock, remnant vegetation and revegetated), and then to determine differences between only two sites (remnant vegetation and revegetated). The reason for these separate comparisons was that there was a distinction between the three-site versus two-site data, in that soil texture and chemistry data were only available for the remnant and revegetated sites, so could not be used as covariates when including the paddock as a comparison site. Even though soil texture and chemistry data were taken from the paddock, samples were not taken from the exact same plots as mycorrhizal plant roots because of later weed-poisoning in some plots, which may have influenced results if mycorrhizal roots were taken from these plots. Note that nitrogen, molybdenum, chlorine, and boron were not included in the statistical analysis as laboratory results of these nutrients were not available until later.

**Results**

**Staining procedure**

Staining roots in trypan blue produced sufficient results to be able to identify mycorrhizal structures (arbuscules, vesicles, and hyphae) in all plant species (see Appendix 5). The best procedure for the determination of endomycorrhizal fungal structures in *Medicago minima* roots was 30 minutes in 10% KOH solution, followed by 20 minutes in 0.05% trypan blue solution. The native species *Sclerolaena obliquicuspis*, *Enchylaena tomentosa*, *Stipa nitida*, *Melaleuca lanceolata*, *Atriplex stipitata*, *Rhogodia parabolica*, *Pittosporum angustifolium*, *Acacia sclerophylla*, and *Disphyma crassifolium* required longer clearing in 10% KOH solution to remove the cytoplasm of the roots: sufficient staining results were produced when cleared in 10%
KOH solution for eight hours, followed by staining in 0.05% trypan blue solution for 20 minutes.

**Mycorrhizal colonisation among ten plant species, and among three site types (agricultural, remnant vegetation and revegetated): without soil chemistry as predictors**

A stepwise GLM of mycorrhizal colonisation (without accounting for soil chemistry) indicated no significant site differences in total, arbuscular and vesicular mycorrhizal colonisation between remnant and revegetated sites (Figs. 5.1a, 5.2a, and 5.3a). However, there was significantly less total ($P<0.0001$), arbuscular ($P<0.01$), and vesicular ($P<0.05$) mycorrhizal colonisation in the paddock than in both the remnant and revegetated sites (Figs. 5.1a, 5.2a, and 5.3a). As shown by Figures 5.1c, 5.2c, and 5.3c, soil bulk density had a positive correlation with all types of mycorrhizal colonisation, and soil porosity also had a positive correlation with vesicular colonisation (Fig. 5.3d).

There were significant plant species differences in mycorrhizal colonisation, with the introduced species *Medicago minima* having significantly higher total, arbuscular and vesicular colonisation than all other species (Figs. 5.1b, 5.2b, and 5.3b). Total mycorrhizal colonisation of the introduced *Medicago minima* ranged from 16% in the paddock to 58% at remnant vegetation sites (Fig. 5.4a). All Chenopod species, apart from *Rhagodia parabolica*, which had medium mycorrhizal colonisation, had low or no mycorrhizal colonisation (Fig. 5.4 [b, c, d]). *Enchylaena tomentosa* was the only species in the survey to not show any mycorrhizal colonisation. Survey results for non-chenopod native species indicated that all species had low mycorrhizal colonisation apart from *Stipa nitida* and *Pittosporum angustifolium*, which had medium mycorrhizal colonisation (Fig. 5.4 [e, f, g, h, i]).

**Soil chemistry as predictors of mycorrhizal colonisation: ten plant species and two site types (remnant vegetation and revegetated)**

