Investigating factors affecting restoration of native grassland in ex-cropland

Shakir Bahaddin SHAKIR

This thesis is submitted in total fulfilment
of the requirements for the degree of
Doctor of Philosophy

School of Health and Life Sciences
Environmental Sciences
Federation University Australia
PO Box 663

Mt. Helen, Ballarat, Victoria 3356, Australia

February 2019

ABSTRACT

Native grasslands are one of the most endangered ecosystems in Australia. Approximately 99% of native grasslands have been disturbed for agriculture and pastoralism. Today, however, many agricultural and grazing lands have been abandoned. Restoring abandoned areas to native grassland is a challenge that must be met if these systems are to persist. This thesis sought to gain a better understanding of the biotic and abiotic barriers to restoring native grasslands in ex-cropping land, and to investigate novel techniques to overcome them in degraded native grassland in the Victorian Volcanic Plains.

Firstly, I compared ex-cropland to high-quality remnant grassland, and showed that ex-cropland supports a high number of exotic weeds, a lack of native species propagules, high nutrient levels (especially phosphorus and nitrogen), and an absence of organic carbon—all barriers which must be overcome if native grassland restoration is to succeed. I conducted a replicated field experiment in ex-cropland, to investigate four restoration approaches to overcoming barriers to restoration: (i) adding urban green waste to heat and kill the exotic seed bank (ii) adding sugar and/or mulch to promote microorganism and draw down soil nitrogen, (iii) using a modified clay product called Phoslock to reduce soil phosphorus levels, and (iv) scalping of topsoil 10 cm on ex-cropland site to remove the exotic seed bank and high nutrient soil. After each treatment, native grass seed was added, and the vegetation, seed bank, soil nutrients and microbial activity were monitored over 2.5 years. The hot mulch, scalping and sugar treatments all achieved significantly greater cover of native grasses than the control treatments. The hot waste treatment also effectively eliminated the exotic weed seed bank, but the soil N levels increased dramatically, which is counterproductive to the long-term goals of grassland restoration. Scalping out-performed all other treatments with regard to reducing soil N and P. All treatments suffered from reinvasion by exotic species, suggesting that any grassland restoration technique needs to be coupled with ongoing exotic weed management.

High soil phosphorus is a difficult barrier to restoration of native grassland. A possible way to address this is to use plants with high P uptake to help draw down soil P. Native grassland taxa from the genus *Ptilotus* have been shown to have high P-uptake. I conducted two studies of *Ptilotus macrocephalus* and *Ptilotus polystachyus* to investigate their potential in this role. The first of these was an examination of techniques to break their seed dormancy, and to find their optimum germination conditions. I tested their response to smoke water, heating shock, cold stratification and gibberellic acid. The highest germination rates (62% and 38% for *P. microcephalus* and *P. polystachyus*, respectively) were achieved when the seeds were pre-treated with GA500 and exposed to a temperature range of (20/18^{O}C) and a 12h dark/12h light regime. Smoke water, heat shock and the removal of floral bracts also improved germination rates, but not at the same magnitude as GA.

The second study of *Ptilotus* was a glasshouse trial that examined the effectiveness of the two taxa at reducing available soil phosphorus. This trial included a third high P-uptake species (*Lupinus albus*) for comparison, and also investigated if the addition of Phoslock® could bind soil P into insoluble forms. *P. macrocephalus* and *P. polystachyus* accumulated high amounts of soil P. Thus, several years of seeding and harvesting of these plants is anticipated to provide a useful option for soil P reduction. Phoslock® reduced soil available P, but only at high concentration of Phoslock 1500 g/m^2 and at very high soil P concentrations; it was less effective at levels that typically expect in ex-cropping paddocks.

The findings of this thesis have advanced our current knowledge of the restoration of ex-cropland. The research has tested methods to overcome biotic and abiotic barriers to restoration of the Victorian Volcanic Plains grasslands, and has demonstrated some practical approaches to begin the treatment. It was suggested that many of the methods and techniques used in this study could be useful technique in broad areas of grassland restoration within Australia as well as in similar situations in temperate climate conditions across the globe.

STATEMENT OF AUTHORSHIP

Except where explicit reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been relied upon or used without due acknowledgement in the main text and bibliography of the thesis.

Signed:_____ **Signed:**_____

Dated:_____ **Dated:**_____

Shakir Bahaddin SHAKIR **Singarayer K. FLORENTINE**

Candidate ***Principal Supervisor***

ACKNOWLEGEMENTS

I am grateful to my principal supervisor, Assoc. Prof. Singarayer Florentine, for his ideas, guidance, support and professional development. I would like to thank my co supervisors: Dr. Nicholas Schultz for his advice and input whenever I needed it and for help with plant identification, Dr. Grant Palmer for his feedback, Assoc. Prof. James Sillitoe for excellent feedback on chapters, and Dr. David McLaren for his help and advice on the field study.

I would like special thanks to Dr. Steve Sinclair who assisted me with plant identification in the field in the early stage of my thesis and of supply native plants. Special thanks goes to Dr. Kim Butler for his assistance and help with the most statistical analysis program. Dr. Janis Webb, for her support and assistance in early drafts of my chapters. I would like to thank Dr. Tony Dugdale for his help and support at Agribio, La Trobe University. I would also like to thank Kevin Banner and Simone French (Organic Recyclers) for their contribution in the field management by providing materials for trials.

I would like to thank the technical staff at the university, particularly Wendy Cloke for her assistance with providing tools and laboratory materials. I would also like to thank Dr. Roy Schrieke for his assistance and suggestions in chemistry laboratories. I also would like to thank Bruce Armstrong for his help and assistance in biomedical laboratory. I would like to thank the post grad students at Federation University Australia who provided much needed support, friendship and advice, especially Ako Mahmood, Majid Sabri, Rashidul Islam and Sandra Weller.

Shakir Bahaddin Shakir was supported by an Australian Government Research Training Program (RTP) Fee-Offset Scholarship through Federation University Australia.

Finally, I would like to thank all my family members, especially my nice daughter Basma and my cute son Mateen. Completion of this thesis would not have been possible without the encouragement, assistance and patience of my wife Rugash Bakr, for which I am grateful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1. Introduction

1.1 General overview

Grasslands occupy about half of the land mass in the ice-free world, and they constitute approximately 70% of the world's agricultural areas (Suttie *et al.,* 2005). As a consequence, they are an integral and central part of the global ecosphere, and thus remaining native grasslands need to be protected. Further, degraded grasslands should be, wherever possible, restored to be a fully functioning ecosystem. Healthy native grassland is a complex soil-based community that contains native grasses and a range of other native species that includes herbs and shrubs. This community both supports and interacts with a wide range of native animals and insects, and supports, and is supported by, microbial species in the soil matrix (Radford *et al.,* 2007).

Most of the world's native grasslands that receive enough rainfall to support crops have been converted to agricultural lands. Ellis *et al.* (2010) calculated that 90% of global grasslands have been converted to human land-uses, and less than 3% can be considered 'wild'. In accordance, Australian grassland ecosystems that are in regions of high agricultural activity, have been highly fragmented from the perspective of native flora and fauna (McIntyre & Hobbs, 1999).

In Victoria, southeast temperate Australia, native grasslands are one of the most endangered ecosystems. Approximately 99% of these grasslands have been destroyed in the last 150 years, (Scarlett *et al.,* 1992), and as a result of decreasing this once extensive native vegetation to small isolated remnants, the complex soil, which are a key feature of healthy ecospheres, have been significantly disturbed, leading to concomitant negative consequences for the landscape (Mitchell, 2003).

The Victorian Volcanic Plains (VVP) are located in Western Victoria, and cover over 2.3 million ha, which represents 10.36% of the state (Figure 1.1). They extend from Portland in the west to Mernda in the east, and from Clunes in the north to Colac in the south (Traill & Porter, 2001). These grasslands are an integral ecological element of the VVP, and are

one of the most endangered vegetation formations in Australia (Muir, 1994). The grasslands have consequently been listed as 'critically endangered' under the Federal Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) (TSSC, 2008), and thus require immediate and sustained restorative attention. Figure 1.2 shows the modelled extent of plains grasslands in the VVP as of 1750, prior to the commencement of European agriculture.

There are two broad classes of barriers which prevent the recovery of degraded grassland (i) biotic (exotic weed infestation, decreased soil microbial activity and lack of native seeds) and (ii) abiotic (soil nutrient enrichment and altered soil structure). This thesis will investigate some novel methods for restoring degraded native grassland ecosystems in the VVP, with a focus on overcoming these barriers to restoration. This chapter will review current literature pertinent to these barriers in grassland restoration, and review previous attempts at grassland restoration which have been reported. Following the literature review, this chapter will identify knowledge gaps that appear to be impeding successful grassland restoration attempts. Finally, the chapter will present the key research questions driving this investigation and the structure of this thesis.

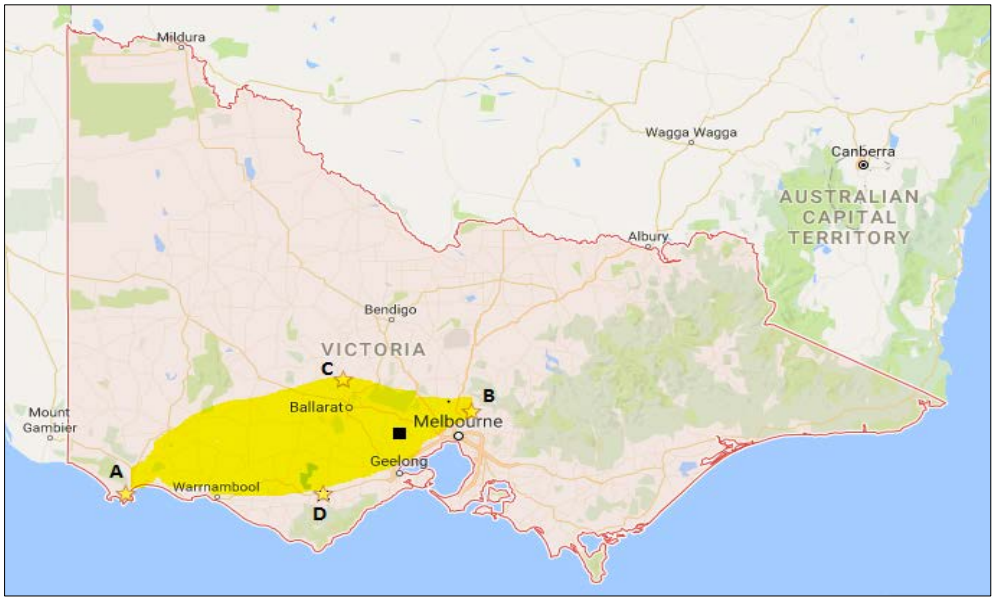

Figure 1.1 State map of Victoria, Australia. The yellow shaded area represents the Victorian Volcanic grassland Plain, and the black colored square is the study site. (A) is Portland in the west, (B) is Mernda in the east, (C) is Clunes in the north and (D) is Colac in the south.

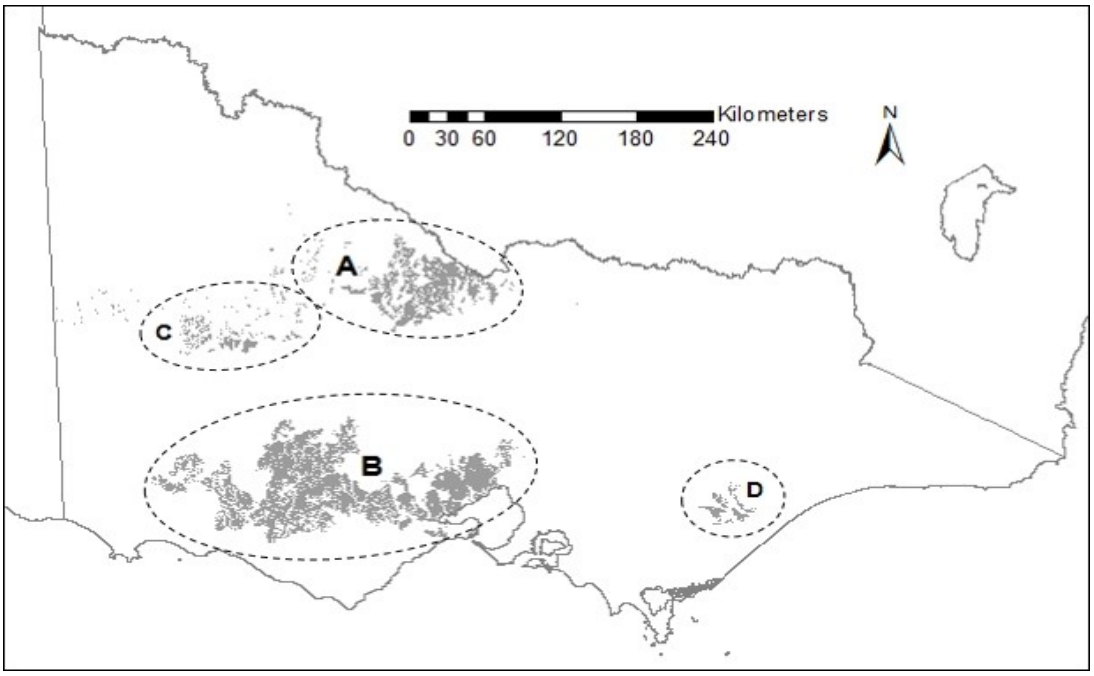

Figure 1.2 Areas of plains grassland in Victoria. The two major areas are the plains grasslands of the Victorian Riverina (**A**) and the Victorian Volcanic Plains (**B**). Plains grasslands also occur in the Wimmera Plain (**C**) and the Gippsland Plain (**D**). The grey shaded areas show the modelled 1750 distribution of the Plains Grassland ecological vegetation class (DELWP, 2008).

1.2 Models for conceptualising ecological restoration

To some degree, the nature of a proposed restoration approach will depend on which type of threshold has been crossed. For example, if the system has degraded mainly because of biotic factors such as vegetation cover change, restoration efforts may need to focus on biotic manipulations, which address the degrading factor by improving management and adjusting the biotic composition. This process may not require active modifications to the physical environment. However, if the system has degraded due to abiotic changes such as soil physical features (e.g. nutrient level, compaction, loss of soil), restoration efforts may need to focus on removing the degrading factors and repairing the physical conditions (Figure 1.3).

Whisenant (1999) has proposed that biotic and abiotic factors represent two thresholds to restoring a self-sustaining native ecosystem. The model suggests that as ecosystems move to more advanced states of degradation, ecological thresholds are crossed that make it hard for a system to return to its previous state. In the model, the first threshold is

a biotic one; exotic weeds invade and displace native species, and soil microbes are depleted or changed. The physical structure of the system remains intact, but active restoration is needed to overcome the biotic changes to the community. The second threshold is a change in the abiotic components of the system, and this represents a more advanced state of degradation and another, more difficult threshold to overcome. For grasslands (and as represented by the modified model shown in Figure 3.1), crossing this abiotic threshold usually means dramatic changes to soil structure and soil nutrient status, such that any attempts to correct the biotic component of an ecosystem will fail if the abiotic component of the system has not been fixed first. For example, the reintroduction of native perennial grasses into degraded grasslands of south eastern Australia will not be successful if the nutrient status of degraded land is not addressed first. As such, this model provides a suitable framework to conceptualise the changes that are needed if ex-cropping land in the VVP is to be returned to native grassland. However, Whisenant's model may be too simplistic, and it is important to appreciate that biotic and abiotic barriers are not always independent and can interact profoundly, as highlighted by Hobbs and Harris (2001). Management practices need to be aware of these interactions when trying to overcome barriers to grassland restoration.

A feature that is common to most models of ecological restoration is the concept of ecological barriers. It is recognised that this concept is particularly important in grassland ecosystems, and in the following sections, each of these barriers to ecological restoration have been reviewed, and those that are important to VVP grassland have been specifically identified.

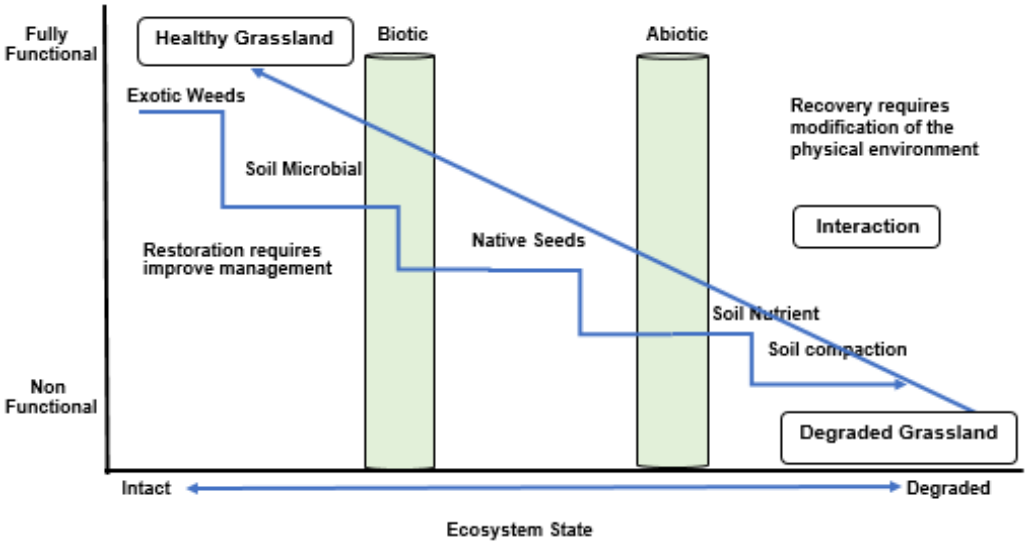

Figure 1.3 The two types of restoration threshold. Modified from Whisenant (1999).

1.3 Barriers to ecological restoration - biotic factors

1.3.1 Exotic weeds

The invasion of exotic plant species into natural ecosystems is a major threat to biological diversity across nearly all bio-geographical regions of the world (Adair & Groves, 1997). The invasion of weeds into natural areas has been associated with human movement throughout our evolutionary development, but, as is now widely recognised, the extent of this process has been accelerated considerably in recent years (Adair & Groves, 1997). Exotic species invasion is one of the main components of universal environmental change and represents a significant threat to global biodiversity and sustainability (Vitousek *et al.,* 1997).

Invasive species are one of the biggest threats to native biodiversity in Australia and present one of the largest costs to the Australian agriculture industry (Sinden *et al.,* 2004). Numerous species arrived with the first phases of European colonisation of Australia, and the number has continually increased. In many cases, these species have been deliberately and widely introduced as forage for agricultural livestock and other horticultural purposes. In addition, however, many grasses of less forage value have also been dispersed without deliberate human intervention (Bennett, 2014). In many

circumstances, these introduced species, due to their aggressive nature, have significantly modified natural environments by competing and displacing native plant species and creating conditions more suited to their own establishment in Australian grasslands (Mack & Lonsdale, 2001). As a consequence, these species may also cause secondary impacts within the ecosphere such as (i) alterations to hydrological cycles and fire regimes, (ii) significantly altering critical soil properties in terms of nutrients and microbial colonies, and (iii) changing the micro-climate in the area of colonisation to that which is more suited to their growth (Vidler, 2004). In addition, they have negative effects on native animals and plants since they can be unpalatable, thus decreasing available food sources, and can affect the growth and survival of competing native species (Vidler, 2004).

Weeds that significantly change ecosystem functions inevitably affect the habitat condition of other species in the system, and thus have the most profound effects on biodiversity (Adair & Groves, 1997). Competition from invasive plants has been shown to impact severely on native plant diversity (Levine *et al.,* 2003; Grice, 2004). Exotic species usually have no natural enemies to control their spread (MacDougall & Turkington, 2005), they have large and invasive root systems, and they produce huge quantities of seeds (Cronk & Fuller, 2001). With these attributes, they represent aggressive competition for native species for soil nutrients and available moisture (Carr *et al.,* 1992; Cole & Lunt 2005), and at the same time, because they are often not palatable to fauna or livestock, they are not controllable by grazing (Seabloom *et al.,* 2003).

The mechanisms by which exotic species can replace native species are rarely confirmed (Seabloom *et al.,* 2003). However, one is that invasive weeds have greater uptake of nitrogenous material and, particularly in areas which have been treated with nitrogen-rich fertiliser, they use the absorbed nitrogen with greater efficiency (Rossiter *et al*., 2006). Exotic plant species can alter ecosystem nutrient cycles. For instance, systems occupied by non-native plants tend to have greater crop biomass, and they have rapid litter decay

and changes in the timing of nutrient cycling. This leads to greater inorganic nitrogen levels and increased amounts of nitrogen mineralization, a soil condition which is not favourable to the growth and sustainability of native species (Ehrenfeld, 2003).

Agricultural practices too, can provide exotic species with a further competitive advantage over native plants through cultivation and fertiliser application (Flory & Clay, 2010). Of particular interest here is Morgan's (1998) study, which showed that VVP grasslands, which are naturally dominated by *Themeda triandra*, has an unexpectedly large exotic vascular plant component, even in those areas that have only been slightly disturbed and are otherwise rich in native species. For example, 41% of 102 species found in the study plots in the grassland areas in Sunbury were exotics (Morgan, 1998). Similarly, Lunt (1990) found that 24 of 41 species in the soil seed bank at Derrimut Grassland Reserve in the VVP were exotic. This is not just a local phenomenon; Sharp's (1997) study also found that 41% of species recorded in surveys of 39 natural grasslands in the ACT, were exotics. This high proportion of exotic species is attributed largely to the elevated nutrient status of native grasslands, which will be discussed further below.

1.3.2 Decreased soil microbial activity

Changes in the composition of microbial species in the soil after land use conversion and changes in vegetation can contribute to changes in the ecosystem function (Copley, 2000; Steenwerth *et al*., 2002). Cavigelli and Robertson's (2000) study showed important links between the microbial community composition and healthy soil processes. Soil microbes can play a significant role in grassland restoration of former agricultural land through (i) their effects on soil porosity, (ii) organic material storage through decomposition, (iii) increased water holding capacity, and (iv) natural nutrient cycling (Jastrow *et al.,* 1998), all of which begin to favour native plant species. In addition, microbes play a crucial role in the development and maintenance of soil structure by binding soil elements and organic matter to create healthy aggregates (Jastrow *et al.,* 1998; Six *et al*., 2000).

It has been noted that microbial activity in terrestrial ecosystems is also often negatively effected by the availability of phosphorus and nitrogen (Güsewell, 2004; Cleveland & Liptzin, 2007; Elser *et al*., 2007). High levels of nitrogen and phosphorus decrease microbial activity in the soil, which can then lead to mineralization of organic carbon and release it to the atmosphere as carbon dioxide. However, an increase in perennial root systems and plant litter, which is characteristic of grasses, will increase microbial biomass and soil organic carbon and nitrogen over the time of restoration. (McKinley *et al*., 2005; Matamala *et al*., 2008).

1.3.3 Lack of native seeds

The unassisted reestablishment of grasslands requires a suitable quantity and diversity of native seed. The absence of such represents another barrier to grassland restoration. Many native species are characterised by low production and distribution of seeds (Mac Dougall & Turkington, 2007), and this factor may lead to partial or complete failure of grassland restoration (Munzbergova *et al.,* 2005). In contrast, exotic invasive plants generally produce very large seed numbers and have mechanisms for wide dispersal. As such, they can overwhelm the establishment of native species by numerical competition (Donath *et al.,* 2007). Furthermore, other studies have indicated that lack of seed accessibility seems to be the main cause of restoration failure of native species, rather than it being a matter of direct weed competition or climatic stress (McDougall & Morgan, 2005, Madsen *et al.,* 2016).

It has been established that none of the native perennial inter-tussock species in current native temperate grasslands are obligate seeders, almost all being of limited seed production and generally unable to set seed within 12 months of restoration (Lunt, 1990; Morgan, 1996; Lunt & Morgan, 2002). Morgan (1998) investigated five areas of western Victorian grassland and has found that only 12% of species have persistent seed banks, and all of these were annuals with most native hemicryptophytes and perennials having transient seed banks.

1.4 Barriers to ecological restoration - abiotic factors

1.4.1 Soil nutrient enrichment

Australian soils generally have been characterized as having a low level of nutrient content, especially in relation to nitrogen, phosphorus and minor nutrients, though they have relatively high organic matter (Leeper, 1970). Studies by Wijesuriya and Hocking (1999) have shown that most soils which support Australian native grasslands usually have low levels of nitrogen and phosphorus, which implies that these conditions are the most suitable for the establishment and sustainability of these native species.

It is well known that agricultural activity on previous grassland sites significantly affects the soil's chemical levels, especially because of the continual addition of mineral-rich fertilizer (Dorrough *et al.,* 2004). In Australia, superphosphate has been the most common agricultural fertilizer used over the last century, and this increases both phosphorus and nitrogen levels in the soil. This raised level of minerals has led to an increase in the exotic weed population, partly because it facilitates their competitive advantage (Brereton & Bakhouse, 2003). This has caused large changes in grassland plant species composition (Grime, 1974), and, in addition, concomitant reduction of native plant growth and microbial activity in the ecosystems of these ex-agricultural areas has resulted, due to significant accumulation of inappropriate soil nutrients over the time (Güsewell, 2004; Cleveland & Liptzin, 2007; Elser *et al.,* 2007; Van Daele *et al.,* 2017).

Studies have also shown that nitrogen and phosphorus fertilization of grassland increases the above ground biomass of native grasses in the short term, but in the long term, it leads to an irreversible overall reduction in their productivity (Haddad *et al.,* 2000). Other studies have shown that the application of nitrogen fertilizers, even in small amounts, leads to a decrease in species number of native grasses (Willems *et al.,* 1993). Long-term N fertiliser application is also associated with increased soil acidity (Aguiar, 2005), which in turn is likely to have detrimental effects on native grassland species (Sharp, 1997).

Low availability of soil phosphorus is argued to have played a significant role in the evolution and distribution of Australian flora (Beadle, 1954; Orians & Milewski, 2007). It has been predicted that the application of phosphorus fertilisers in temperate Australia will have negative impacts on native plant species (Yates & Hobbs, 2000; Kirkpatrick *et al.,* 2005). For example, it has been observed that the species richness of native forbs declines with increasing phosphorous levels (McIntyre & Lavorel, 2007; Dorrough *et al.,* 2004).

It is widely believed that the degradation of native grassland is a natural result of increases in mineral fertilizer application, mainly because of the negative effect on biodiversity of native grasses and the concomitant preparation of a good environment for exotic weed species. This allows exotics to grow faster and compete favourably with native species for water, light and space (Seabloom *et al.,* 2003).

1.4.2 Soil compaction

Grassland soil compaction is thought to contribute to poor growth of native grasses and forbs, and this barrier consequently affects the maintenance of grassland biodiversity (Palmer *et al.,* 2004; Ruser *et al*., 2006). Soil compaction can be defined as the decrease in the volume to weight ratio of the matrix due to external influences, and this reduction, probably because of the increased density of the soil and its lower aeration, will lead to lower soil productivity. The extent and impacts of soil compaction also increase as the size and weight of farm machinery equipment increases (Soane & Van Ouwerkerk, 1994).

It is observed that topsoil is compacted when grassland is converted to crops because of the common use of agricultural machinery for sowing, fertilising and harvesting (Jorajuria *et al.,* 1997). The focus on soil compaction in this study arises from the belief that grassland soils are only stable because of the contributions of perennial plant cover and root reinforcement. Indeed, Cofie *et al.'s* (2000) study showed that the perennial landscape and root structure of grassland vegetation improves the stability and bearing

capacity of its soil, and acts to reduce the stresses on grassland being transmitted to greater depths (Krebstein *et al.,* 2013).

However, this stabilising role provided by surface vegetation can be severely compromised by soil compaction in grasslands. Soil properties in healthy grasslands critically depend on several important factors, including soil structure stability which is directly related to open soil texture, high organic matter content and continual nutrient accumulation (Horn & Rostek, 2000), and each of these factors are degraded by soil compaction.

An immediate effect of soil compaction on natural grass development is reduced root growth, water infiltration and seed germination, due to the reduction of resources such as oxygen, water and nutrients to the roots. This can severely limit plant growth and lead to a decline in production of perennial forages as well as native grasses (Lipiec & Hatano, 2003). Jorajuria *et al.'s* (1997) study showed the vegetation cover of grassland decreased by 52 to 76% where machinery tyres compacted the soil. In addition, soil compaction leads to the destruction of soil structure and an increase in the bulk density of soil, which then effects the nature of the soil's organic matter and causes a decline in the porosity of the soil.

1.5 Previous grassland restoration efforts

Most previous studies of grassland restoration have focused on conversion of ex-agricultural land and have mainly investigated (i) the effect of soil nutrients on species-richness (Janssens *et al.,* 1998), (ii) methods of reducing the nutrient status of soils to improve success in natural grassland establishment (McCrea *et al.,* 2001) and (iii) multi-site experiments comparing soil pre-treatments, natural regeneration, nurse crops, and seed mixes (Pywell *et al.,* 2002).

An important consideration is the reduction of introduced nutrient concentrations, mainly nitrogen and phosphorus, which are found in the soil. In this respect, physical topsoil removal (scalping) seems to be the most efficient method to create a more desirable nutrient-poor situation (Gibson-Roy, 2000; Gibson-Roy *et al.,* 2010a). In addition, whilst most studies on weed control methods prior to sowing have focussed on agricultural crops, there is a limited research that reports specifically on weed control for direct sowing of complex mixes of grasses and forbs for the re-establishment of temperate grassland populations in south-eastern Australia (Gibson-Roy, 2000; Allison & Ausden, 2004; Cole & Lunt, 2005).

Many European legislations that are aimed at increasing the connection of existing nature conservation areas, have attempted to improve areas of former agricultural land that are available for ecological restoration (Smolders *et al.,* 2008). The major problem that has to be overcome in these areas, similar to the Australian experience, is that they have been heavily fertilized in recent decades, resulting in the accumulation of huge amounts of phosphorus and nitrogen in the soil (Barberis *et al.,* 1995), and it is anticipated that results of this investigation will be of interest to the European situation.

In the following review, attempts at grassland rehabilitation through scalping, carbon addition, and manipulation of phosphorus levels through chemical and natural means, will be discussed.

1.5.1 Scalping

In terrestrial systems, topsoil (up to 10cm) removal is most effective measure to achieve a low nutrient status within a relatively limited time span (Smolders *et al.,* 2008). Several studies on topsoil removal have been conducted in Europe. Of these, Jaunatre *et al.* (2014) asserts that removing topsoil partly restored soil conditions and significantly reduced the non-target seed bank. Moreover, species richness and similarity to the reference steppe was significantly increased with topsoil removal. Scalping has also provided favourable results in former agricultural areas favouring low productive plant populations (Gibson-Roy, 2000; Allison & Ausden, 2004). It is, however, an expensive measure - in Australia the cost is around $3000 per hectare (Gibson-Roy *et al.,* 2010b). Sometimes it is not possible to remove enough soil to limit P, as deeper soil layers still contain high phosphorus concentrations, and additional measures have to be taken (Smolders *et al.,* 2008). This will be further discussed below. Scalping also requires other considerations, such as the need for relocating nutrient-rich topsoil, and restricted access due to surface rocks. However, these nutrient enriched soils have been spread on areas planted with crops in some cases. Removing the topsoil also results in the disappearance of the diaspore bank, although in agricultural soils, this mainly contains seeds from eutrophic species and not from rare target species (Smolders *et al.,* 2008).

1.5.2 Carbon addition

Increased availability of nitrogen promotes the invasion by exotic plants into natural grasslands (Burke & Grime, 1996; Wedin & Tilman, 1996) and this leads to a decrease in species richness of native grasses (Willems *et al.,* 1993), since exotics can out-compete native grasses. If this increased nitrogen availability can promote invasion into grasslands, then it is postulated that declining nitrogen accessibility might help stop exotic invasion, especially in systems where nitrogen enrichment is taking place (Alpert & Maron, 2000). It has also been shown that addition of biologically available carbon (for example as sugar or sawdust) can increase microbial activity which helps to draw down the nitrogen level

(Morghan, & Seastedt, 1999; Prober *et al.,* 2005; Faithfull *et al.,* 2010). Carbon addition has also been shown to reduce inorganic nitrogen and exotic plant biomass (Blumenthal *et al.,* 2003), and studies have shown that addition of sugar has a significant impact on reducing the germination of exotic weed seeds within the seed bank (Prober *et al.,* 2009). The source of carbon added to the soil is important as its influence on soil condition and plant growth is governed by the rate at which the carbon becomes available to microorganisms (Eschen *et al.,* 2006). For instance, a readily available carbon source, such as sugar, may stimulate microbial activity within hours (Dalenberg & Jager, 1981), however other sources, such as more complex molecules, have structures that need more time to decay (Magill & Abel, 2000).

Eschen *et al*. (2007) showed that carbon addition during grassland restoration is a useful management technique to reduce nitrogen availability on ex-cropping land. This action alters the vegetation composition by creating gaps in the plant cover that facilitates the establishment of late-succession plant species, and is most effective when started immediately after the abandonment of arable fields and is applied over several years. Ex-arable land soils are often categorized by high inorganic nitrogen levels, which lead to the rapid formation of annual and fast-growing perennial species during the preliminary phase of habitat creation (Eschen *et al*., 2007).

1.5.3 Manipulation of Phosphorus

1.5.3.1 Previous use of Phoslock® in lake water and terrestrial ecosystems

Phoslock® is the registered brand of a phosphorous-absorbing material made by Phoslock® Water Solutions Ltd., Sydney, which distributes this material globally. Phoslock® is a natural product, produced from modified bentonite clay initially developed by the Land and Water Division of Australia's CSIRO (Commonwealth Scientific and Industrial Research Organisation). It was an attempt to significantly reduce the amount of Filterable Reactive Phosphorus (FRP) present in the water column and in the sediment pore water of a water body, and in this context, FRP is an important growth factor for blue

green algae and other algae (Douglas, 2002). Aqueous trials with Phoslock® have previously been performed in Australia, where it has been applied to two Western Australian waterways in the summer of 2001/2002 (Robb *et al.,* 2003).

For our terrestrial purposes, there is a very stable mineral formed by reaction between available soil phosphorous and Phoslock®, called rhabdophane ($La_3PO_4.n\ H_2O$). The complex formed here has a very low solubility constant of $K_S < 10^{-23}$, therefore the bound phosphate is no longer bioavailable (Douglas *et al.,* 2000). Furthermore, Phoslock® is insensitive to changes in pH, redox potential and oxygen concentrations (Afsar &Groves, 2009; Ross *et al.,* 2008), implying it will be stable in many soil conditions.

For our purposes, the addition of Phoslock® is expected to be especially effective in flooded soils, because it can form an active layer on top of the soil that reduces phosphate mobilization to the water layer (Geurts *et al.,* 2011).

1.5.3.2 Planting of *Lupinus albus* (White lupin) and the native species *Ptilotus macrocephalus* (Tall mulla mulla) and *Ptilotus polystachyus* (Long-tails)

Because high phosphorus availability in soils has been recognized as a barrier to restoration of semi-natural vegetation (Pywell *et al.,* 2007) a number of studies have investigated the use of plants to reduce soil phosphorus concentration (Bates, 1990; Gilbert *et al*,. 2009). For example, Islam *et al.* (1999) examined the phosphorus uptake by Amaranthaceae (*P. exaltatus*, *P. macrocephalus*, and *P. aerovoides*), *Dysphania kalpari* (Chenopodiaceae) and *Abutilon oxycarpum* (Malvaceae) on soil with low phosphorus concentration in subtropical, semi-arid Australian grassland. The study showed that the concentration of phosphorus in shoots was greatest in the *Ptilotus* species, ranging up to 1.75 mg.

Ryan *et al.* (2009) investigated the growth response of *P. polystachyus* and *Cichorium intybus* (Asteraceae) to phosphorus and nitrogen addition in a sandy soil of extremely low bicarbonate-extractable phosphorus and mineral nitrogen. *Ptilotus* species grow much better and produce more biomass than chicory in soil with low bicarbonate-extractable

phosphorus or mineral nitrogen. The concentration of phosphorus in shoots kept steadily increasing in *Ptilotus* when there was an increase in the concentration of phosphorus in the soil.

The biology of legumes indicates that they are adaptable to a wide range of phosphorus levels, where a special root structure called cluster (proteoid) roots are formed in response to phosphorus deficiency (Shane *et al.,* 2005). Indeed, a number of plants, native to Australia, possess a range of adaptations to maintain adequate phosphorus nutrition when growing in soil with low availability of labile inorganic phosphorus (Handreck, 1997), and root-system adaptations to enhance phosphorus uptake that are commonly found in Australia include symbiosis with mycorrhizal fungi (Johnston & Ryan, 2000).

The genus *Ptilotus* (family Amaranthaceae) includes approximately 100 species, most of which are endemic to Australia (Lee *et al.,* 2007). Most species are found in arid and semi-arid regions, where they range in growth form from prostrate to erect herbs, and in some cases, small, woody shrubs (Lee *et al.,* 2007). *Ptilotus polystachyus* has long been thought to be a promising candidate for domestication as a short-lived perennial forage herb for acid soils in the cropping zones of southern Australia (Gardner, 1934). The nutritional response of a short-lived perennial herb native to the deficient soils of Australia*,* *Ptilotus polystachyus*.

The ability of *Ptilotus* to accumulate high concentrations of phosphorus in its shoot tissues whilst maintaining biomass production, makes it highly suitable for such a "phytoremediation" role (Ryan *et al.,* 2009), reducing the phosphorus concentration in soil when there is the potential to harvest material from grasslands in order to remove phosphorus from the system.

1.5.4 Direct seeding with native species

Early restoration efforts were often focused on the elimination of nutrients, rewetting the soil and modifying exotic species management. In many cases, such measures alone were unsuccessful due to a lack of seeds or propagules of the target community, even

though environmental conditions were favourably changed (Bakker & Berendse1999; Walker *et al.,* 2004).

Because of this problem, during the past decades numerous methods for propagule transfer have been tested across the world in a broad range of habitats (McDougall & Morgan, 2005; Prober *et al.,* 2009). The lack of propagules is a major obstacle to the restoration of native grasslands on former agricultural sites (Walker *et al.,* 2004), particularly because of abiotic constraints and related management issues (Kiehl *et al.*, 2010). It has been suggested that this problem could be overcome by native seed sowing (Morgan, 1996; Morgan, 1999; Lunt & Morgan, 2002; Madsen *et al.,* 2016; Kiss *et al.,* 2018) if other conditions, such as nutrient and bacterial population, are suitable (Wong *et al.,* 2015).

The main features of direct seeding are the ability to sow large areas rapidly by hand or with broadcasting machinery, which involves lower cost compared with transplanting seedlings, it is still expensive and has high cost effects (Douglas *et al.,* 2007). The seeds for restoration can be purchased from commercial sources or collected by local harvesting (Török *et al*., 2011).

Many past restoration efforts have required not only the control of the above-ground biomass, but also a high level of control of the weed-dominated soil seed banks (Hitchmough, 1994). To achieve this latter condition, practitioners have employed a variety of site preparation and control techniques, including (i) the elimination of standing vegetation, (ii) killing emerging seedlings, (iv) removing, burying or destroying soil-borne propagules, and (iv) reducing nutrient levels in the soil (Hitchmough, 1994). Under these conditions, it has been stated that direct seed addition will be favourable (Cole *et al.,* 2004; Gibson-Roy *et al.,* 2010a). In essence, the superiority of these control methods is based on providing a competitive advantage for the sown species, either by suppressing or removing weed propagules, or by selectively targeting weed species. The method chosen will be advised by budget and time constraints (Coor, 2003; Gibson-Roy *et al.,* 2010b).