Analysis of all plant species surveyed among remnant and revegetated sites (not including the paddock, as previously explained) found the following soil chemistry effects. Total, arbuscular, and vesicular mycorrhizal colonisations were significantly and negatively associated with calcium levels ($P<0.05$) (Figs. 5.5f, 5.6h, and 5.7f). Total and vesicular mycorrhizal colonisations were also significantly and negatively
Fig. 5.1. Stepwise GLM, showing fitted mean and 95% confidence intervals, of total mycorrhizal colonisation (ten species among three site types: without soil chemistry as predictors). The scale for percentage mycorrhizal colonisation is adjusted (see Figure 5.2 for actual mycorrhizal colonisation percentages). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchylaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Rhagodia parabolica), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.2. Stepwise GLM, showing fitted mean and 95% confidence intervals, of arbuscular mycorrhizal colonisation (ten species among three site types: without soil chemistry as predictors). The scale for percentage mycorrhizal colonisation is adjusted (see Figure 5.2 for actual mycorrhizal colonisation percentages). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchylaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Rhagodia parabolica), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.3. Stepwise GLM, showing fitted mean and 95% confidence intervals, of vesicular mycorrhizal colonisation (ten species among three site types: without soil chemistry as predictors). The scale for percentage mycorrhizal colonisation is adjusted (see Figure 5.2 for actual mycorrhizal colonisation percentages). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchylaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Rhagodia parabolina), S.n (Stipa niutta) and S.o (Sclerolaena obliquicuspis).
Fig. 5.4. Arbuscular, vesicular, and total mycorrhizal colonisation for all species surveyed at remnant vegetation, revegetated and grazed sites. Error bars indicate standard errors of the mean.
Fig. 5.4. (cont’d.) Arbuscular, vesicular, and total mycorrhizal colonisation for all species surveyed at remnant vegetation, revegetated and grazed sites. Error bars indicate standard errors of the mean.
Fig. 5.4. (cont’d.) Arbuscular, vesicular, and total mycorrhizal colonisation for all species surveyed at remnant vegetation, revegetated and grazed sites. Error bars indicate standard errors of the mean.
Fig. 5.5. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between total mycorrhizal colonisation and soil properties (ten species at two site types with soil chemistry as predictors). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchylaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Rhagodia parabolica), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.6. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between arbuscular mycorrhizal colonisation and soil properties (ten species at two site types with soil chemistry as predictors). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchylaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Rhagodia parabolica), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.7. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between vesicular mycorrhizal colonisation and soil properties (ten species at two site types with soil chemistry as predictors). Plant species are labelled as: A.sc (Acacia sclerophylla), A.st (Atriplex stipitata), D.c (Disphyma crassifolium), E.t (Enchyelaena tomentosa), M.l (Melaleuca lanceolata), M.m (Medicago minima), P.a (Pittosporum angustifolium), R.p (Ragodia parabolica), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
associated with levels of aluminium and zinc (P<0.05) (Fig. 5.5 [h, i] and 5.7 [h, i]); in addition, all but arbuscular colonization were negatively correlated with magnesium (P < 0.003) (Figs. 5.5g and 5.7g). Organic carbon was significantly and positively associated with vesicular and arbuscular colonisation (P<0.05), but not so for total colonisation (Figs. 5.6f and 5.7e). Phosphorus (as determined by the Olsen method) was significantly and negatively correlated with arbuscular, vesicular and total mycorrhizal colonisation (P<0.05) (Figs. 5.5e, 5.6e and 5.7d). Sulphur, potassium, sodium, copper, manganese, and iron had no significant association with mycorrhizal colonisation.

Soil texture and structure as predictors of mycorrhizal colonisation: ten plant species and two site types (remnant vegetation and revegetated)

Soil bulk density and porosity were significantly and negatively correlated with total and arbuscular mycorrhizal colonisation (p<0.005) (Figs. 5.5 [b, c] and 5.6 [b, c]). Note that in the analysis for ten species among three sites, without taking soil chemistry into account, mycorrhizal colonisation was actually positively correlated with soil bulk density and porosity. Sand content was significantly and negatively correlated with total and arbuscular mycorrhizal colonisation (p<0.01) (Figs. 5.5d and 5.6d). However, no significant sand, soil bulk density or porosity effects were found for vesicular colonisation.