1.6 Synthesis

Despite the broad recognition that nitrogen, phosphorus and exotic weeds are barriers to restoration of grasslands, there is a lack of understanding of the relative importance of these factors for the grassland restoration. Exotic weeds effect the habitat conditions of native grass species in the system, by competing with other plants for nutrients, space, light and water for their survival (Grice, 2004). Imbalance of soil microbial activity will affect native grassland restoration on former agriculture land because of the flow-on effects of decreased organic material storage through decomposition, together with interrupted nutrient cycling (Elser *et al*., 2007). Also, by comparison with the high fecundity features of exotic weeds, native species are generally characterised by low germination rates and modest distribution of seeds (MacDougall & Turkington, 2007). Their inability to compete with exotic weeds to colonise ecological niches, may contribute to the partial or complete failure of many grassland restoration attempts based on reseeding (Munzbergova *et al.,* 2005).

The widespread and long-term use of superphosphate fertilizer has had a negative residual effect on attempts to recover converted grassland, because the increased phosphorous levels interfere with the growth of many native plant species and the essential microbial activity in these land-based ecosystems (Güsewell, 2004; Cleveland & Liptzin, 2007; Elser *et al.,* 2007; Dorrough & Scroggie 2008). In addition, sustained agricultural activity with heavy machinery in converted grassland areas has led to significant soil compaction, and the resultant impact on native grasses is seen in reduced root growth and function, together with reduced oxygen and nutrient resource availability to developing root systems. In consequence, these factors act to inhibit plant growth and lead to weakened production of perennial forages as well as native grasses (Lipiec & Hatano, 2003).

Previous studies in restoration of native grasslands have focussed on removing nitrogen, phosphorus and exotic weed infestations together in one scalping treatment. Because of

the cost and other practical considerations it has become clear that novel methodologies other than scalping are required to reduce soil nutrient levels and thus benefit indigenous grassland species and, simultaneously, reduce the competitiveness of exotic weeds. The aim of this project is to examine the establishment of grassland species under a range of soil / mulch manipulations and to assess the influence of carbon and other management techniques on the control of soil nutrients and soil microbial activity. In addition to gaining an understanding of the individual effects of N, P and exotic weeds as barriers to restoration success, the project aims to develop potential methods for grassland restoration and weed control, utilizing green waste products that may fast track weed seed bank decline.

In this work, 'green waste' is defined as the bark, wood and other larger materials sieved out from recovered organic material which has been used for commercial composts. The material has been composted with consequent heat treatment of 60^{O}C for 70-90 days, which is sufficient to kill any weed propagules or weed reproductive organs consistent with available Australian standards for recycled materials (Moral *et al.,* 2009).

Chemical analysis showed that this material will cause nitrogen drawdown, much like the results of using of sawdust and woodchips (Chan *et al.,* 2008). Nitrogen drawdown occurs through increased microbial activity that facilitates removal of excess nitrogen. Whilst this is not a desirable soil process for agriculture, it has obvious benefits in reducing native seed competition with exotic weeds in croplands where grassland restoration is planned. It is hoped that this unique technique can lead to landscape-scale weed management with benefits to indigenous grassland restoration, and also to reduce weed spread into surrounding prime agricultural lands. If successful, it will offer potentially huge benefits to the restoration and sustainability of the Australian environment.

Secondly, the project will investigate a unique method to reduce phosphorus level in the terrestrial system by using Phoslock®, a product which has previously been used to reduce phosphorus levels in the lakes and sedimentary layers (Geurts *et al.*, 2011). This will be

the first trial to assess the use of Phoslock® for removing available soil phosphorous in Australia.

It is anticipated that, overall, this study will enhance our theoretical understanding of the relative effects of nitrogen, phosphorus and exotic weeds as barriers to restoration, the results of which may be applied to similar ecosystems globally in which previously used agricultural land has been abandoned.

1.7 Research focus and objectives

The objective of this study are, first, to identify the biotic and abiotic barriers to grassland restoration, and second, to investigate techniques for overcoming these observed barriers.

The anticipated outcomes of this thesis are to determine: (i) the current state of factors that prevent restoration of native grasslands in ex-cropping land in VVP grassland and (ii) what mitigation steps might be taken to restore soil properties, reduce exotic weed competition and establish native species to allow native ecosystems to develop.

Finally, this thesis will present an evaluation of the impacts of both biotic and abiotic barriers to the restoration of grasslands in the VVP. It is anticipated that the findings will help to improve the scope and focus of potential management regimes that might be applied to VVP grassland systems to enhance the richness and abundance of native grassland species. It is also intended to help identify gaps in our understanding of the interaction of controlling practices with climatic and soil variability, and thus to help direct future experimental work in this area.

1.8 Knowledge gaps and contributions from this work

To our knowledge, this is the first experimental attempt at manipulating nitrogen, phosphorus and exotic weeds individually, and observing the influence of each on grassland restoration. Previous studies in restoration of native grassland have focussed on removing nitrogen, phosphorus and exotic weed infestations together in one treatment, known as scalping. The topsoil was removed and buried in order to destroy soil-borne

propagules of exotic weeds and to reduce nutrient levels in the remaining soil. Although this method is ultimately effective, it involves a high cost, and may be more suitable for small areas where no rocks or trees are present. Scalping also creates a large amount of soil that needs disposal, although this may be spread over agricultural cropping land which is a desirable outcome due to its innate high nitrogen and phosphorus levels.

This project has used novel approaches to reduce elevated soil nutrient levels in order to benefit indigenous grassland species and reduce competitiveness of exotic weeds. A potential source of carbon substrate for this purpose is the large particulate material left over after composting urban green waste. In addition, I also used a method to reduce the individual effect of phosphorus by using a treatment based on a commercial product called 'Phoslock®', which has previously been used to reduce the concentration of phosphorus in lake water (Reitzel *et al.,* 2013). Phoslock® is a commercial product created using bentonite that has been modified by ion exchange, and contains lanthanum ions as the active ingredient (Afsar & Groves, 2009).

1.9 Research questions

1.9.1 Principal question

The primary aims of this research were to gain a better understanding of the biotic and abiotic barriers that prevent restoration of native grasslands in ex-cropping land, and to investigate economically feasible and sustainable ways to overcome them. Specific factors which have been suggested to interfere with the restoration of grasslands as a result of previous work include: high soil nitrogen, high soil phosphorous content; low levels of microbial activity (Güsewell, 2004; Cleveland & Liptzin, 2007; Elser *et al.,* 2007; Wong *et al.,* 2015); soil compaction caused by external factors (Lipiec & Hatano, 2003); the presence of aggressive exotic weeds (Adair & Groves, 1997) and the lack of native seeds, either in resident seed banks or through natural dispersal (Lunt, 1990; Morgan, 1996; Morgan, 1998; Lunt & Morgan, 2002; McDougall & Morgan, 2005). Although each of these factors have been previously identified, the relative importance of each of these

as barriers to restoration success has not been addressed. For instance, it is not clear if all of these barriers need to be addressed, or if some might be less crucial to restoration success. As such, the principal research question is: *What are the relative influences of nitrogen, phosphorus and exotic weeds as barriers to grassland restoration in former cropland?* This question will be addressed in Chapters 2, 3 and 5.

In order to address this question, I have designed experiments to manipulate each of these factors individually. To our knowledge, this is the first such attempt to manipulate each of these factors separately in the context of grassland restoration, and I anticipate that successfully addressing this question will advance our theoretical understandings of the barriers to grassland restoration, which will prove crucial to the development of new and viable restoration techniques.

1.9.2 Sub questions

In addition to primary research questions, it was necessary to also address a number of additional questions related to grassland restoration. As a consequence, the current condition of vegetation cover, the composition of the soil seed bank, soil nutrient levels and the bulk density of the topsoil were assessed at the ex-cropland (experiment site) and the native grassland (reference site). This allowed several sub-questions to be addressed which are contained in the overall statement: *What are the differences in some of the variables such as: (vegetation cover, soil seed bank emergence, current level of nutrient and soil bulk density) between ex-cropland grassland and untouched native grassland*? This suite of questions will be addressed in Chapter 2.

Indeed, it appears that grassland restoration has rarely been attempted, and identified trials have been on relatively small scales (Suding& Hobbs, 2009). Perhaps the most successful current method of grassland restoration in Victoria to date is the previously mentioned removal of topsoil (scalping) and subsequent sowing of native seeds into the subsoil. This physical removal of the soil matrix reduces soil nitrogen, phosphorus and the exotic seed bank (Gibson-Roy, 2000; Gibson-Roy *et al.,* 2010a). Whilst it is realized that

this method, although useful, has its limitations, and it is important that the new methods which have been suggested are subjected to detailed investigation. By including scalping as a parallel treatment in the main field experiments, I can address the subsidiary question: *How do novel methods for manipulating barriers to restoration compare to scalping?* This question will be addressed in Chapter 3.

Another significant barrier to grassland restoration is the problematic re-colonisation of sites by native plant species. Whilst this re-colonisation is generally approached by attempts at direct seeding of native species, this is an expensive and labour intensive aspect of grassland restoration. As a consequence, it is essential that understandings are developed about which species (if any) will naturally recolonise in a grassland site. In order to establish clear yardsticks, our main field experiment will involve a comparison of restoration techniques that include seeding of native grasses with techniques that do not include seeding of native grasses; this leads to the question: *Do native species recolonise without the need for additional seeding? If so, which species are most effective?* This question will be addressed in Chapter 3.

It is well known that agricultural activity on previous grassland sites has significantly effected the soil's chemical structure, especially due to the addition of fertilizers which lead to significant nitrogen increases. In an effort to overcome this nutrient imbalance (when compared to native grassland conditions), I examined the effect of adding available carbon sources to ex-cropland soils, and will ask the question: *What is the impact of addition of carbon sources such as sugar and mulch (cold green waste) on soil microbial activities and soil N levels in ex-cropland areas?* This question will be addressed in Chapter 3.

In the main field experiment, the use of Phoslock®, a modified clay product, will be used in an effort to manipulate soil phosphorus levels. This is a relatively expensive and time consuming technique, and consequently a parallel study using plant species with high uptake of phosphorus will be attempted, which may be another viable option for reducing

phosphorus in ex-cropping land. A glasshouse pot trial was invoked to address the question: *How does the use of Phoslock® to reduce soil available phosphorus compare to the use of a range of high-phosphorus binding plant species?* This question will be addressed in Chapter 5.

Because of the innate barriers to germination of many native species, an additional investigation was carried out in order to overcome the seed dormancy of two species (*P. macrocephalus* and *P. polystachyus*). These species will be used in a glasshouse study to examine the extent of the reduction of phosphorus levels in samples of ex-cropping land. The seed germination study was conducted in Federation University's laboratories in order to address this question: *What is the best pre-treatment to overcome the seed dormancy in P. macrocephalus and P. polystachyus and what is the ideal temperature regime and the most effective light exposure period, for the optimum germination of these seeds*? This question will be addressed in Chapter 4.

1.10 Thesis structure

This thesis contains five chapters, diagrammed in Figure 1.4. It includes the Introduction and Literature Review as Chapter 1. Chapter 2 will cover experimental and reference site description and look at the similarities and differences between these areas. Chapter 3 will describe the field experiments which involved *in situ* investigation of the factors that affect the restoration of native grassland. It will describe methods and ecological approaches to the restoration of native grassland such as the addition of green organic waste (hot and cold green waste), sugar addition, Phoslock® and scalping treatments. It will also describe the results of field experiments such as green waste and sugar addition treatments and compare their effects with scalping and Phoslock® treatment. Chapter 4 will cover a range of laboratory experiments to investigate different methods to breaking seed dormancy of *P. macrocephalus* and *P. polystachyus* seeds. Chapter 5 will cover a range of glasshouse experiments, giving description of the methods conducted to investigate techniques to reduce available soil P by binding or extraction of P using native

plants. Chapter 6 will describe the main findings of Chapters 2, 3, 4 and 5, summarises and explores the implications of the research findings presented in this thesis and also provides some future research direction in this critical area of research.

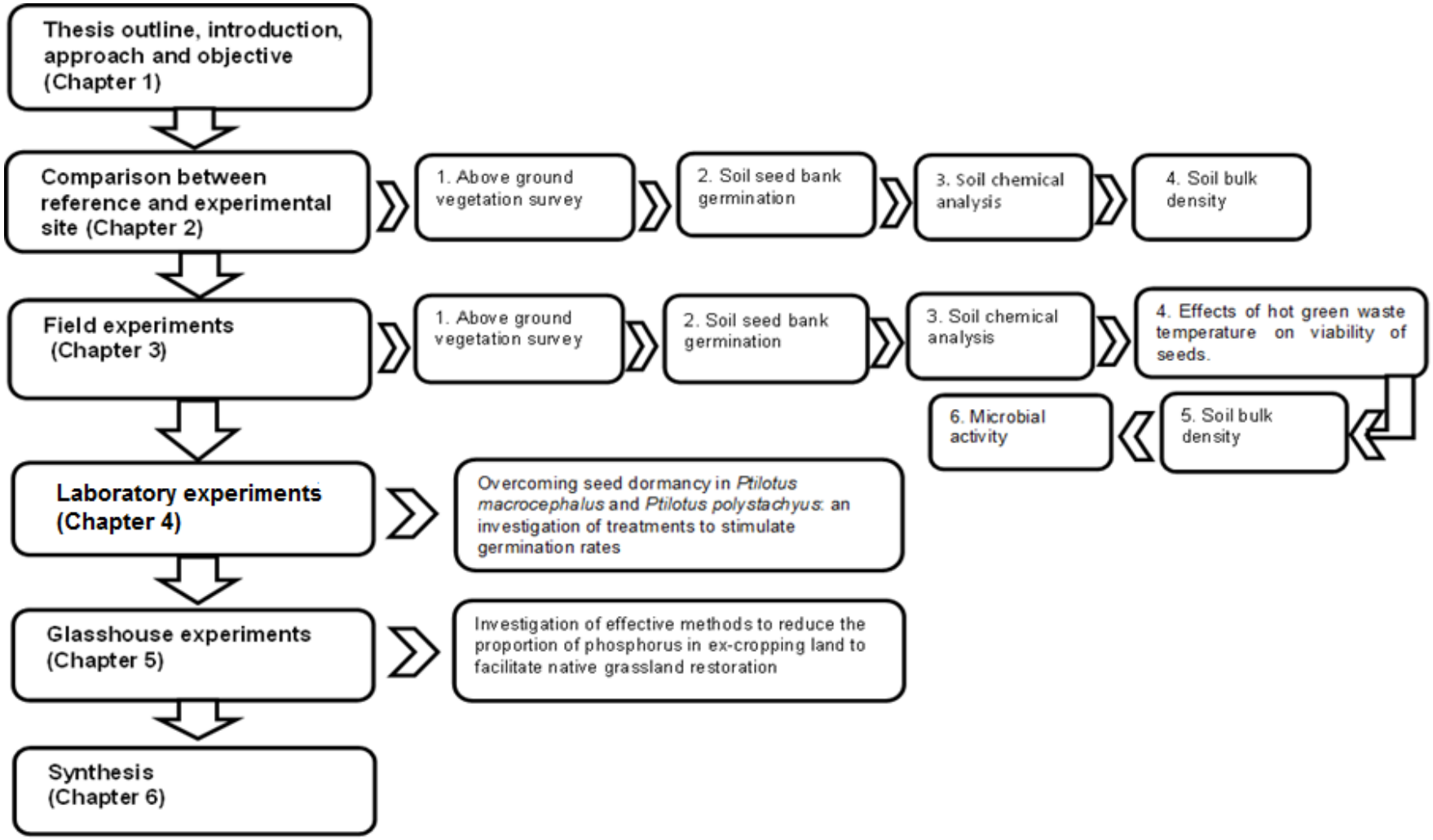

Figure 1.4 Conceptual framework of thesis structure.

Chapter 2. Physical and vegetative comparisons between reference native grassland and ex-croplands

2.1 Introduction

The Victorian Volcanic Plains grasslands (VVP) are one of the most endangered vegetation formations in Australia (Muir, 1994). Consequently, these grasslands have been listed as 'critically endangered' under the Federal Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) (TSSC, 2008), and thus require immediate and sustained restorative attention. This investigation will consider a number of alternative attempts to restore such grasslands to their former state. Experiences from previous restoration attempts reported in the literature have indicated that there are no simple solutions to such a task, and that it is likely that a number of sequential or simultaneous treatments may be required (Whisenant, 1999; Hobbs & Harris, 2001).

However, before discussing potential approaches, an examination of the ex-croplands site, in relation to a native grassland area, will be undertaken. This exercise will establish a number of useful benchmarks, including an appreciation of quantitative and qualitative soil composition changes, physical changes to the soil structure, the current vegetation community, and changes to the seedbank. Such analyses will usefully inform the nature and intent of our restoration attempts, and provide a clear benchmark for the evaluation of individual and conjoint treatments during the project.

2.2 A brief description of the state of disturbed grassland

It has been shown that conversion of grassland into cultivated land causes numerous alterations in the physical and chemical properties of the soil, and also to the bacterial and mycorrhizae processes necessary for healthy support of flora and fauna. This scenario implies that several interventions, including both physical and chemical approaches, may be necessary for establishing any long-term restoration initiative. In particular, it is clear that conventional cultivation has been shown to harm soil horizons, increase soil erosion, change nutrient accessibility and mineral compositions, compact soil, and decrease the

percentage and formation of soil macro aggregates (Tisdall & Oades, 1982; Tisdall, 1994; Six *et al.,* 2000; McLauchlan, 2006), and it is likely that all of these issues will need to be specifically addressed in some form. In addition to these immediate issues, cultivation also increases average soil temperature because of less shading from plant matter, and it causes extreme wet and dry cycles in the soil due to poor irrigation practices and a general decline of water penetration into the degraded soil matrix (Mann, 1986). The additive effect and legacy of these impacts on the soil will also need to be considered in any holistic approach to restoration. It should be noted, in terms of establishing the importance of this and similar projects, that these harmful outcomes of cultivation of grassland areas are not confined to this country, and across the world, degradation of grasslands has become a huge ecological problem (Harris, 2010; Day & Buckley, 2013).

In Australia, studies have consistently shown a negative relationship between introduced soil nutrients and native species, mainly with respect to phosphorous and nitrogen, with the result that exotic species tend to dominate altered grasslands that carry an excess of these minerals (Morgan, 1998; Dorrough *et al.,* 2004; Prober *et al.,* 2005; McIntyre & Lavorel, 2007). The many years of fertiliser application during the cropping history of the experimental site thus represents a significant problem for reestablishment of native species. In this respect, the level of phosphorus enrichment provides a relatively stable indicator of the altered state of the soil. Phosphorus is comparatively stable in the soil matrix, and thus phosphorus levels change slowly if left to natural processes (Marrs 1985; McIntyre & Lavorel 2007). It is well known that super phosphate has been a common agricultural fertilizer in Australian farming practice, and this has significantly increased both phosphorus and nitrogen levels in the soil. The result of these mineral increases is both an increase in the exotic weed population and a boost to their individual competitiveness (Brereton & Bakhouse, 2003), which has now caused large alterations in the overall grassland flora construction (Grime, 1974). This suggests that any sensible restoration initiative will have to squarely address this issue of raised phosphorous levels.

A complicating factor for restoration attempts for native grassland areas, is that most Australian indigenous grassland species have short-lived seed banks, with the result that few native species will be able to regenerate from available soil seed bank sources without specific intervention (Morgan, 1998). This means that that removal of the standing population of native plants, due to agricultural practices rather than by natural cases such as periodic fire events, is likely to have been a state change factor which will not be reversible by natural mechanisms alone.

This chapter will describe the current state of the study sites to allow an assessment of the magnitude of the restoration processes that will be needed to support grassland reclamation. Specifically, it will: (i) document the differences of vegetation cover between a nearby area native grassland and the ex-cropland experimental site, (ii) examine and contrast the nature of the current soil seed banks in the two situations by numerating and identifying species which appear in the vegetation survey and in a controlled soil seed bank germination, (iii) assess the differences in the current level of soil nutrients and (iv) observe soil bulk density variables in reference and ex-cropland site grasslands. These steps are necessary in order that a systematic and informed research design can be developed.

2.3 The study sites [1]

2.3.1 Reference site

The reference site, located at the nearby Truganina cemetery, Victoria (37° 82' 39" S, 144° 72' 25" E), contains relatively undisturbed native grassland. It is a one hectare remnant of the western plains grassland, and is approximately 23 km west of Melbourne. The soil is a red-brown clay-loam, and is dominated by the tussock-forming grass *Themeda triandra* (Morgan, 1995). Its proximity to the ex-cropland site and its relatively

[1] Quoted rainfall and temperature data were taken from the RAAF (Royal Australian Air Force) Laverton station records, (37.88 °S, 144.76 °E),which is the nearest recording station for both sites, 22 km from ex-cropland and 13 km from reference site.

untouched history, make it an ideal reference site. However, notwithstanding this relatively isolated existence, there may have been incursions of exotic plant material from random animal and human interventions, and from natural processes such as wind and water-borne materials. This initial comparative vegetation survey will give some indication of this spontaneous invasion trend, which will help to demonstrate the aggressive nature of exotic weeds in this area.

2.3.2 Ex-cropland site

The ex-cropland [2] site is located within the Victorian Volcanic Plains grassland, near the Werribee River, northwest of Werribee, Victoria (37° 49' 20" S, 144°34'23" E) at an altitude of 66 m. The distance between both sites is about 17 km. Soils were classified as a clay loam, and the site was fertilized and sown with barley during 2011-2012, but was taken out of production during 2013. This means that, at the time of the vegetation survey, the land had been fallow for one year. This is a typical site situated within the 15,000 hectare "Western Grassland Reserves", and it is anticipated that results of this investigation will be transferrable throughout the region. Also, in selected cases of climate and soil similarity to these grassland sites, results may be of interest to overseas regions.

2.3.3 Climatic conditions

The average annual rainfall in 2014 for both sites was approximately 367 mm. The highest total monthly rainfall in 2014 was recorded in April (62 mm), while the lowest was recorded in January (8 mm). The mean minimum daily temperature was 5.9^{O}C (August) and the mean maximum daily temperature was 28^{O}C (January) (Figure 2.1). No major fire event had been noted in this area for some years. This selected year was not rare in terms of climate extremes, and 50-year records show that the 2014 median rainfall was also not unusual.

[2] Permission to access the study site was given by Victorian Government Western Grassland Reserve, to whom grateful thanks are recorded.

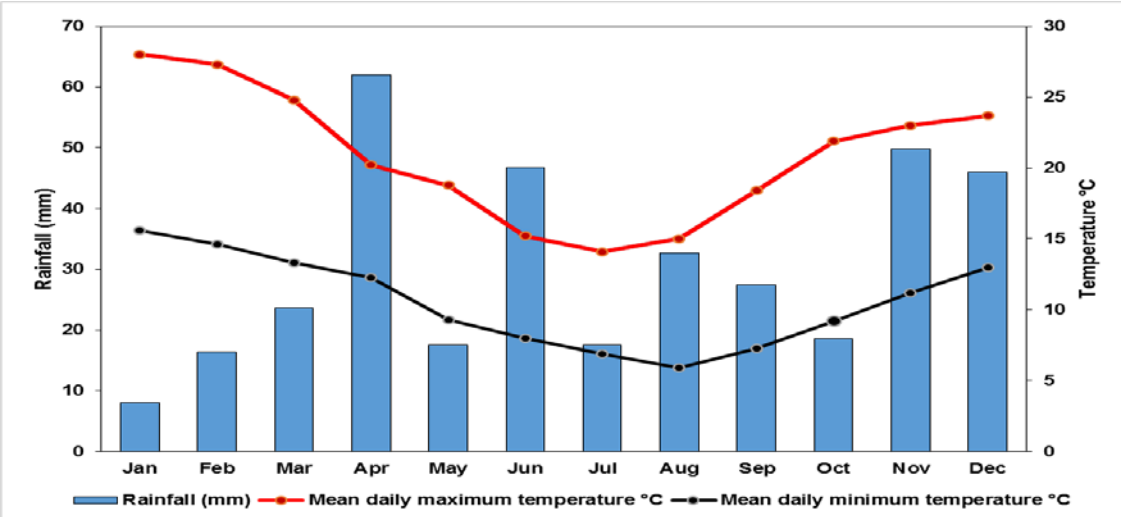

Figure 2.1 Total monthly rainfall, maximum and minimum temperatures for both sites recorded during 2014.

2.4 Above-ground vegetation survey

2.4.1 Sampling methods

Observational vegetation measurements were made in the experimental and the reference sites, the physical arrangements of which are described in more detail in Chapter 3. However, for the purposes of this vegetation assessment, each experimental and reference plot was divided into four 2.5 m x 2.5 m quadrats. Within each quadrat, vegetation cover was estimated by (i) recording of all vegetation presence and (ii) making an assessment of the surface density of each species by using the Braun-Blanquet cover-abundance scale (Wikum & Shanholtzer, 1978). To help systematise and record life-form cover estimates, the observed vegetation was classified as (i) perennial or annual, (ii) native or exotic and (iii) grass or 'other'.

2.4.2 Results

2.4.2.1 Reference site

The reference site was, as expected, dominated with native species, which were estimated to be 74.4% of the total plant cover. Of these, 41.2% were native grasses, and the amount of native 'other' (which includes herbs and forbs) was recorded as 33.3%. The highest proportion of native grass recorded was *Themeda triandra* grass, with 24%.

However, as presaged earlier, there was evidence of invasion of the site by exotic species, the percentage cover of which was 25.6%. Of those 18.2% and 7.3% were exotic grass and exotic others respectively (Table 2.1).

Table 2.1. The number and types of individual plants in the reference site, showing the percentage cover, for native species (native grass and native other) and exotic species (exotic grass and exotics others).

Categories	Number of species types	Total number of plants	Percentage Cover (%)
All natives individual	22	297.8	74.4
Native grass	5	164.6	41.2
Native other	17	133.2	33.3
All exotics individual	9	102.2	25.6
Exotic grass	4	73.0	18.2
Exotic others	5	29.3	7.3

In addition to this quantitative assessment of the number and type of species present in the site, a further examination of the status of the plants, together with their identification, life form, family and individual percentages was carried out. These results are given in Table 2.2.

Table 2.2. Status, identification, life form and percentage (%) of aboveground species that are present at reference sites.

Scientific name	Common name	Life cycle	Family	Percentage
Native grass				
Austrostipa bigeniculata	Stipa	Perennial	Poaceae	2.17
Poa sieberiana	Grey tussock-grass	Perennial	Poaceae	5.96
Rytidosperma spp.	Wallaby grasses	Perennial	Poaceae	4.07
Schoenus apogon	Common Bog-rush	Annual	Cyperaceae	2.17
Themeda triandra	Kangaroo grass	Perennial	Poaceae	26.02
Native other				
Asperula conferta	Common woodruff	Perennial forb	Rubiaceae	3.25
Brachyscome multifida	Rocky daisy	Perennial herb	Asteraceae	0.81
Calocephalus citreus	Lemon beauty-heads	Perennial herb	Asteraceae	2.71
Convolvulus angustissimus	Pink bind weed	Perennial herb	Convolvulaceae	1.08
Convolvulus erubescens	Australian bindweed	Perennial herb	Convolvulaceae	2.17
Crassula sieberiana	Australian Stonecrop	Annual/ perennial	Crassulaceae	0.27
Dianella admixta	Black anther	Perennial	Hemerocallidaceae	0.27
Geranium retrorsum	Common cranesbill	Perennial	Geraniaceae	4.34
Goodenia pinnatifida	Scrambled eggs	Annual/perennial herb	Goodeniaceae	1.63
Oxalis perennans	Oxalis	Perennial Forb	Oxalidaceae	2.71
Pimelea curviflora	Curved Rice-flower	Perennial shrub	Thymelaeaceae	2.17
Pimelea spinescens	Spiny Rice-flower	Perennial shrub	Thymelaeaceae	2.44
Plantago gaudichaudii	Narrow plantain	Annual/perennial	Plantaginaceae	5.42
Rutidosis leptorhynchoides	Button Wrinkle wort	Perennial herb	Asteraceae	0.27
Senecio quadridentatus	Cotton Fireweed	Annual/ Short live perennial	Asteraceae	0.27
Stackhousia subterranea	Grassland candles	Perennial herb	Celastraceae	2.44
Wahlenbergia spp.	Royal blue bill	Perennial herb	Campanulaceae	1.90
Exotic grass				
Conyza bonariensis *	Fleabane	Perennial	Asteraceae.	1.63
Lolium perenne *	Perennial Rye-grass	Perennial	Poaceae	5.96
Nassella trichotoma	Serrated Tussock	Perennial	Poaceae	0.54
Romulea rosea	Onion grass	Perennial herb	Iridaceae	9.76
Exotic others				
Arctotheca calendula	Capeweed	Perennial	Asteraceae	0.54
Hypochoeris radicata *	Flatweed	Perennial	Asteraceae.	0.27
Oxalis pes-caprae	African wood sorrel	Perennial	Oxalidaceae	1.08
Trifolium arvense	Rabbit foot clover	Annual	Fabaceae	2.44
Trifolium resupinatum *	Persian clover	Annual	Fabaceae	3.25

* These four species were identified at both reference and experimental sites (cross reference with Table 2.5)

2.4.2.2 Ex-cropland (experimental) site

The experimental site was, as again expected, dominated with exotic species, which were estimated to be 98.6% of the total plant cover. Of these, 43.9% were exotic grasses, and the exotic 'other' (which includes broadleaves) was recorded as 54.6%. There were very few native plants in the site, with a total of only 1.4%. Of these, 0.4% were native grasses and 1.0% were other native species. Clearly, the overwhelming dominance of exotic species indicates that significant disruption to the vegetative population has occurred, and observations are presented in Table 2.3.

Table 2.3. The number and types of individual plants in the experimental site, showing the percentage cover of native species (native grass and native other) and exotic species (exotic grass and exotics others).

Categories	Number of species types	Total number of plants	Percentage Cover (%)
All natives individual	2	52.0	1.4
Native grass	1	14.2	0.4
Native other	1	37.7	1.0
All exotics individual	25	3548.0	98.6
Exotic grass	4	1581.2	43.9
Exotic others	21	1966.8	54.6

A comparison of Table 2.1 and Table 2.3 gives a clear quantitative indication of the effect on vegetation caused by extensive land tillage and fertilization. To further explicate this difference, Table 2.4 presents the status, identification and life form of aboveground species that are present at the experimental site.

Table 2.4. Status, identification and life form of aboveground species that are present at ex-cropland sites.

Scientific name	Common name	Life cycle	Family	Percentage
Native grass				
Chloris truncata	Windmill grass	Annual	Poaceae	0.51
Native other				
Euchiton collinus	Euchiton	Perennial/ Biennial	Asteraceae	1.37
Exotic grass				
Bromus catharticus	Brome/ prairie grass	Annual/ perennial	Poaceae	15.07
Conyza bonariensis *	Fleabane	Perennial	Asteraceae.	6.27
Lolium perenne *	Perennial Rye-grass	Perennial	Poaceae	12.25
Triticum aestivum	Wheat	Annual	Poaceae	8.38
Exotic others				
Brassica rapa	Turnip mustard	Annual	Brassicaceae	0.66
Cirsium vulgaris	Spear thistle	Perennial	Asteraceae.	0.23
Chamaesyce drummondii	Caustic weeds	Annual	Euphorbiaceae	0.23
Galenia pubecum	Galenia	Perennial	Aizoaceae	4.05
*Hypochoeris radicata**	Flatweed	Perennial	Asteraceae.	4.05
Lactuca virosa	Wild lettuce	Annual	Asteraceae.	0.03
Lepidium graminifolium	Peppercress	Perennial	Brassicaceae	9.66
Lytherum hysophilum	Hyssop losestrife	Perennial/ Biennial	Lythraceae	0.71
Malva parviflora	Mallow	Perennial	Malvaceae	0.74
Medicago sativa	Alfalfa	Annual	Fabaceae	0.09
Medicago truncatula	Barrel medic	Annual	Fabaceae	3.79
Picris echioides	Oxtongue	Annual/perennial	Asteraceae.	1.60
Polygonum aviculare	Wire weed	Annual	Polygonaceae	4.53
Pseudognaphalium luteoalbum	Jersey Cudweed	Annual	Asteraceae	1.60
Rumimx pulcher	Fiddle Dock	Perennial	Polygonaceae	6.81
Silibium marianum	Vargeted thistle	Biennial	Asteraceae.	0.11
Solanum nigrum	Black-berry nightshade	perennial	Solanaceae	0.34
Sonchus asper	Spiny sow thistle	Annual	Asteraceae	1.03
Sonchus oleraceus	Milk Thistle	Annual	Asteraceae.	0.57
Taraxacum officinale	Dandelion	Perennial	Asteraceae	4.84
Trifolium resupinatum *	Persian clover	Annual	Fabaceae	9.60

* These four species were found to exist at both sites (cross reference with Table 2.3)

2.4.3 Discussion

The significant degradation, in terms of the native vegetation cover, of the experiment study area is not a surprise, and has resulted mainly from the intensive conversion of the grassland to cropland (Yates & Hobbs, 2000; Kirkpatrick *et al.,* 2005). Of the five native grasses present in the reference site, none were seen in the experimental site. Only one native grass species was observed in the experimental site, which suggests that in the one year of fallow conditions at the experimental site, this was the only species that was reintroduced by natural means, and which could establish in the new soil conditions. There

were 17 ‘native other’ species found in the reference site, but again only one such species was observed in the experimental site. Interestingly, neither of these two native species in the experimental site were found in the reference site, strongly suggesting that any natural seeding mechanisms of the original species may have been thwarted by the changed soil conditions and by the aggressive nature of the exotic population. This implies that in any restoration attempt, new seed stocks will be needed after significant clearing and soil reclamation attempts have been made. The seedbank analysis will be used to affirm that the natural seedbank has been exhausted, and that reestablishment of native species will only be possible by introduction of selected species.

Also of interest was that of the four exotic grass species evidenced in the reference site and the experimental site, only two were common. It would appear that competition between exotic grasses also occurs, and there is a concentration of the fittest species for the new soil conditions which would make reestablishment of native grasses even more difficult. There were five exotic others in the reference site, compared with 21 species in the ex-cropland site, with only two common species. The three exotic others that initially were able to become established in the reference site, have clearly been shown to not flourish in the new conditions or compete with the more aggressive exotic species.

Whilst exotic species arrived with the first phases of European colonization of Australia, in the new environment their numbers have continually increased. In many circumstances, these introduced species, due to their aggressive nature, have significantly modified the natural environment by competing and displacing native plant species and creating conditions more suited to their own establishment in Australian grasslands (Mack & Lonsdale, 2001). This was shown by the results of the vegetation survey of the reference site, where nine species had already established a foothold. It is also known that exotic species generally produce very large seed numbers, they have mechanisms for their wide dispersal and, in the case of exotic broadleaf species, they can shadow out a considerable area, thus preventing other species’ establishment. By these means, exotic plants can

overwhelm the establishment of native species by numerical competition and by physically blocking their reseeding and germination (Donath *et al.,* 2007).

2.5 Identification of the composition of soil seed bank in experimental and reference sites

2.5.1 Sampling methods

In our attempts to establish a clear understanding of the current state of the ex-croplands site which are the target for restoration, an accurate description of the seedbank status was deemed appropriate. Of interest was to determine the range of exotic species present, particularly those with the potential to reside for some years in the seedbank, and also to see if there were any native species which had survived agricultural activities.

The general composition of the soil seed bank was assessed by germinating the soil samples collected from the experimental site and the reference site at Truganina Cemetery. The objective of this part of the study was not only to compare the seed bank composition at the two sites, but to provide a baseline for assessing the effect of our restoration treatments. Seeds were germinated under conditions of (i) no pre-treatment and (ii) a pre-treatment which involved heating the seedbank sample to 80^{O}C, and watering with smoke water. This pre-treatment was incorporated since stimulation of germination of some native plants is, during natural conditions, by fire events.

On 2^{nd}-4^{th} June 2014, a total of 80 soil samples were collected from the study and reference sites. These soil samples were taken from all four plots in the reference site and from eight selected plots in the experimental site. Two plots were chosen from each block, ensuring that the 'green waste' and 'control plots' for the next phase of the work (Chapter 3) were selected. A composite of five soil samples were collected within each 5 m x 5 m plot using 100 mm x 100 mm corer. From all five samples which were taken within each plot, surface litter was included as this layer is potentially an important seed source (Rotundo & Aguiar, 2005). Collected soil samples were placed in labelled plastic zip bags, kept cool, and transferred to the Federation University Australia, Mount Helen Campus,

glasshouse, Mount Helen, where the soil was passed through a 4 mm diameter sieve to remove unwanted parts of the litter and stones.

The soil seed bank germination commenced on 2nd August 2014. The samples were divided into two equal portions. To facilitate the application of the heat and smoke pre-treatment, 40 soil samples were placed in an aluminium foil punnet, then heated to 80^{O}C in an oven for two minutes. All soil samples were then spread over 2 cm layer of sterilised sand in a plastic punnet (13 cm x 8 cm x 5 cm) lined with a paper towel, and placed in a glasshouse at approximately 25^{O}C. Punnets were individually labelled, and placed in large plastic tray (44 cm x 28 cm x 5.5 cm). To minimise disturbance of the buried seeds, the trays were watered from below the base on an as-required basis. The first dose of smoke water was applied on pre-heated soil samples by using a 1% concentration of smoke water (100 mL/m^2). The second and third doses of smoke water were applied during the second and third weeks after the study had commenced.

The non-treatment samples were not heat-treated, and were watered only with rain water. The physical provision of the germination apparatus for these samples was as described above, and the following procedures were applied to both sets of experiments.

The position of germination trays were changed every two weeks to avoid confounding variations from differences in light exposure. Emerging seedlings were identified, counted and removed periodically. Unidentified seedlings were transplanted in individual pots and watered regularly to grow until identification of species was possible (Diaz-Villa *et al.,* 2003), and emergent seedlings were maintained and checked over a 5-6 month period (Baskin & Baskin, 1998).

2.5.2 Results

2.5.2.1 Reference site

In Table, 2.5a and Table 2.5b, a quantitative estimate of the emergent species at the reference site are given. In Table 2.5a, treatment with heat and smoke water has been carried out, and in Table 2.5b, only rain water was used. As can be seen, a considerable

difference in the results has occurred, emphasising the importance of heat and smoke on the germination of native seeds and the negative effect of heat on the germination of exotic seeds. These results will inform the experimental restoration treatments in the next Chapter.

Table 2.5a. Total number of emergence seeds after application of pre-treatments (heat and smoke), number of seeds emerging per square metre, and percentage of seeds emerging from reference site.

Categories	Total number of seeds emergence	Total number of seeds emergence (m^{-2})	Percentage (%)
All native individuals	91.0	218.8	54.2
All exotics individual	77.0	185.1	45.8
Native grass	20.0	48.1	11.9
Native other	71.0	170.7	42.3
Exotic grass	67.0	161.1	39.9
Exotic other	10.0	24.0	6.0

Table 2.5b. Total number of emergence seeds without pre-treatment (water only), number of seeds emerging per square metre, and percentage of seeds emerging from reference sites.

Categories	Total number of seeds emergence	Total number of seeds emergence (m^{-2})	Percentage (%)
All native individuals	76.0	182.7	74.5
All exotics individual	26.0	62.5	25.5
Native grass	33.0	79.3	32.4
Native other	43.0	103.4	42.2
Exotic grass	24.0	57.7	23.5
Exotic other	2.0	4.8	2.0

A more detailed examination of the seedbank, in terms of status of emergent plants, identification life form and percentages for treatments with water only and heat and smoke water, are given in Table 2.6.