Mycorrhizal colonisation among four common plant species, and among three site types (agricultural, remnant vegetation and revegetated): without soil chemistry as predictors

A stepwise GLM of mycorrhizal colonisation (without accounting for soil properties) of only the plant species common to all sites (Medicago minima, Sclerolaena obliquicuspis, Enchylaena tomentosa and Stipa nitida) indicated no significant site differences in total, arbuscular and vesicular mycorrhizal colonisation between remnant and revegetated sites (Figs. 5.8a, 5.9a and 5.10a). However, there was significantly less total (P<0.0001) and vesicular (P<0.05) mycorrhizal colonisation in the paddock than in both the remnant and revegetated sites (Figs. 5.8a and 5.10a). Arbuscular colonisation was only significantly lower in the paddock when compared to the remnant vegetation sites (P<0.001) (Fig. 5.9a).
Fig. 5.8. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between total mycorrhizal colonisation and soil properties for the three site analysis with four common plant species (without soil chemistry as predictors). Plant species are labelled as: E.t (*Enchylaena tomentosa*), M.m (*Medicago minima*), S.n (*Stipa nitida*) and S.o (*Sclerolaena obliquicuspis*).
Fig. 5.9. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between arbuscular colonisation and soil properties for the three site analysis with four common plant species (without soil chemistry as predictors). Plant species are labelled as: E.t (*Enchylaena tomentosa*), M.m (*Medicago minima*), S.n (*Stipa nitida*) and S.o (*Sclerolaena obliquicuspis*).
Fig. 5.10. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between vesicular colonisation and soil properties for the three site analysis with four common plant species (without soil chemistry as predictors). Plant species are labelled as: E.t (*Enchylaena tomentosa*), M.m (*Medicago minima*), S.n (*Stipa nitida*) and S.o (*Sclerolaena obliquicuspis*).
Mycorrhizal colonisation among four common plant species, and two site types (remnant and revegetated): with soil chemistry as predictors

Analysis of the four common plant species surveyed among remnant and revegetated sites (not including the paddock) found the following soil chemistry effects. Total and vesicular colonisation were significantly and negatively associated with calcium levels ($P<0.02$) (Figs. 5.11g and 5.13f), and with levels of aluminium and zinc ($P<0.03$) (Figs. 5.11i, j and 5.13g, h). In addition, total colonisation was significantly and negatively correlated with magnesium ($P<0.006$) (Fig. 5.11h). The negative impacts of sulphur on total and arbuscular mycorrhizal colonisation were revealed when analysing the four common species, rather than all 10 species (Figs. 5.11f and 5.12h). Whilst sulphur had a significant negative impact ($P<0.05$) on total and arbuscular mycorrhizal colonisation, phosphorus (Olsen) was significantly and negatively correlated with total and arbuscular mycorrhizal colonisation ($P<0.02$), but not with vesicular colonisation (Figs. 5.11e and 5.12g) (this result is similar to the GLM results of the ten-species analysis). Organic carbon was not significantly associated with any type of mycorrhizal colonisation (this differs from the ten species results where organic carbon was significantly and positively associated with arbuscular and vesicular colonisation). Sodium, copper, manganese, and iron had no significant association with mycorrhizal colonisation, in agreement with the results from the ten-species analysis.

Soil texture and structure as predictors of mycorrhizal colonisation: four common plant species and two site types (remnant and revegetated)

Soil bulk density and porosity were significantly and negatively correlated with both total and arbuscular mycorrhizal colonisation ($P<0.05$) (Figs. 5.11b, c and 5.12c, d). Sand and silt content were also significantly and negatively correlated with arbuscular mycorrhizal colonisation ($P<0.04$) (Fig. 5.12e, f), and just sand with total mycorrhizal colonisation ($P<0.05$) (Fig. 5.11d). No significant sand, silt, soil bulk density, nor porosity effects were found for vesicular colonisation.
Fig. 5.11. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between total mycorrhizal colonisation and soil properties for the two-site analysis on the four common species (with soil chemistry as predictors). Plant species are labelled as: E.t (Enchyela tomentosa), M.m (Medicago minima), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.12. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between arbuscular mycorrhizal colonisation and soil properties for the two-site analysis on the four common species (with soil chemistry as predictors). Plant species are labelled as: E.t (Enchylaena tomentosa), M.m (Medicago minima), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Fig. 5.13. Stepwise GLM with fitted mean and 95% confidence intervals, showing significant correlations between vesicular mycorrhizal colonisation and soil properties for the two-site analysis on the four common species (with soil chemistry as predictors). Plant species are labelled as: E.t (Enchyela tomentosa), M.m (Medicago minima), S.n (Stipa nitida) and S.o (Sclerolaena obliquicuspis).
Discussion

Overall mycorrhizal colonisation was lowest in the grazed paddock, which was expected from the literature. The low colonisation rates in the paddock could have been caused by sheep trampling or heavy machinery, which can damage fine roots, fungal propagules and the hyphal network (Miller & Lodge 2007). However, soil nutrient availability and soil texture may have also had some influence on mycorrhizal establishment and growth.

Increased mycorrhizal colonisation was correlated with increased organic carbon when analysing all ten plant species, which was not unexpected as carbon is the main growth benefit mycorrhizae gets from its plant host (Tinker et al. 1994). However, the statistical analysis provided some conflicting results between the four species analysis and ten species analysis. Organic carbon was not significantly associated with mycorrhizal colonisation when analysing only the four common species. There were also conflicting results when analysing the association between soil bulk density and mycorrhizal colonisation. Without taking soil chemistry into account in the statistical analysis, soil bulk density was positively correlated with mycorrhizal colonisation, but when soil chemistry was accounted for, bulk density was negatively correlated with mycorrhizal colonisation. These results suggest that it would be difficult to determine the exact effects of soil properties, especially soil chemistry on mycorrhizal colonisation without setting up more controlled experiments.

Sand content was the soil variable that had the most significant correlation with mycorrhizal colonisation, all analyses having a negative relationship with sand content. This finding agrees with the literature that reports high rates of mycorrhizal colonisation tend to be associated with clay soils rather than sand; however, mycorrhizal colonisation may form more rapidly in sandy soils (Land & Schönbeck 1991).

There was an overall negative correlation between mycorrhizal colonisation, and calcium, aluminium, zinc, magnesium, sulphur and phosphorus. These results agree with the hypothesis of Treseder (2004), that when soil nutrients are depleted plants allocate more of their carbohydrates to the formation of mycorrhizae than when soil nutrient levels are high. However, as mentioned above, it would be difficult to
determine the exact effects that soil nutrients have on mycorrhizal colonisation without setting up more controlled experiments.

Mycorrhizal colonisation rates varied among plant species, the introduced *Medicago minima* being highly mycorrhizal, and all of the native species having no to medium colonisation. The literature suggests that many chenopod species are non-mycorrhizal, and endomycorrhizae is not crucial for their growth; therefore, low mycorrhizal colonisation of *Sclerolaena obliquicuspis* and *Atriplex stipitata*, and no colonisation of *Enchylaena tomentosa* was considered normal. It is common for non-mycorrhizal plants such as chenopods to dominate arid habitats (Brundrett 2002), which may explain the abundance of chenopods throughout the semi-arid study site. However, overall colonisation of chenopod species was much higher than expected. Interestingly, *Rhagodia parabolica* had medium mycorrhizal colonisation, and only one species (*Enchylaena tomentosa*) was non-mycorrhizal.

All mycorrhizal species contained hyphae, vesicles and arbuscules, apart from *Acacia sclerophylla* having no arbuscules, *Atriplex stipitata* at remnant sites having no arbuscules, *Melaleuca lanceolata* at remnant sites having no arbuscules or vesicles, and *Sclerolaena obliquicuspis* at revegetated sites having no arbuscules or vesicles. In the absence of arbuscules, the mycorrhizal association may not significantly contribute to the uptake of nutrients such as phosphorus by the plant roots (O'Connor *et al.* 2001). Since *Acacia sclerophylla* is a legume, it also has another symbiotic relationship known as rhizobia, which allows the plant to fix atmospheric nitrogen. Therefore arbuscules may not be as crucial for nutrient uptake in the legumes as in non-leguminous species. However, arbuscules and vesicles may have occasionally occurred in these plant species but were not detected because of limitations in the number of plants being able to be sampled.