Table 2.6. Soil seedbank composition, status, identification, life form and total number of seeds emergence per metre square of each species emergence using pre-treatments (H & S) and without pre-treatment (W) of emergence species from soil seed bank samples of reference site. The symbols C_3, C_4 and CAM represent the plant photosynthetic pathways.

Scientific name	Common name	Life cycle	Family	C3/C4 CAM	H&S (m^2)	W (m^2)
Native grass						
Rytidosperma spp.	Wallaby grasses	Perennial	Poaceae	C3	24	46
Themeda triandra	Kangaroo grass	Perennial	Poaceae	C4	24	34
Native other						
Convolvulus erubescens *****	Australian bindweed	perennial herb	Convolvulaceae	C3	5	0
Crassula sieberiana	Australian stonecrop	Annual/ perennial	Crassulaceae	CAM	26	24
Einadia nutans	Climbing saltbush	Perennial	Chenopodiaceae	C4	41	14
Oxalis perennans	Oxalis	Perennial Forb	Oxalidaceae	C3	99	65
Exotic grass						
Lolium perenne *****	Perennial rye-grass	Perennial	Poaceae	C3	12	0
Nassella trichotoma *****	Serrated tussock	Perennial	Poacaciae	C3	149	58
Exotic other						
Cirsium vulgaris *****	Spear thistle	Perennial	Asteraceae	C3	0	5
Hypochaeris radicata *****	Flatweed	Perennial	Asteraceae	C3	19	0
Medicago truncatula *****	Barrel medic	Annual	Fabaceae	C3	5	0

*****These six species were present at reference and experimental sites (Cross reference with Table 2.8).

2.5.2.2 Experimental site

In a parallel investigation to that presented for the reference site, a quantitative estimation of the emergent species at the experiment site is presented in Table 2.7a and Table 2.7b. In Table 2.7a, treatment with heat and smoke water has been carried out, and in Table 2.7b, only rain water was used. As can be seen, a considerable difference in the results has occurred, emphasising the importance of heat and smoke on the germination of native and exotic seeds.

Table 2.7a. Total number of emergence seeds after application of pre-treatments (heat and smoke), number of seeds emergence per square metre, percentage of seeds emergence from ex-cropland site.

Categories	Total number of seeds emergence	Total number of seeds emergence (m^{-2})	Percentage (%)
All native individuals	2.0	4.8	0.2
All exotics individual	963.0	2314.9	99.8
Native grass	0.0	0.0	0.0
Native other	2.0	4.8	0.2
Exotic grass	471.0	1132.2	48.8
Exotic other	492.0	1182.7	51.0

Table 2.7b. Total number of emergence seeds without application of heat and smoke (water only), number of seeds emergence per square metre, percentage of seeds emergence from ex-cropland site,

Categories	Total number of seeds emergence	Total number of seeds emergence (m^{-2})	Percentage (%)
All native individuals	0.0	0.0	0.0
All exotics individual	613.0	1473.6	100.0
Native grass	0.0	0.0	0.0
Native other	0.0	0.0	0.0
Exotic grass	297.0	713.9	48.5
Exotic other	316.0	759.6	51.5

A more detailed examination of the seedbank, in terms of status of emergent plants, identification life form and percentages for treatments with water only and heat and smoke water, is given in Table 2.8.

Table 2.8. Soil seedbank composition, status, identification, life form and total number of seeds emergence per metre square of each of each species emergence using pre-treatments (H & S) and without pre-treatment (W) of emergent species from soil seed bank samples of the ex-cropland site. The symbols C3, C4 and CAM represent the plant photosynthetic pathways.

Scientific name	Common name	Life cycle	Family	C3/C4 CAM	H& S (m^2)	W (m^2)
Native grass						
Nil						
Native other						
Convolvulus erubescens *	Australian bindweed	perennial herb	Convolvulaceae	C3	5	0
Exotic grass						
Avena sativa	Oats	Annual	Poaceae	C3	310	151
Conyza bonariensis	Fleabane	Perennial	Asteraceae.	C3	5	29
Lolium perenne *	Perennial rye-grass	Perennial	Poaceae	C3	356	248
Nassella trichotoma *	Serrated tussock	Perennial	Poacaciae	C3	466	315
Exotic others						
Chenopodium oahuense	Hawaiian goosefoot	Perennial	Chenopodiaceae	C3	60	70
Cirsium vulgaris *	Spear thistle	Perennial	Asteraceae	C3	166	77
*Hypochaeris radicata**	Flatweed	Perennial	Asteraceae	C3	233	118
Malva parviflora	Mallow	Annual/ perennial herb	Malvaceae	C3	68	43
Medicago truncatula *	Barrel medic	Annual	Fabaceae	C3	14	12
Polygonum aviculare	Wire weed	Annual	Polygonaceae	C3	115	77
Sonchus oleraceus	Sow thistle	Annual	Asteraceae	C3	154	120
Taraxacum officinale	Dandelion	Perennial	Asteraceae	C3	216	108
Trifolium resupinatum	Persian clover	Annual	Fabaceae	C3	156	106

* These seven species were found at both sites (cross reference with Table 2.7)

Clearly, the numbers of native seeds contained in the seedbank of the experimental site are negligible, even when heat and smoke water treatments were used. This was expected given the history of the land usage. In addition, it was seen that the pattern of seed emergence in the reference site was substantially different to that present in the experimental site, which further reinforces the complex relationships between the soil and the available seed sources.

2.5.3 Discussion

Interestingly, in this study there was one native species and five exotic species which were present at both ex-cropland and reference sites (Table 2.6 and Table 2.8). These similarities may have resulted because of introduction of seeds of these species caused by animal ingress, from carriage of light seed by prevailing winds, or possibly from human intervention through unclean machinery or footwear.

Whilst there were exotic grasses and exotic others emergent at the reference site, there were also emergent native seedlings (native grass and native others). Two perennial grasses and four perennial herbs and forbs were observed (Table 2.6), and it is encouraging that the high numbers of emerging native grass and forb seedlings indicate that the soil seed bank can be a key factor in a restoration program where only moderate disruption to the soil has occurred (Major & Pyott, 1966; Kiss *et al.,* 2018). As expected, there were substantial differences in the mean number of native others (native herb and forb), exotic grasses and exotic others seedlings emergent from the soil seed bank under heat-smoke sub-treatments rather than with water only. Only one broadleaf weed (*Cirsium vulgaris*) species did not respond to heat-smoke sub-treatments. However, somewhat surprisingly there was no effect of heat-smoke sub-treatment on the mean number of native grasses (Table 2.6). These findings suggest that, at least, emergence of some native perennial herb and forbs (*Convolvulus erubescens*) from the soil seed banks can be enhanced by using heat-smoke methods.

The failure of native seed emergence at the ex-cropland site is probably caused by two factors; first, continual tillage of the soil will have exposed any residual native seeds in the original seedbank leading to their removal, and second, replacement from natural sources would not have been possible because seeding from mature plants would have been prevented by competition with non-native species (Hobbs & Harris, 2001). It is reported that establishment of native seeds in disturbed sites is limited to opportunities where chance seed availability and conditions for germination coincide with the absence of aggressive exotic competitors (Hurtt & Pacala, 1995). Indeed, the study of Dobson (2005) has shown that the overall relative density of native species is typically lowest in areas with a high exotic weed invasion level. The results of this current study have shown that there was considerable variation in the mean number of exotic weed seedlings (both exotic grass and exotic others) that emerged from the soil seed bank of the ex-cropland site in response to the heat-smoke sub-treatments applied (Table 2.8). This is in contrast to heat-smoke sub-treatments of soil seed banks which have previously shown significant increase in emergent seedlings (Read *et al,* .2000). There was no native species emergent at the ex-cropland site with water sub-treatment, and there was only one native species counted when using heat-smoke sub-treatment (Table 2.8).

In this respect, Bellairs and Bell's (1993) study has indicated that perennial grasses and forbs showed positive responses to heat-smoke sub-treatments. This improved germination rate and seedling emergence following such sub-treatment has important implications for effective restoration and rehabilitation of grasslands. It is also reported that the seed banks may be important in the long-term survival of individual species, as well as plant communities. However, as a reminder of the complex nature of this area, (i) not all species noted in the vegetation community are represented in the seed bank, and (ii) some species present in the seed bank do not appear in the existing vegetation survey, an observation noted also by Baskin and Baskin (1998).

2.6 Current soil nutrient conditions in reference and ex-cropland sites

2.6.1 Sampling methods

Pre-treatment soil samples were taken on the 2nd -3rd June 2014, from all plots in the four block replication of the ex-cropland site. The samples were collected from five different points inside each plot with a (10 cm x 10 cm) corer. A similar sampling method was used for the reference site, which included the four 5 m x 5 m plots during the gathering of soil samples. Soil samples were placed in labelled plastic zip bags, then transferred to the Federation University Australia, Mount Helen Campus, glasshouse. All soil samples were air-dried and stored under greenhouse conditions until used. The five samples from each individual plot were mixed together to get a composite sample to increase the homogeneity of soil samples and to reduce the variation inside each treatment. Soil samples were passed through 2mm mesh then a weighed amount of 200 gm of soil placed into a zip plastic bag stored at approximately 25^{O}C. These were then sent to the Soil and Plant Analysis Laboratory (CSBP) for analysis of ammonium nitrogen, nitrate nitrogen, phosphorus (Colwell method), potassium (Colwell method) (Colwell, 1963), organic carbon and pH level.

Chemical analysis readings were obtained for each of 32 experimental plots (which excluded the four scalping plots since this was not of interest) and the four Cemetery plots which represented the reference site. Prior to statistical analysis, soil chemical values of each plot were logarithmically transformed so that the residual variation in the analyses did not systematically change as the mean changed. One plot was deleted from the pH (H_2O) as it had uncharacteristic measured values which may have arisen from a specific event. The soil colour and structure of this plot were observed to be visually different to all other plots in the study, suggesting that some interfering intervention had occurred.

The two sites were compared using a Wald F test, suitable for a mixed model analysis. The analyses were carried out using the REML directive of GenStat 16 (Payne, 2013).

2.6.2 Results

The results of the analysis of current soil nutrient levels and soil pH of the reference and ex-cropland sites are given in Table 2.9.

Table 2.9. The value of soil nutrient concentrations of reference and ex-cropland sites Values are predicted means, on the transformed scale.

Soil Nutrient	Reference site	Ex-cropland site
Ammonium Nitrogen (ppm)	7.5	10.8
Nitrate Nitrogen (ppm)	6.6	8.90
Phosphorus (Colwell) (ppm)	8.1	87.5
Organic Carbon (%)	2.33	3.35
pH (H_2O)	6.05	6.11
Potassium (Colwell) (ppm)	352	564

Table 2.10. Standard error of differences, degree of freedom and p value between reference and ex-cropland sites.

Soil Nutrient	*sed	Numerator degrees of freedom	p value
Ammonium Nitrogen (ppm)	2.330	3.6	0.240
Nitrate Nitrogen (ppm)	0.261	4.8	0.640
Phosphorus (Colwell) (ppm)	0.038	5.6	**0.001**
Organic Carbon (%)	0.253	4.8	**0.011**
pH (H_2O)	0.133	4.1	0.680
Potassium (Colwell) (ppm)	0.091	3.8	0.091

*** Standard error of differences**

2.6.3 Discussion

The current condition of soil nutrient levels at the experiment and reference sites demonstrate that the level of soil phosphorus and soil organic carbon were significantly higher at the experiment site than at the reference site. However, the value of ammonium nitrogen, nitrate nitrogen, potassium were not significantly higher at the experiment site than the reference site at the 0.5 level. There was no significant differences on soil pH level (Table 2.9 and Table 2.10). It is assumed that the high value of soil nutrient at the experiment site is related to agriculture practice activities over the last 60 years, which is of interest because it has been observed that the species richness of native forbs declines with increasing phosphorous levels (Dorrough *et al.,* 2004; McIntyre & Lavorel, 2007). The reference site at Cemetery Truganina was not subjected to any changes through agricultural practices, thus soil nutrient levels obtained here can be used as a baseline to

assess the effect of proposed treatments for restoration of native grassland at ex-cropland sites.

2.7 Soil bulk density

2.7.1 Methods

To assess the value of soil bulk density in the reference and ex-cropland sites, soil samples were collected at 4th Jun 2014 using a cylindrical steel core cutter approximately 7.5 cm long and 7.5 cm diameter. Three samples were randomly collected from each 5 m x 5 m plot for each of the four replications. The corer was inserted into the topsoil by placing a piece of wood over the cylinder body, and then hammering it to the required depth. The cylindrical corer with the soil sample was gently lifted to collect the soil, which was placed in plastic zip bag and kept cool. The collected soil samples were immediately transferred to Federation University Australia, Mount Helen Campus, laboratories, then placed in a 5^{O}C fridge to keep the samples fresh until used.

The soil samples were placed in an aluminium foil punnet, 19.5 cm x 80 cm x 5.5 cm, and weighed'. These weighed samples were placed in oven at 80^{O}C for 48 hours to dry, then the dried samples were again weighed. The soil bulk density was calculated by the formula below:

$$\textit{Bulk Density (g/cm}^3\textit{) = Mass of dry soil (g) ÷ Volume of corer (cm}^3\textit{)}$$

Similar to the soil nutrient readings, the statistical analysis of soil bulk density were obtained for the reference (n=4) and ex-cropland (n=36) sites. These reference and ex-cropland site means were compared using a mixed model restricted maximum likelihood (REML), with a fixed effect for site (reference or ex-cropland). The 40 plots were the units of analysis. Results at the two sites were compared using a Wald F test, which is suitable for a mixed model analysis. The analyses were carried out using the REML directive of GenStat 16 (Payne, 2013).

2.7.2 Results

Results indicated that there was significant differences in soil bulk density between the reference and ex-cropland sites as shown in Table 2.11. The soil bulk density was higher at the reference site than the ex-cropland site, where the mean values were 1.19 g/ cm^3 at the reference site and 1.01 g/ cm^3 at the ex-cropland site (Table 2.11).

Table 2.11. Effects of treatment of site on soil bulk density. Values are predicted means, on the transformed scale. Standard error of differences, degrees of freedom and p value indicating the difference between reference and ex-cropland sites are given.

Variable	Reference site	Ex-cropland site	*sed	Numerator degrees of freedom	P value
Soil bulk density (g/cm^3)	1.19	1.01	0.035	22.4	**0.001**

***Standard error of difference**

2.7.3 Discussion

The mean value of soil bulk density at the reference site was higher than at the ex-cropland site, with the difference being shown to be significant. These values were 1.19 g/cm^3 and 1.01 g/cm^3 respectively. These results were not expected because of the history of agricultural activity practices at the experimental site, where heavy machinery was used for crop production. Usually, the soil bulk density under undisturbed native vegetation is less than that under cropping, but in the current study the results clearly demonstrate an opposite trend. However, there are several factors which effect the value of soil bulk density. For instance, a higher value of organic carbon can result in a smaller bulk density in some cases, because organic carbon has a lower particle density than mineral particles (Logsdon & Karlen, 2004). In our case, the measured value of organic matter in the experiment site soil was much higher than at the reference site. Also, soil bulk density usually increases with soil depth since subsurface layers are more compacted and have less organic matter, less aggregation, and less root penetration compared to surface layers. These subsurface layers therefore contain less pore space. In addition, soil texture is another important factor which affects the soil bulk density, and the effect of the presence of sand content on soil bulk density is important since clay soils

tend to have lower bulk densities and higher porosities than sandy soils (Chaudhari *et al.,* 2013). The agricultural practice such as tillage prior to planting temporarily decreases bulk density on the surface but increases at the depth of tillage. The soil bulk density of both sites were in the ideal range for plant growth in sandy clay loam and clay loam soils.

2.8 Synthesis

The results of the vegetation cover and current condition of soil nutrients at reference and ex-cropland sites suggest a strong relationship between the presenting species and levels of soil nutrients. In the reference site, where the phosphorus was found to be at 8.1 ppm, the percentage of native species was 74.4% and exotic species was 25.6%. By sharp contrast, in the ex-cropland the phosphorus level was 87.5 ppm, and the percentage of native species was 1.4% and exotic species 98.6% (Table 2.1, Table 2.3 and Table 2.9). Other studies have also consistently shown a negative relationship between introduced soil nutrients and native species, mainly with respect to phosphorous and nitrogen, with the result that exotic species tend to dominate altered grasslands that carry an excess of these minerals (Morgan, 1998; Dorrough *et al.,* 2004; Prober *et al.,* 2005; McIntyre & Lavorel, 2007). The many years of fertiliser application during the cropping history of the experimental site thus represents a significant problem for reestablishment of native species, particularly in terms of raised phosphorous and nitrogen levels. This study only examined the biophysical environment of one site in each condition, and hence has not been replicated. However, the two sites were good representatives of the condition states, and the study serves to highlight the broad differences between the grassland conditions, and the thresholds that must be overcome if an ecosystem resembling the remnant condition is to be restored.

With respect to observed vegetation cover and soil seed bank germination at both reference and ex-cropland sites, the number of species that appeared in the vegetation cover assessment were 31 (reference) and 27 (ex-cropland) species, while the number of species from soil seed bank germination were 11 species at both sites. This low

correspondence between the composition of the above-ground vegetation and the seed bank has been commonly reported in seed bank studies (e.g. Williams, 1984; Wisheu & Keddy, 1991; Lunt, 1997). In addition, our results showed that only eight species were similar at each site, which indicates the level of influence that cropping activities have on the soil's propensity to sustain vegetation growth.

The soil bulk density found in undisturbed native vegetation conditions is less than that for areas which have been exposed to cropping activities. There is a negative relationship between organic carbon and soil bulk density values, where a higher value of organic carbon can cause a lower bulk density, because organic carbon has a lower particle density than mineral particles (Curtis, 1964; Logsdon & Karlen, 2004). This relationship was also demonstrated by the study of Chaudhari *et al*., (2013), which showed the strong relationship between soil bulk density and soil macro-micro nutrients. Their work also showed a strong negative correlation of bulk density with available total nitrogen, phosphorus and potassium levels.

In conclusion, the data obtained by our investigations of the vegetation cover, soil seed bank germination, current soil nutrient conditions and the level of soil bulk density of the reference and ex-cropland sites, shows that the ex-cropland site is currently harmfully effected in relation to its potential to be restored to its original ecological condition. There is a high number of aggressive exotic weeds, a lack of native species, very high nutrient levels especially for phosphorus and nitrogen, and an absence of organic carbon due to conversion of grassland into cultivated land. Each of these conditions have been shown to mitigate against the introduction and sustainability of native species.

Rectifying these issues will be addressed in the following chapters. Approaches will use some unique restoration techniques such as the loading of hot and cold green waste on to the site, and the introduction of the commercially available phosphorus sequestering material *Phoslock®,* into the soil. The effects of current methods such as soil scalping and sugar treatments will also be evaluated. Included in the management techniques for

achieving restoration of the ex-cropland site will be introduced grassland seeding of some native grass species, and the use of plant species such as *Ptilotus* and *Lupin* to reduce phosphorus levels in the soil.

Chapter 3. Field experiments: Investigating and evaluating alternative techniques for restoring abandoned crop land

3.1 Introduction

Chapters 1 and 2 have contextualised the focus of this research, and have established the current nature of abandoned crop land, comparing it with conditions in a native site. In addition, the formal research question underpinning this thesis was developed, and this question will be linked to (i) for restoration approaches and (ii) a number of testable research hypotheses as outlined in the specific project details. These hypotheses have been designed to address key restoration aspects arising from historical and disruptive disturbances to the land. This Chapter introduces and describes the series of field experiments which were introduced in an attempt to address these historical disturbances in the land, and to restore it to its original condition.