Revegetation success appears to be associated with endomycorrhizal colonisation, as all revegetated sites had significantly higher mycorrhizal infection levels compared with the recently grazed paddock. Similar mycorrhizal colonisation among revegetated and remnant vegetation sites indicates that mycorrhizal colonisation at revegetated sites, without specific treatment such as mycorrhizal inoculation, is likely to be sufficient for restoration. The remnant vegetation (control) sites gave an idea of the desired level of mycorrhizal colonisation for the semi-arid vegetation type occurring in the Port Wakefield region. The revegetated sites were established approximately eight years ago, indicating that eight years may be
sufficient for mycorrhizae to effectively colonise the roots of plants planted in soil previously used for agriculture. Based on the results of very similar mycorrhizal colonisation among remnant and revegetated sites, revegetation techniques such as inoculation of plants with mycorrhizae may not be necessary. However, sites with higher levels of disturbance than sheep grazing, or non-arid sites, might require techniques such as mycorrhizal inoculation.
References


R Development Core Team (2007) *R Version 2.6.0*, NZ.


CHAPTER 6: General discussion and conclusions

Discussion

Effects of agricultural land disturbance on physical and chemical soil properties

The most noticeable effect of agricultural land use on soil chemistry was the very high salinity level at the agricultural site, four times higher than at remnant vegetation and revegetated sites. High salinity was possibly caused by the removal of native species and replacement with shallow-rooted crop species, resulting in a rising water table (Wolf & Snyder 2003). However, it is difficult to determine the more widespread significance of the salinity problem because only one paddock was available to use at the Defence Force’s site in the Port Wakefield region. It is suggested that a number of different paddock sites be sampled in order to be able to draw stronger conclusions on agricultural impacts for the land and vegetation type of the Port Wakefield region.

High levels of sulphur, nitrogen, potassium, and magnesium at the agricultural site were also evident, probably because of the addition of fertilizer and animal excreta to the soil (Smith & Wheeler 1979). These high nutrient levels may have had some effect on micro-organism abundance (Chapter 4) and mycorrhizal colonisation (Chapter 5). However, as mentioned above, it is difficult to determine the more widespread influences of agriculture on soil nutrient levels in the Port Wakefield region because there was only one available agricultural study site.

Interestingly, all sites had highly porous soils, with porosity being over 50% at all sites. High soil porosity at the agricultural site gives an indication that sheep trampling and machinery may not have significantly affected the soil structure. All soils had a high sand content, which may explain the high soil porosity. However, the agricultural site was slightly siltier than the remnant vegetation and revegetated sites. This result was not unexpected because sandy soils are less suitable for agricultural uses, drying rapidly and easily losing nutrients which drain away in percolating water (Gardiner 2001), so sandy sites would have been less favorable when local farmers were selecting land to cultivate.
Effects of agricultural land disturbance on soil micro-organism abundance and biodiversity

Agricultural land use did not appear to have significantly reduced the abundance of micro-organisms (Chapter 4). Micro-organism abundance was more related to soil texture than external factors such as land use, with sandy soils having significantly lower abundance of micro-organisms than silty soils. One suggestion is that finer sized soil particles provide a protective habitat for micro-organisms through pore size exclusion of predators such as protozoa (Sessitsch et al. 2001). However, biodiversity may have been affected by agricultural land use, as biodiversity, because of an over-abundance of Proteobacteria, was slightly lower in the grazed paddock.