It is noted that, in general, the ecological barriers to restoring ex-cropping land to native grassland conditions include having compacted soils that now contain high levels of nitrogen and phosphorus, together with the existence of an extensive seed bank of aggressive exotic weeds (Török *et al.,* 2012). These factors, singly and together, give exotic species a competitive advantage over native species, an advantage which can seriously hinder the restoration of abandoned cropland to its original state (Brereton & Bakhouse, 2003). The current study area is no exception to this significant and widespread problem, and an underlying aim of this investigation is to both assess the biotic and abiotic barriers (sections 3.2 and 3.3) to grassland restoration, and investigate possible techniques (sections 3.4 and 3.5) for overcoming these barriers.

The outcomes of the interventions will be assessed in section 3.6 in terms of (i) the change in vegetation cover observed each season after various treatments (3.6.1), and (ii) the change in the soil seedbank composition (3.6.2). Additionally, because effects of cropping have altered the physical and chemical properties of the soil, any amelioration of these

effects by the interventions are presented in section 3.6.3. Section 3.7 outlines the management implications which arise from a consideration of these interventions.

3.2 Biotic barriers

Exotic weeds are a significant biotic barrier to grassland restoration, affecting the habitat conditions for native grass species by aggressively competing for nutrients, space, light and water (Grice, 2004). In addition, weeds can also cause changes to soil microbial activity, and this can affect native grassland restoration on former agricultural land due to decreased organic material storage through decomposition, and will lead to interrupted nutrient cycling (Elser *et al*., 2007). Chapter 2 provided a comprehensive census of the overwhelming preponderance of exotic species currently existing in the land as germinated plants and as a seed bank, and an essential part of the restoration procedures will be to address this issue.

3.3 Abiotic barriers

There are also well known abiotic factors which also represent barriers to restoration of previous grassland conditions. The process of conversion of grassland into cultivated land causes numerous modifications to the physical properties of the soil in parallel to the interference with bacterial and mycorrhizae processes necessary for healthy support of flora and fauna. In particular, conventional cultivation harms soil horizons, increases soil erosion, changes nutrient accessibility, compacts soil, and decreases the formation of soil macro aggregates (Tisdall & Oades, 1982; Tisdall, 1994; Six *et al.,* 2000; McLauchlan, 2006). In addition, native Australian soils have generally been characterised as nutrient poor, and native species have adapted to these low nutrient conditions which are characterised by low phosphorus, nitrogen, minor nutrients and organic matter (Leeper, 1970). With the advent of European farming in Australia, there was a clear need for the extensive use of chemical fertilisers over the period of crop farming of the land to suit exotic crop species. As a consequence, in these temperate Australian conditions which have been used for farming, exotic grasses proliferate in these heavily fertilised areas of

native grassland (Lunt, 1990). It has been observed, for example, that the historical addition of high-phosphorus fertilisers favours the emergence of cool season annual grasses such as *Hordeum* spp. (Groves *et al.,* 2003), and thus exotic pasture grasses and exotic weed species tend to dominate grasslands that evidence these elevated phosphorous levels (Morgan,1998; Dorrough *et al*., 2004; Prober *et al.,* 2005; Dorrough *et al.,* 2011). It is the presence of these high mineral and nutrient levels in the soil of ex-arable land that constitute the abiotic barriers to restoration of native species, and steps need to be taken to redress these levels in order to restore the land to its original condition.

3.4 Restoration techniques

A recent novel method of Australian grassland rehabilitation is the removal of both the nutrient rich topsoil and the resident seedbank by scalping off the topsoil, followed by direct seeding of the exposed earth with native grassland species (Gibson-Roy *et al.,* 2010 a). Although this method, generally termed ‘scalping’, has been shown to be effective, it involves high labour costs, and also creates a large amount of nutrient and mineral rich soil that needs to be relocated. This can, of course, be conveniently spread over other areas of cropping land, which is a desirable outcome due to its high nitrogen and phosphorus levels, but it is clear that significant transport costs are involved. Also, whilst scalping would seem to be a suitable management technique to facilitate native grassland restoration in nutrient-enriched areas, there are some practical problems such as the occurrence of rocky patches of soil, the need to preserve valuable remnant vegetation and the physical hurdle of inaccessible areas; all of which make it important to investigate alternative restoration methods which allow the retention of topsoil. An added advantage of *in situ* remediation is that topsoil is a potentially valuable resource, and also the cost and physical difficulty of soil removal are mitigated.

Alternative restoration techniques involve focus on specific elements of concern in the soil. For example, the addition of carbon (in the form of sugar, wood chips or sawdust) to soil has been investigated as means to increase microbial populations, which will, in turn,

reduce soil nitrogen (Jonasson *et al.,* 1996; Eschen *et al.,* 2007). In particular, because the addition of sucrose to soil can rapidly stimulate microbial activity, probably mostly of gram-negative bacteria (Nottingham *et al.,* 2009), sugar applications have frequently been used to effectively to decrease above-ground biomass production and reduce the competitive ability of invasive plants, which tend to proliferate in soils with high nutrient levels (Morghan & Seastedt, 1999; Eschen *et al*., 2007).

Another technique to suppress weeds is the use of large amounts of plant residue and mulches placed on the soil surface (Hutchinson & McGriffen, 2000; Teasdale & Mohler, 2000; Chalker-Scott, 2007). A potential source of cheap carbon substrate for this purpose is the large-particle material (termed 'oversize mulch') which remains after composting of urban curbside green organic waste. Mulches which are commonly applied in urban/natural areas provide aesthetic, economic and environmental benefits to landscapes, but these large scale composting mulch piles, are a little unsightly and are not suitable for urban use, However, in industrial applications, this mulch can reach temperatures exceeding 60^{O}C (Mathur, 1998), and it is anticipated that such temperatures could significantly reduce the resident seedbank in abandoned crop land soil, limiting competition for rehabilitation of indigenous grassland when treated with direct seeding.

A novel method which will be introduced in an attempt to reduce soil phosphorus, is using a commercial product called Phoslock® (Geurts *et al.,* 2011). This product was originally designed to reduce the concentration of phosphorus in lake water (Douglas *et al.,* 1999; Robb *et al.,* 2003; Reitzel *et al.,* 2013), but there are suggestions that it has potential for use in soils. Phoslock® is a bentonite clay that has been modified by ion exchange, with lanthanum (La) replacing some hydrogen atoms in the clay structure (Afsar & Groves, 2009). The lanthanum in the clay structure binds strongly with phosphate when in solution, creating a highly insoluble complex which prevents phosphorous being taken up by plants.

Informed by these previous observations, the investigations described in this chapter have been designed to compare four methods of overcoming biotic and abiotic barriers to

restoration initiatives, and to allow the re-establishment of native grassland species. These experiments are: (1) using urban green waste (oversize) as a compost to heat up and physically destroy the resident exotic seed bank; (2) the use of carbon (in the form of cold green waste and sugar) to stimulate microbial activity and temporarily reduce soil nitrogen; (3) the application of Phoslock® to the topsoil to reduce available phosphorus by complexing; and (4) scalping. As implied earlier, these four treatments each deal with a different combination of ecological barriers to restoration, and it is anticipated that:

- Scalping will remove the exotic seed bank and reduce N and P levels;
- The use of hot mulch will remove the available seed bank;
- The addition of carbon (using sugar or cold mulch) will reduce N levels;
- The use of Phoslock® will decrease P levels.

The experimental design included a large number of treatments, and hence there were a large number of treatment combinations and interactions between treatments and the measured environmental variables. In this chapter, I focus on those interactions that are most pertinent to evaluating the pathways demonstrated in Figure 3.1. To streamline a study that is already large, I have omitted the evaluation of some treatment combinations that were of less ecological significance to the overarching research questions.

Presented in this way, it is clear that these treatments allow us to separately test the four major approaches described in Figure 3.1 below. This approach will allow us to assess whether one of the barriers to restoration might be more intractable than others, and thus require further strategies to be introduced. To allow this level of analysis, the study will involve measurement and evaluation of the effect of each of the proposed management techniques on the above-ground vegetation cover, the composition of the soil seed bank, and the soil's bulk density and microbial activity.

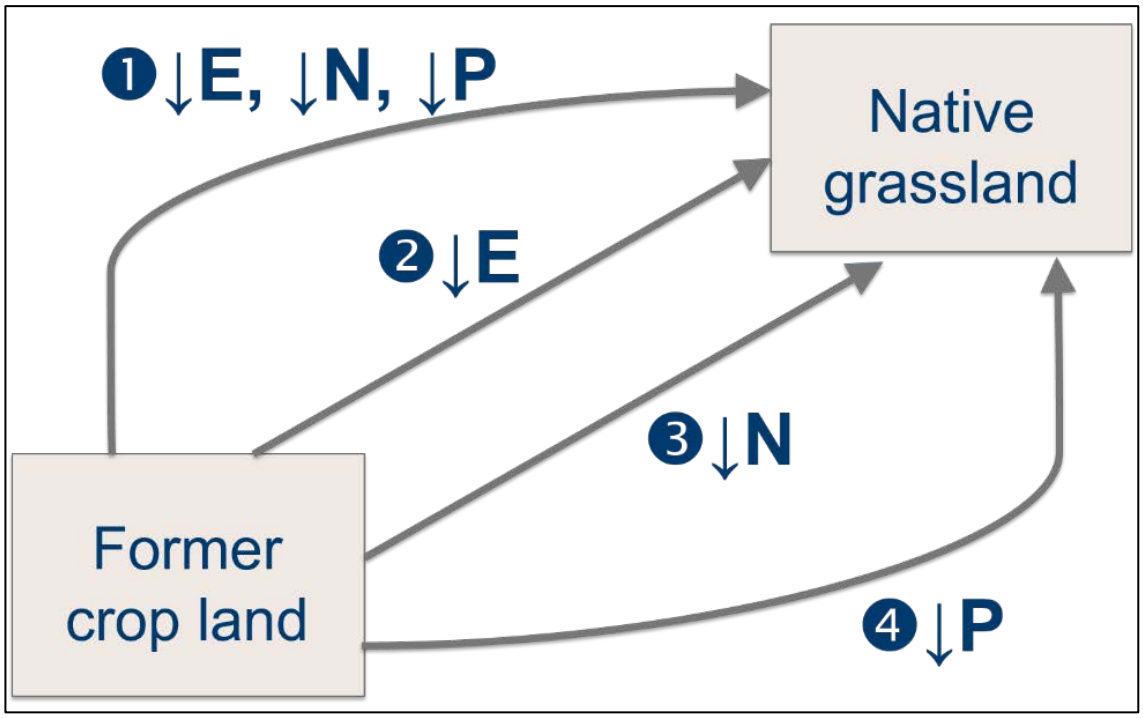

Figure 3.1 Four major approaches about the barriers to restoration of native grassland from former cropping land, where E = Exotic weeds, N = Nitrogen level, P = Phosphorus level. Approach ❶ posits that all three barriers must be addressed to re-establish native grasslands, whereas approach❷,❸ and ❹ posit that either exotic weeds, nitrogen or phosphorus, respectively, are singly the main barrier to restoration success.

3.5 Methods

3.5.1 Preparation of the study site

To remove weed competition before application of treatments to the study site (Figure 3.2) and in order to remove one possible confounding barrier to the evaluation of the efficacy of the restoration evaluation, a broad spectrum non-residual herbicide (glyphosate) was applied to the plots on 22nd May 2014. Glyphosate is absorbed by plant leaves and green stems, then translocated through the plant to the root system, thus removing above ground plants but not affecting the seed bank. The application rate was 9 mL/L water, which was applied using a boom sprayer. During the herbicide application, temperature was 17^{O}C and the prevailing wind was 18 km/h.

Figure 3.2 Study site at the Victorian Volcanic Plains grassland Werribee, Victoria. The white squares show the replication blocks 1, 2, 3 and 4. The black dots within each square are the hot green waste plots that are visible from the satellite image.

3.5.2 Climate

The mean annual rainfall for the experimental site during the research (2014-2016) was 420 mm yr^{-1}, the mean minimum daily temperature was 6.3^{O}C (August) and the mean maximum daily temperature was 26.8^{O}C (January). There was above-average rainfall in 2016. The highest total monthly rainfalls were recorded for September, January and July 2016, with 80.8 mm, 66.4 mm and 66.2 mm rainfall, respectively (Figure 3.3.)

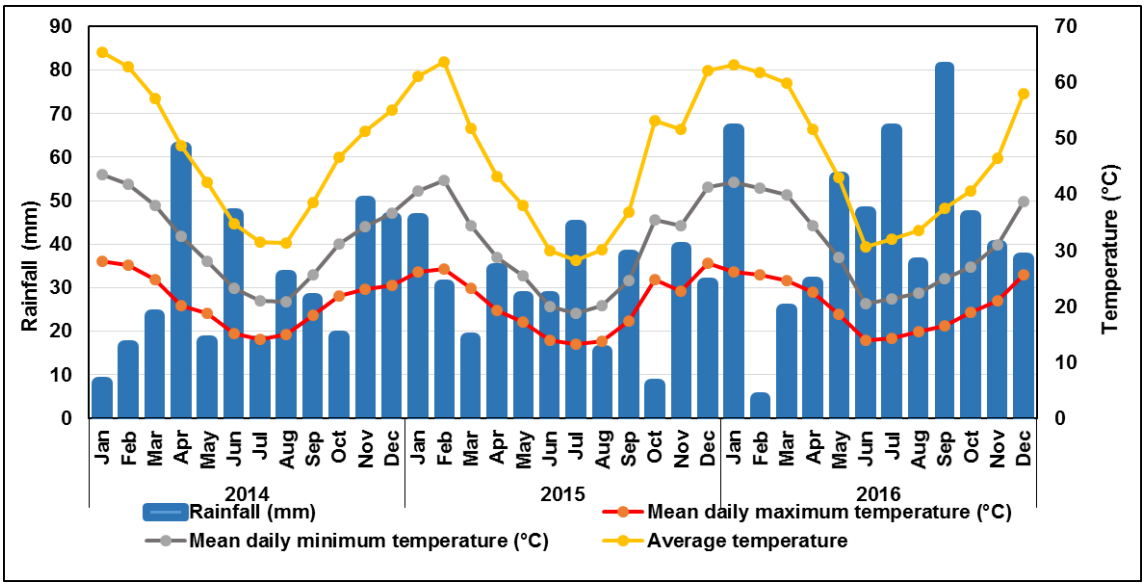

Figure 3.3 Total monthly rainfall, maximum, minimum daily and average temperature for the experimental site across three years. Latitude: 37.86°S, Longitude: 144.76°E, Elevation: 20 m. © Copyright Commonwealth of Australia 2017, Bureau of Meteorology. Prepared using Climate Data Online, Bureau of Meteorology http://www.bom.gov.au/climate/data

3.5.3 Experimental design

The experiment area was about 6082 m^2. The experimental design involved closely situated four blocks used for replication of treatments. Each separate block area was 1521 m^2, and contains nine experimental plots (Figure 3.4. A). These plots were 13 m x 13 m (169 m^2), which comprised a 5 m x 5 m area at the centre for application of the treatment, and an 8 m buffer zone for equipment transport (Figure 3.4. B). The corners of each 5 m x 5 m experimental plot were labelled with metal pegs painted with permanent paint.

The nine treatments which were applied in each experimental block, are as follows:

1. **Scalping**: Approximately 10 cm of topsoil were removed. This treatment reduces soil nitrogen and phosphorus, and removes the seed bank which includes exotic weeds. Native seed mixture was then added.
2. **Sugar addition:** Carbon, in the form of sugar, was added to the ground to increase microbial activity and to consequently decrease nitrogen level in the soil in the short term. Native seed addition was applied after a small period.

3. **Hot green waste (Hot):** Hot composting mulch (oversize) was used to create high temperatures to destroy the resident seed bank of exotic weeds. Native seed mixture was applied after hot composting had been completed.
4. **Hot green waste with mulch (Hot + mulch):** Following the decomposition of the hot green waste, cold mulch was added to increase soil carbon and microbial activity in order to decrease nitrogen levels in the soil. This was done to potentially reduce competition from nitrophilous exotic weeds. Native seeds were then added after a period of stabilisation.
5. **Hot green waste with sugar (Hot + sugar):** Post the hot green waste decomposition, application of sugar was used to increase soil carbon and decrease nitrogen levels in the soil. Native seed mix was applied after decomposition had completed and sugar had been applied.
6. **Cold green waste (Cold):** Cold green waste was added to plots as a carbon source, in an attempt to increase microbial activity and decrease nitrogen levels in the soil over the long term prior to native grass seeding.
7. **Cold green waste with no seed addition (Cold + no seed):** A second cold green waste treatment was implemented that had no subsequent seeding of native grasses. This was to test the effectiveness of native grass seeding on the establishment of native grasses.
8. **Phoslock®**: A commercial clay product, Phoslock®, was applied to plots in an attempt to reduce available soil phosphorus, followed by addition of native seeds.
9. **Control:** There were two control treatments related to each block. The first of these treatments (named ‘control’ hereafter) which was within the experimental area was treated with herbicide at the beginning of the experiment and broadcast with the mixed native seeds**.** The second control treatment, outside the experimental area, had no herbicide applied, and was not broadcast with native seeds – this treatment will hereafter be called ‘control + no seed’.

3.5.4 Native seed addition

Four species of native seed were broadcast to all treatments except the cold + no seed (Figure 3.4 A) and the control + no seed areas. The seeding rate has been suggested by *Flora Victoria* in relation to the native species *Themeda triandra* (Kangaroo grass), *Bothriochloa macra* (Red grass), *Rytidosperma* spp. (Wallaby grass mix seed) and *Austrostipa semibarbata* (Bearded spear grass). The volume of seed measured out for the individual species was judged to be the amount required for each to fully occupy the experimental area. The weight of seeds calculated depended on the plot areas; this was 800 m^2, which included 32 plots, with each plot measuring 25 m^2. All seeds of the native species were thoroughly mixed, then 0.375 kg per plot of native mixed seed was put in separate nylon zip bags ready for sowing.

Table 3.1. The main experimental field treatments, and the biotic and abiotic barriers to restoration addressed by each.

	Barriers											
Treatments	**Nitrogen**		**Phosphorus**		**Exotic weeds**		**Lack of native seeds**		**Low microbial activity**		**Compaction**	
	D	**I**	**D**	**I**	**D**	**I**	**D**	**I**	**D**	**I**	**D**	**I**
Scalping	x		x		x		x				x	
Sugar addition		x				x	x		x			
Hot green waste		x			x		x		x			
Hot green waste + mulch		x			x		x		x			
Hot green waste + sugar		x			x		x		x			
Cold green waste		x				x	x		x			
Cold green waste + no seed		x				x			x			
Phoslock®			x				x					
Control							x					

All treatments involve the addition of native seed (and hence address the lack of native seed' barrier) except the cold green waste + no seed treatment. **D** and **I** letters indicate where there were either direct or indirect effects intended by each treatment.