It was difficult to determine the extent to which soil chemistry was influencing micro-organism abundance and biodiversity, even though the statistics suggested highly significant correlations with soil chemistry (Chapter 4). The reason for this uncertainty was that many soil variables were interacting with each other, shown by conflicting statistical results between two-site (remnant vegetation and revegetated) and three-site (paddock, remnant vegetation and revegetated) analyses. The most significant results common to three-site and two-site analysis were that Proteobacteria abundance was positively correlated with aluminium and zinc; Cyanobacteria abundance was positively correlated with magnesium; and Chlorophyta abundance had positive correlations with magnesium, aluminium and zinc. It is difficult to determine the exact influences of these nutrients on micro-organisms without setting up more controlled experiments to look at the ‘before’ and ‘after’ effects of adding nutrients to the soil.

Effects of agricultural land disturbance on endomycorrhizal colonisation of plant roots

Low mycorrhizal colonisation was evident in the paddock when compared to the remnant vegetation and revegetated sites (Chapter 5). It has previously been suggested that the use of machinery and trampling can cause damage to mycorrhizal fungal propagules and the hyphal network, resulting in a loss of mycorrhizal colonisation in plant roots (Miller & Lodge 2007). The remnant and revegetated sites were quite similar to each other in mycorrhizal colonisation, indicating that mycorrhizal colonisation can recover at revegetated sites without specific intervention such as inoculation of seedlings with mycorrhizal fungi.
A very interesting result was that only one chenopod species, *Enchylaena tomentosa*, was non-mycorrhizal, and one chenopod species, *Rhapodia parabolica*, had medium mycorrhizal colonisation, when it is widely assumed that most chenopods are either non-mycorrhizal or rarely mycorrhizal (Brundrett *et al.* 1996). The remaining chenopod species, *Sclerolaena obliquicuspis* and *Atriplex stipitata* had low mycorrhizal colonisation levels, but had evidence of arbuscular, vesicular, and hyphal colonisation. The other interesting result was that *Acacia sclerophylla* was the only species (apart from the non-mycorrhizal *Enchylaena tomentosa*) to have no sign of arbuscular colonisation, indicating that the mycorrhizal association may not significantly contribute to the uptake of certain nutrients by the plant roots (O’Connor *et al.* 2001). However, since *Acacia* are legumes, they also have another symbiotic relationship known as rhizobia which allows them to fix atmospheric nitrogen.

The interactions between soil variables and mycorrhizal colonisation were complex. However, it was found that almost all soil nutrients had a negative correlation with mycorrhizal colonisation, supporting the literature that suggests plants produce less mycorrhizal associations when available nutrients are high. It is thought that when plant-available nutrient levels are high plants allocate less of their carbohydrates to the formation of mycorrhizal fungi, because they do not depend so much on mycorrhizae for nutrient uptake (Treseder 2004). The most significant mycorrhizal association was a negative correlation with sand content, which confirms reports of lower rates of mycorrhizal colonisation in sandy soils than in clay soils (Land & Schönbeck 1991).

**Options for future research**

Future research should include experiments to examine the impacts of various levels of agricultural disturbance on soil biodiversity and mycorrhizal colonisation, for example different intensities of sheep or cattle grazing. The monitoring of soil at revegetated sites over time may also be useful in determining the length of time required for the improvement of various indicators of soil health. The soil components that should be monitored over time are micro-organism abundance, mycorrhizal colonisation levels, soil pH and salinity levels, and the levels of essential plant nutrients found in the soil.
To gain a more accurate idea of the impacts of certain soil nutrients on mycorhizal colonisation and micro-organism abundance, controlled experiments analysing the ‘before’ and ‘after’ effects of adding specific nutrients to the soil could be carried out. These experiments would give a clearer idea of the impacts of soil nutrients on soil biodiversity.

**Conclusion**

The overall results of this study suggest that agriculture in the sheep-grazed paddock has had impacts on soil biota and soil chemistries. There was low mycorrhizal colonisation in the paddock, probably at least partly a result of agricultural activities, and also increased soil nutrient levels, probably because of high nutrient input (fertilizer and animal excreta) from agricultural activities. Remnant vegetation and revegetated sites did not appear to be substantially different to each other in levels of most soil nutrients, mycorrhizal colonisation, and micro-organism biodiversity and abundance, indicating that the revegetated sites after eight years have regained the soil properties of a natural vegetation site.

The length of time required for adequate restoration success may vary, depending on the extent of soil disturbance (Burke *et al.* 1995). This study suggests that after a 77-year history of grazing and cropping in rotation, in the semi-arid region of Port Wakefield, eight years following revegetation may be sufficient for the return of natural mycorrhizal colonisation levels, but nutrient recovery may be a much longer process.
References


Appendix 1.1: Photographs of eight study plots located within the grazed paddock adjacent to Port Wakefield Firing Range.

GPS location: 54H 0239802, UTM 6207357. Date: 26 July 2008.

GPS location: 54H 0239792, UTM 6207156. Date: 26 July 2008.
GPS location: 54H 0239934, UTM 6206950. Date: 26 July 2008.

GPS location: 54H 0239736, UTM 6206959. Date 26 July 2008.
GPS location: 54H 0239964, UTM 6206548. Date: 26 July 2008.

GPS location: 54H 0239564, UTM 6206567. Date 26 July 2008.
Appendix 1.2: Photographs of eight remnant vegetation study sites located within the Defence Force’s Firing Range (photos 1-4) and along roadsides (photos 5-8) at Port Wakefield.

GPS location: 54H 0246302, UTM 6199214. Date: 13 September 2008.

GPS location: 54H 0246117, UTM 6199294. Date: 13 September 2008.
GPS location: 54H 0246664, UTM 6199488. Date: 13 September 2008.

GPS location: 54H 0246367, UTM 6200905. Date: 13 September 2008.
GPS location: 54H 0240059, UTM 6211154. Date: 5 October 2008.

GPS location: 54H 0239319, UTM 6211973. Date: 5 October 2008.
GPS location: 54H 0244141, UTM 6206220. Date: 5 October 2008.

GPS location: 54H 0242991, UTM 6208156. Date: 5 October 2008.
Appendix 1.3: Photographs of eight revegetated sites located within the Defence Force’s Firing Range at Port Wakefield.

GPS location: 54H 0246788, UTM 6200776. Date: 13 September 2008.

GPS location: 54H 0245350, UTM 6203874. Date: 13 September 2008.
GPS location: 54H 0243862, UTM 6205086. Date: 13 September 2008.

GPS location: 54H 0244224, UTM 6205551. Date: 13 September 2008.
GPS location: 54H 0243992, UTM 6206324. Date: 13 September 2008.

GPS location: 54H 0243006, UTM 6207971. Date: 13 September 2008.
GPS location: 54H 0244925, UTM 6204309. Date: 13 September 2008.

GPS location: 54H 0239875, UTM 6206241. Date: 13 September 2008.
### Appendix 2: Pearson correlation matrix of soil characteristics.

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Appendix 3: Micro-organisms as seen under a microscope at 1000 x magnification. Note that Proteobacteria are too small to be seen clearly in the photographs, but can be seen as rapidly moving organisms under a microscope.
Appendix 4: Summary of ANOVA on micro-organism abundance. The stars indicate increasing levels of significance for the relationship between organisms and each of the soil variables (0 ****, 0.001 ***, 0.01 **, 0.05 *)

**Proteobacteria**

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**Cyanobacteria**

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**Chlorophyta**

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**Ascomycota**

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Appendix 5: Photographs of mycorrhizal structures in plant roots. Roots were heated in potassium hydroxide to remove cytoplasmic components, then stained with trypan blue to reveal vesicles (v), arbuscules (a) and hyphae (h).

Medicago minima

Stipa nitida

Disphyma crassifolium
Pittosporum angustifolium

Rhagodia parabolica

Atriplex stipitata

Sclerolaena obliquicuspis
Sclerolaena obliquicuspis