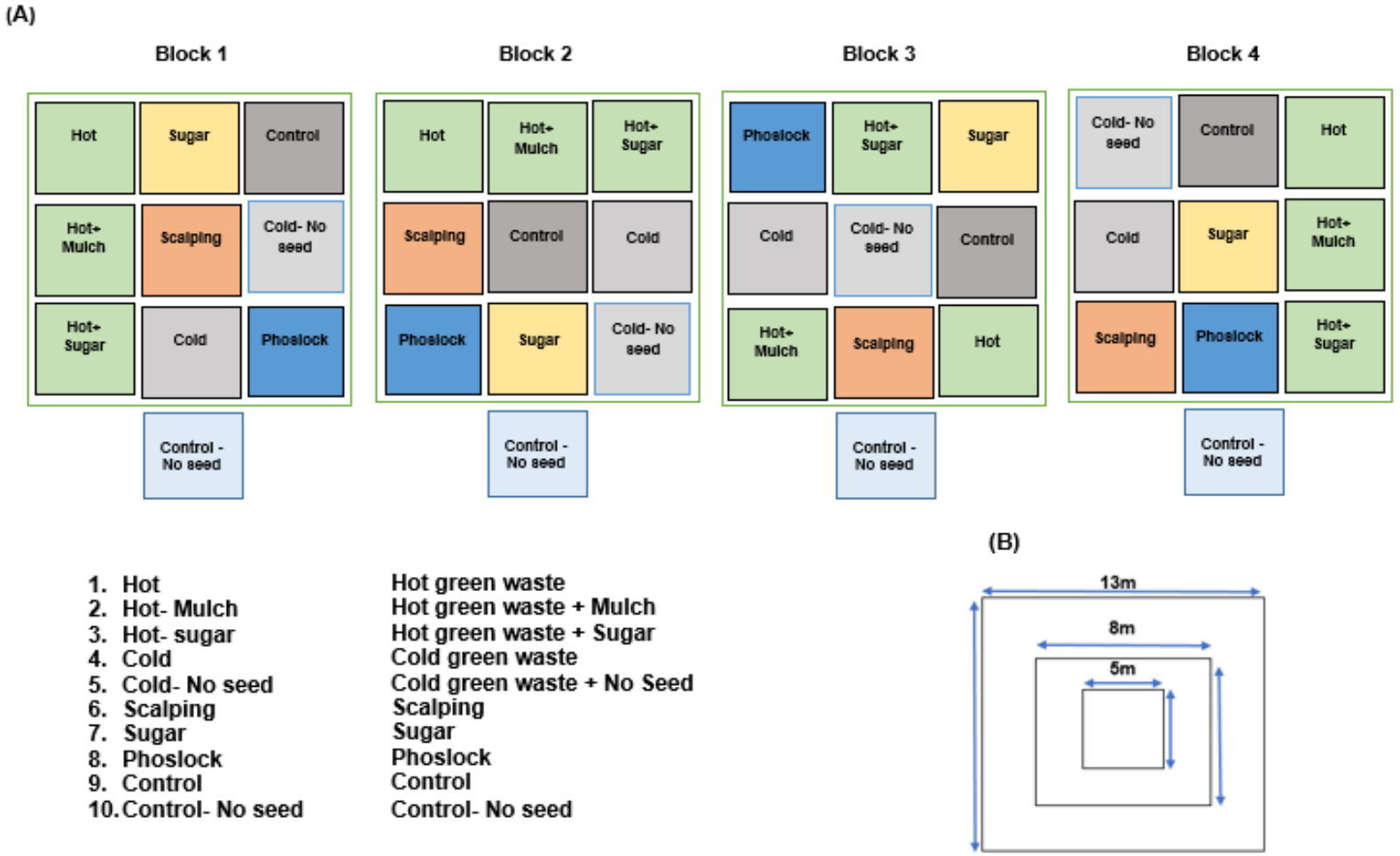

Figure 3.4 (A) Randomised block experimental design for the field study; control + no seed were included for natural vegetation cover assessment only. (B) Experiment plot size and dimensions.

3.5.5 Scalping

The scalping treatment was conducted on the site on 16th June 2014 (25 days after initial herbicide treatment), by removing the topsoil layer of each 5 m x 5 m plot to an average depth of 100 mm using a front end bulldozer. This treatment cleared the soil containing enriched nitrogen and phosphorus as well as removing all exotic weed seed banks (Figure 3.5). The scalping treatments took place simultaneously with the other treatments in the four replication blocks. The addition of seeds was applied on 21st August 2014, where the maximum wind speed was 20 km/h.

Figure 3.5 Topsoil removal (scalping) method.

3.5.6 Sugar addition

The application of sugar was undertaken on four replication plots (Figure 3.6) by using 12.5 kg of white sugar for each 5 m x 5 m plot on three occasions (9th of June, the 8th of September and 5th of December). Seed application took place on 12th September 2014, with a maximum wind speed of 48 km/h.

Figure 3.6 The application of sugar.

3.5.7 Hot green waste

Originally, hot green waste came from green waste which was placed in urban green bins. The material (green waste) was then transported to urban recycling and composting plants such as the Melbourne Metropolitan Waster Group (MMWG) at Epping. The material is composted and pasteurised for 60-70 days where temperatures reach 55-65^{O}C for approximately 45 days. This process significantly reduces the numbers of plant and animal pathogens and plant propagules in the waste material. Successful composting involves balancing several factors to facilitate the composting process. This includes having the correct carbon: nitrogen ratio (25:1-35:1), sufficient moisture (45-60%), oxygen availability (>10%), correct pH (6.5-8.0), correct porosity and bulk density (the material was piled 1.5 – 3.0 m high to minimise the effects of compression). The material used in this trial is referred to as ‘compost oversize material’. This is the coarse fraction which is left over after screening of green organic compost. Typically, the compost industry has a preference to screen the fines from compost at a level of 25 mm or less. This material is acceptable to the market as it contains a lot of fine organics as well as being attractive to work with, to blend with soil preparation and also for aesthetics. This material is high in nitrogen and is sold to nurseries and commercial composting businesses. The oversize

material left after screening is difficult to market as it contains bigger pieces in addition to plastics or contamination, and is usually not reprocessed. It has a low market value and is available in large volumes. There is a carbon: nitrogen ratio of 60:1, with 0.57% total N, 0.59% organic matter and 34.3% total carbon (Farmright Technical Services). This material is capable of nitrogen drawdown capacity, and is not sought after commercially or for agricultural purposes. Throughout Metropolitan Melbourne, there is some 70,000 to 80,000 tons of oversize mulch produced each year from the green organic composting program. There was approximately 700 m^3 of this material delivered to the trial site. Each of the 12 plots of 5 m x 5 m green waste was set up with a pile of approximately 50 cubic metres (around 16 tonne).

The application of hot green waste oversize treatment began on the 16th June 2014 in three treatments: hot green waste (Hot), hot green waste with mulch (Hot + mulch) and hot green waste with 100 kg sugar (Hot + sugar) in 5 m x 5 m plots with four replications (Figure 3.7).

To measure the temperatures generated from the hot green waste oversize, 15 temperature data logger buttons (DS1922L Thermochrom iButton high resolution data logger) were placed under the piles at three different depths: 0, 5 and 10 cm respectively for each of the four hot green waste treatments and control. In order to maintain biological activity and hence temperatures within the mulch piles, it is necessary to physically turn the piles approximately every 2 - 4 weeks. To achieve this, after two weeks the hot green waste oversize piles were turned using a front end bulldozer. This provided an opportunity to recover some of the data loggers and record temperature progress. It was noted that there was a decrease of temperature in the hot green waste oversize piles during the data loggers' download. Consequently, on the 9th July 2014 (25 days after trial initiation), the hot green waste oversize piles were turned for second time, and water was added to all piles to increase the microbial activity as well as increasing the green waste piles' temperature.

In parallel with the insertion of the temperature data loggers, seeds of three species (*Themeda triandra*, *Nassella trichotoma* and *Galenia pubescens*) were buried at the same depth of data loggers, the results of which will be discussed in detail at section 3.6.3 'Effects of hot green waste temperature on viability of seeds'. The oversize piles were physically removed on 15th August 2014 (62 days after initial application), and the data logger and seed bags were collected. The seed addition treatment was conducted on the same day as the pile movement, where the maximum wind speed was (24 km/h). All plots were sowed with seed mix which was prepared after the hot green waste piles were removed, and the hot + mulch treatment plots were covered by 10 cm mulch (green waste).

Figure 3.7 Hot green waste oversize pile, approximately 2 m height.

3.5.8 Cold green waste

To determine the effect of cold green waste on increasing carbon level and microbial activity in the soil for rehabilitation of native grassland, the hot green waste piles were picked up from the hot treatments after exhausting their capacity to reach high temperatures, and used again as a cold green waste treatment (Figure 3.8). The cold green waste treatments include two fragments; cold green waste with seeding (Cold) and cold green waste without seeding (Cold + no seed). The treatments were conducted on the 8th September 2014. Seeding addition treatment was conducted in cold green waste

with seeding treatments, with a maximum wind speed of 69 km/h. All cold green waste treatments (with seeding and without seeding) were covered to a depth of 10 cm by green waste product.

Figure 3.8 Cold green waste application, approximately 10 cm height.

3.5.9 Phoslock®

To investigate whether a novel method to reduce phosphorus in the soil might be effective, Phoslock® treatment was applied on the 13th August 2014, using 12.5 kg of this commercial product per plot (500 g/m^2). 1 kg Phoslock® was placed in 20 litre of water in a watering can (Figure 3.9), which was vigorously mixed, then immediately and evenly applied across the plot. Thin rope was used to divide the 5 m x 5 m plot in each replication into five equal 1 m x 5 m sub-plots to aid even distribution of the product. Two buckets were applied to each 1 m x 5 m sub-plot segment then 0.5 kg Phoslock® + 10 L water applied across the plot. Seed application took place approximately one month later on the 12th September 2014, when the maximum wind speed was 48 km/h.

Soil samples for soil nutrient analysis were collected before and after the treatments were applied.

Figure 3.9 Application of Phoslock®

3.6 Measured variables

The effect of these foregoing treatments on the study site were investigated by using: an above-ground vegetation survey (3.6.1); estimation of the composition of the soil seed banks (3.6.2); measurement of soil nutrient levels (3.6.3); determination of the viability of seeds in the seed bank (3.6.4); measurement of the soil bulk density (3.6.5); and estimation of the microbial activity (3.6.6). The data collection methods used for estimating these variables are described in the sections below.

3.6.1 Above-ground vegetation survey

The above-ground vegetation survey was conducted on the experimental site five times during spring and autumn sessions after application of the treatments to monitor the extent and composition of vegetation cover, and to estimate the effect of the treatments on the species composition during the study. The vegetation surveys were conducted on 18th November 2014, 22th April and 18th November 2015, 20th April and 21th November 2016, where plant basal cover and species composition were estimated by the step-point method (Barabesi & Fattorini, 1998). Within each 5 m x 5 m plot, this procedure was engaged to assess the plant cover and bare ground ratio, and the extent of plant diversity. The step-point method was carried out by marking each plot with four labelled metal

marker pegs. Transect lines were then set out 1 m from the marker pegs to prevent possible interference from edge effects. Point intercepts were made at 20 cm intervals along each of the 5 m transects (Figure 3.10), allowing the recording of species and bare ground basal intercepts (with 100 point intercepts per plot).

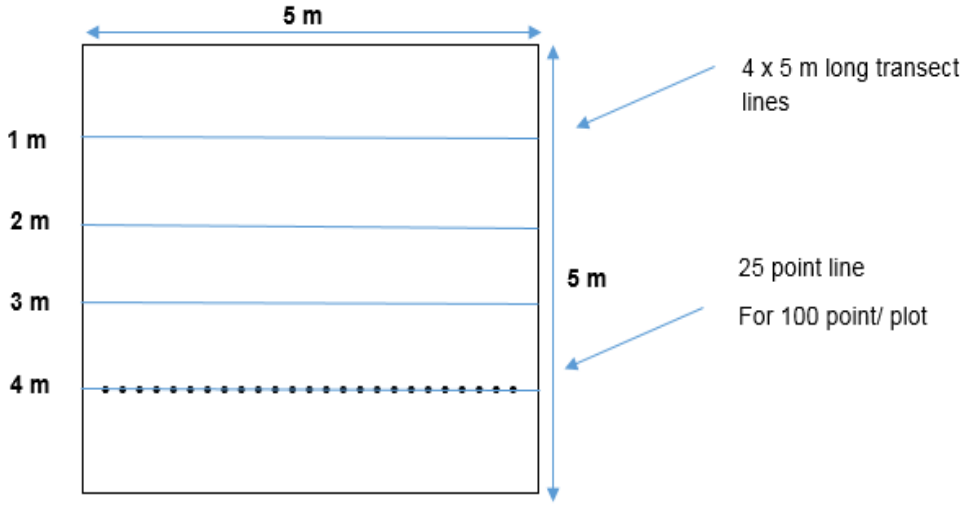

Figure 3.10. Four 5 m lines with transect points at the sampling plot for vegetation assessments.

3.6.1.1 Statistical analysis of above-ground survey

Prior to statistical analysis, all data were angularly transformed so that the residual variation in the analysis did not systematically change as the mean proportions changed (McDonald, 2009). Data were analysed with the MINITAB 17 statistical package (Minitab, 2014) using a one-way ANOVA. For the statistical analyses, the ground cover species were categorised into native species and exotic species cover. The nine treatments in each replication block were: control (planting seed only), control + no seed, hot green waste, hot + mulch, hot + sugar, cold green waste (excluding the cold + no seed treatment), scalping, sugar and Phoslock®. Another one-way ANOVA was run between cold green waste (cold) and cold green waste no seed (cold + no seed) to determine the effect of seeding native mix on basal cover percentage. Tukey's test with a significance level of 0.05 was applied to test for significance between the all factor means and their combinations. The mean data were back transformed.

3.6.1.2 Results of above-ground survey

Figure 3.11 shows the mean foliage cover of native and exotic species in each treatment during the first monitoring period (spring 2014), two months after application of the treatments. Native grass cover was low compared to the levels of exotic cover in all treatments, with only the hot + mulch and hot green waste treatments showing any signs of native grass cover (0.5 and 0.26 %, respectively). However, compared to the control treatment, the cover of exotic species in the first monitoring period was significantly decreased for hot + sugar, scalping, hot + mulch, hot green waste and sugar treatments, which had 3 ,13 ,18 , 22 and 29 % respectively, compared to 68 % cover in the control (Table 3.2). There was no significant effect of Phoslock® and cold green waste treatments on the percentage cover of exotic species when compared to the control.

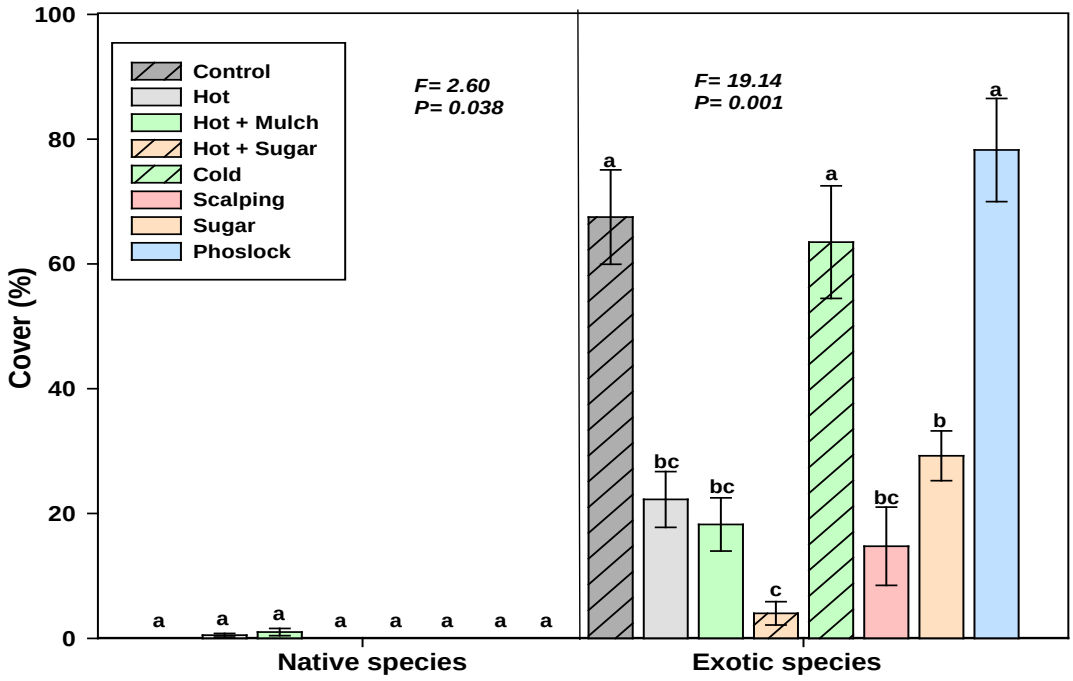

Figure 3.11. Native and exotic species cover for each treatment directly after application of treatment at first assessment period (spring 2014). Vertical error bars represent ± standard error of the means. Letters on the vertical bars represent the significant differences between the treatments using Tukey's test; treatments not sharing a letter are significantly different from one another.

Native species cover in autumn 2015 was higher in all treatments except for the cold green waste treatment (Table 3.2, Figure 3.12). The scalping, hot + mulch, sugar and hot

treatments had higher mean native species cover than the other treatments (15, 14, 13 and 12% respectively). The control + no seed and cold green waste treatment had significantly lower native species cover than the control treatment, while all other treatments did not differ significantly in native species cover from the control.

All treatments (including the control) had significantly less exotic cover in autumn 2015 than the control + no seed treatment. The cold and scalping treatments also had significantly less exotic cover than the control, while all other treatments were not significantly different from the control. The control + seed treatment had significantly greater cover of exotic species than all other treatments (p= 0.001).

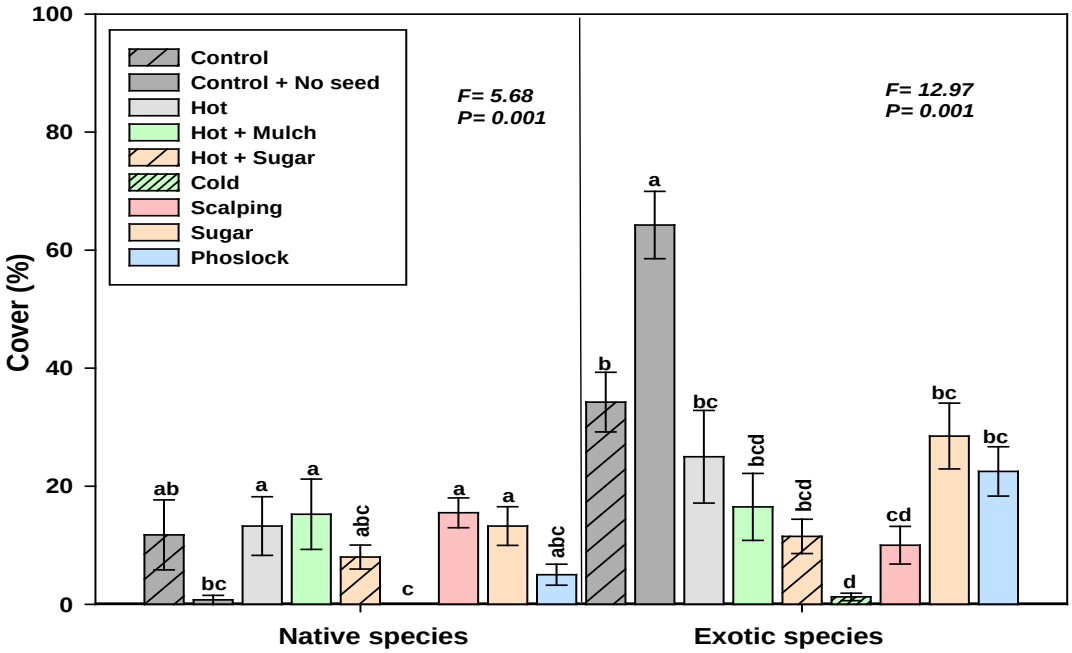

Figure 3.12. Native and exotic species cover for each treatment at second assessment period (autumn 2015). Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test; treatments not sharing a letter are significantly different from one another.

After the spring 2015 assessment (Table 3.2, Figure 3.13) the hot + sugar, hot and hot + mulch treatments had significantly higher mean native grass cover than the control. Native grass cover in the scalping, sugar and Phoslock® treatments was not significantly higher than in the control plot, and there was no native grass cover recorded in the cold green

waste treatments. Exotic cover was high in spring 2015 – up to 71% in the control plots – and it was higher in all treatments than for the previous monitoring period (autumn 2015). However, the three hot treatments and the scalping treatments had significantly less exotic cover than the control treatment.

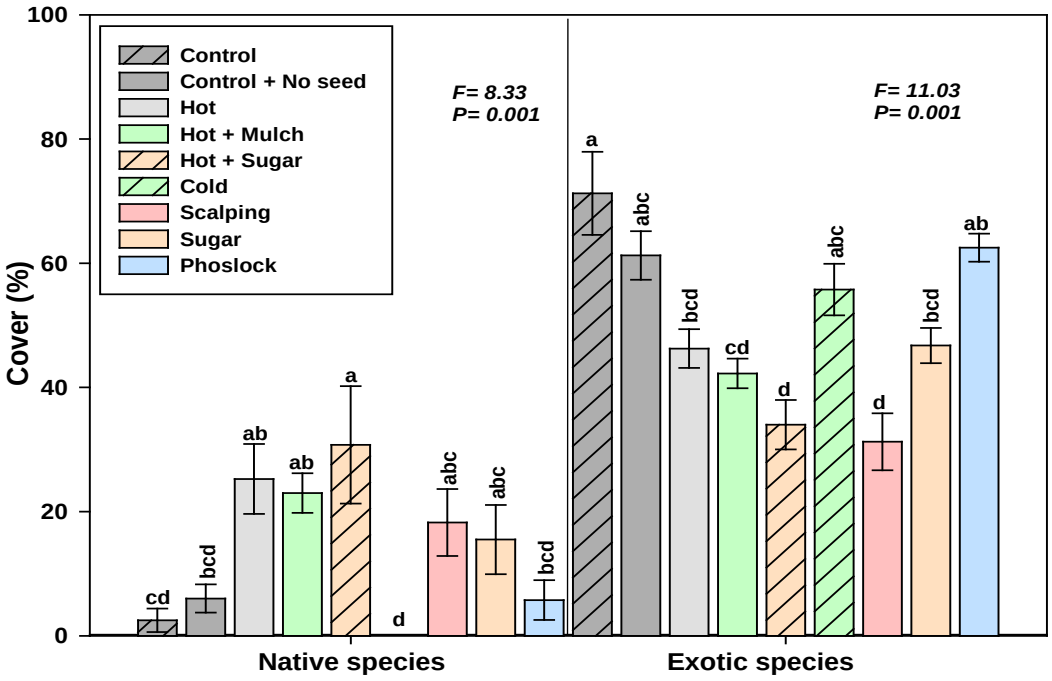

Figure 3.13. Native and exotic species cover for each treatment at third assessment period (spring 2015). Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test; treatments not sharing a letter are significantly different from one another.

The trend of native species cover in the fourth monitoring period (autumn 2016) was similar to the spring 2015 monitoring period (Table 3.2, Figure 3.14). The highest mean cover of native species was recorded in the three hot treatments and the sugar treatment. However, there were no significant differences in native species cover among the treatments. Exotic species cover was much lower than in spring 2015, and was low in all treatments except the 'control + no seed' treatment.

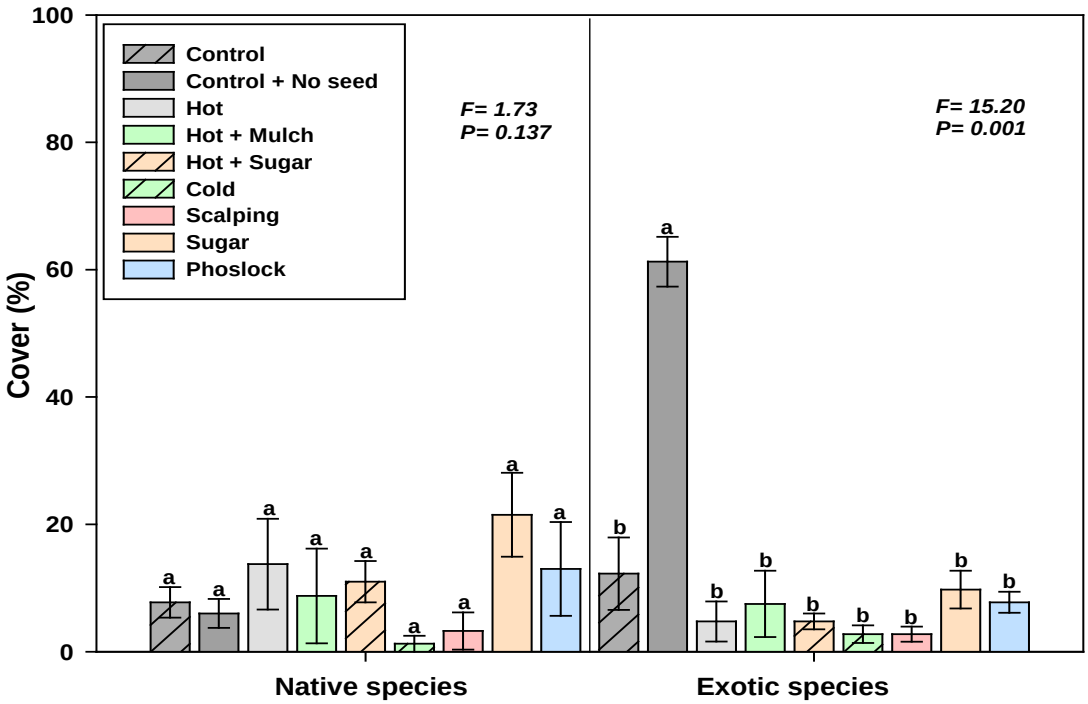

Figure 3.14. Native and exotic species cover for each treatment at fourth assessment period (autumn 2016). Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test; treatments not sharing a letter are significantly different from one another.

For the last monitoring period (spring 2016), the hot + sugar treatment had significantly higher native species cover (19%) (p= 0.001) than the control treatment (1%). While native species cover in the hot, sugar, scalping and hot + mulch treatments was higher than the control, these differences were not significant according to the Tukey test. Native species cover in the Phoslock® and cold treatments did not differ significantly from the control.

There was no significant changes between the treatments and control on the exotic species cover in spring 2016 assessment, and exotic cover was relatively high (ranging from 36–53%). This is a similar to the exotic cover in spring 2015.

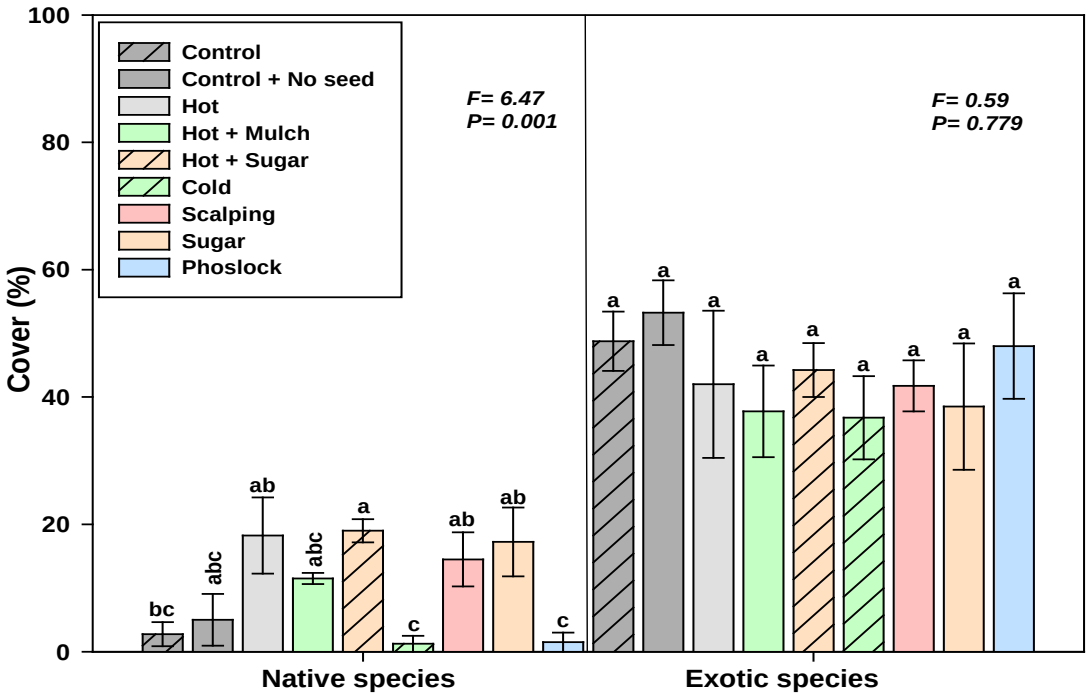

Figure 3.15. Native and exotic species cover for each treatment at last assessment period (spring 2016). Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test; treatments not sharing a letter are significantly different from one another.

There were no significant differences in exotic and native species cover between the cold and cold + no seed treatments across the five assessment periods. However, the exotic species cover fluctuated for both treatments through time, and was high each spring and low in autumn (Figure 3.16). There was no native species cover present at the first three assessment periods for both treatments. There was no native species cover at cold + no seed treatment at the last assessment period (spring 2016) and there was only 0.3% of native species cover for cold green waste treatment (Figure 3.16).

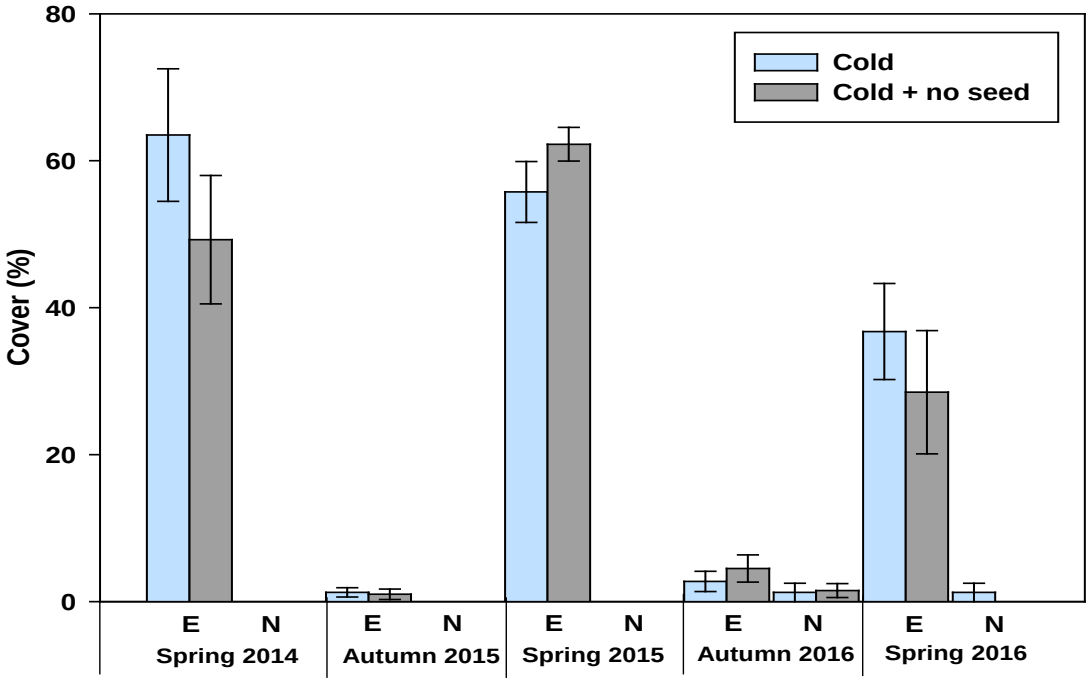

Figure 3.16. Exotic (E) and native (N) species cover for cold and cold + no seed treatments, across five assessment periods. Vertical bars represent ± standard error of the means.

Table 3.2. Native and exotic species percentage cover (%) for each treatment over the five monitoring assessment periods; spring 2014,autumn 2015, spring 2015, autumn 2016 and spring 2016. The letters E and N represent exotic and native species.

Treatments	Spring 2014		Autumn 2015		Spring 2015		Autumn 2016		Spring 2016	
	N	E	N	E	N	E	N	E	N	E
Control	0	68.1	9.2	34	1.1	71.8	7.3	10.6	1.3	48.8
Control + no seed	-	-	0.2	64.6	4.5	61.4	4.5	61.4	2.2	53.3
Hot	0.3	21.6	11.7	22.6	24.6	46.2	11.4	3	17	41.5
Hot + mulch	0.5	17.5	14.1	15.3	22.8	42.2	4.6	5.5	11.5	37.3
Hot + sugar	0	2.9	7.6	10.9	29.6	33.8	10.3	4.5	18.9	44.2
Cold	0	64.5	0	0.9	0	55.8	0.3	1.9	0.3	36.3
Scalping	0	13.3	15.2	9.2	17.2	30.9	1.3	2.4	13.7	41.6
Sugar	0	29	12.6	28	14	46.7	19.6	9.2	16.1	37.9
Phoslock®	0	79.8	3.8	22.2	4.6	62.5	8.1	7.4	0.4	47.7

3.6.1.3 Discussion of above-ground survey

As discussed earlier, the restoration of ex-cropland to its native condition faces a number of biotic and abiotic barriers. Whilst it is known that for successful restoration each of these barriers must be suitably addressed, there is also an issue of ordering the treatments which are used to meet the requirements of specific restoration issues. Hence there arises the practical problems of (i) what constitutes an efficient remediation procedure for a recognised biotic or abiotic barrier? and (ii) what is the most effective and efficient order of application of these remediation procedures? The complexity of this undertaking cannot be overemphasised, and it is likely that the approach made to addressing one barrier will have implications for the treatment of another. In addition, because of the magnitude of the previous impacts made upon the land by agricultural processes, it is also likely that repeated treatments to address some barriers will be necessary.

As a consequence, the statement regarding the primary objective of this study reflects the complexity of this situation. The investigation intends to examine the efficiency and effectiveness of a number of interventions, both in isolation and as a series of restorative actions, to restore ex-arable grassland. To systematize the study, a number of specific hypotheses were proffered and tested, and at the end of the trials, implications and management suggestions have been made.

The restoration methods tested in the study are (i) control area with no treatment except addition of native seeds (control), (ii) control area with no treatment (control + no seed) (iii) application of hot green waste with addition of native seeds (hot), (iv) application of hot green waste plus mulch with addition of native seeds (hot + mulch), (v) application of hot green waste plus added sugar with addition of native seeds (hot + sugar), (vi) application of cold green waste with addition of native seeds (cold) and cold green waste with no native seeds (cold + no seed), (vii) scalping of topsoil with addition of native seeds (scalping), (viii) application of sugar with addition of native seeds (sugar), (ix) treatment with Phoslock® with addition of native seeds (Phoslock®).

To aid systematic evaluation of these treatments, nine hypotheses were posited and tested using field experiments or laboratory investigations, namely:

I. The first hypothesis was that no treatment except addition of native seeds (control) will be sufficient to facilitate the recruitment of native species.

II. The second hypothesis is that no treatment is necessary (control + no seed) to facilitate the recruitment of native species.

III. The third hypothesis was that the high temperature created from the hot green waste treatment followed by seeding the area with a native mix will be sufficient to kill the exotic weed seeds in the soil seed bank and allow recruitment of native species.

IV. The fourth hypothesis was that the high temperatures created from the application of hot green waste followed by the addition of mulch and of native seeds will be sufficient to kill the exotic weeds in the soil seed bank and provide enough nutrient to facilitate the recruitment of native species.

V. The fifth hypothesis was that the high temperature created from the hot green waste treatment in combination with added sugar as a carbon source followed by seeding with a native mix would be sufficient to kill exotic weed seeds in the soil seed bank and provide extra nutrient to facilitate the recruitment of native species.

VI. The sixth hypothesis was that the application of cold green waste followed by the addition of native seeds, and cold green waste without native seed addition will be enough to reduce the exotic weed species and facilitate the recruitment of native species.

VII. The seventh hypothesis was that scalping the topsoil, followed by seeding the denuded area with native mix, is an effective treatment to control the re-emergence of exotic weeds and the subsequent recruitment of native species.

VIII. The eighth hypothesis was that the application of sugar with addition of native seeds will be sufficient to reduce exotic weeds and facilitate the recruitment of native species.

IX. The ninth hypothesis was that treatment of the topsoil with Phoslock®, followed by the addition of native seeds will be sufficient to control the exotic weeds and facilitate the recruitment of native species. This hypothesis will be investigated in laboratory conditions.

The first hypothesis was that no treatment except the addition of native seeds (control) will be sufficient to facilitate the recruitment of native species; the results clearly showed that the exotic weed cover was higher in this case than all other treated plots except the control + no seeds. As a consequence, this hypothesis is rejected, indicating clearly that some intervention other than the addition of native seeds is required for restoration. This is also shown with the second hypothesis which stated that no treatment is necessary (i.e. control + no seed) to facilitate the recruitment of native species. As indicated above, there was high percentage of exotic weed cover and low native species in the control + no seed plots, which again clearly shows that the restoration of native conditions within the VVP grasslands requires active management as it will not simply be restored spontaneously. In a similar study, Morris *et al*. (2016) observed that in the absence of biomass removal and soil nutrient manipulation, control plots remained largely as they were at the start of the experiment, with a dense grass canopy dominated by the same group of grass species.

With regard to the third, fourth and fifth hypotheses, which all suggest that with the use of a novel method for restoration of ex-cropland in VVP grassland involving application of hot green waste (hot), there are more positive indications. The hot green waste (hot) and combinations of hot + mulch and hot + sugar, all led to an increase in the reestablishment of native species *Themeda triandra, Bothriochloa macra, Rytidosperma* spp. and *Austrostipa semibarbata* when seeded. In addition, the hot + sugar treatment was very effective in reducing the exotic species cover directly after its application, and showed significant establishment of native species at the end of the study period (three years). Hot green waste treatments raised the temperature to greater than 60^{O}C down to 10 cm

into the soil for as long as 15 days, and it was clear that this temperature was sufficient to kill most of the seeds of weed species under the hot, hot + sugar and hot + mulch treatments. It should be noted that the hot treatment also reduced the viability of the native grass *Themeda triandra*, and exotic species such as *Nassella trichotoma* and *Galenia pubescens* seeds that were buried under the hot treatments at three different depths (detailed results are given in section 3. 6.3 Effects of hot green waste temperature treatment on viability of seeds). The results of last monitoring assessment (Spring 2016) clearly showed the increased recruitment of the native species at many of these sites, with the greatest native species cover being recorded for the hot + sugar treatment (about 19%) followed by hot, and hot + mulch treatments, which were 17% and 12% respectively. These results gave qualified support to the third, fourth and fifth hypothesis of the study.

In this respect, I concur with the claim that stringent exotic weed control is an important factor in the successful restoration of highly degraded sites to grassland by native species seeding (Cole & Lunt, 2005), and that adding a mixture of native species seeds post-treatment increased species richness and abundance after the exotic species cover has been substantially reduced (Morris & Gibson-Roy, 2018). This was certainly indicated by the hot + sugar treatments in these three series of experiments.

I also agree with the observation that re-establishment of native species during restoration of ex-cropland significantly enhances the resistance of restored vegetation to invasion by exotics (Prober *et al.,* 2005). In this respect, Paschke *et al*. (2000) revealed the positive effects of mulch and sugar addition respectively on growth rates and relative abundances of native species, which is in agreement with the current study. However, other studies reported no benefit of sawdust addition to the establishment of native species (Wilson & Gerry 1995; Morghan & Seastedt 1999; Alpert & Maron 2000; Madsen *et al.,* 2016).

Regarding the sixth hypothesis, which was that the application of cold green waste followed by the addition of native seeds and cold green waste without native seed addition will be enough to reduce the exotic weed species and facilitate the recruitment of native

species, some encouraging results were observed. It was found that cold treatment as a mulch had a positive effect in reducing the infestation of exotic weeds and inhibited seedling emergence, but also had a negative effect on native seed requirement. It appears that this treatment generally made a layer above the ground which inhibited airborne or dropped seeds in reaching the surface (Coleman *et al.,* 2015; Khan *et al.,* 2016). It was noticed, however, that cold treatment was not effective in reducing the exotic species cover as much as expected directly after application of the treatments, but the exotic species cover gradually began to decline, until it was lower than the all other treatments in the spring 2016 assessment.

The seventh hypothesis suggested that scalping the topsoil, followed by seeding the denuded area with native mix, is an effective treatment to control the re-emergence of exotic weeds and the subsequent recruitment of native species. It has previously been reported that the scalping treatment is an effective tool for the restoration of degraded grassland on the Victorian Volcanic Plains (Gibson-Roy, 2010a; Morris & Gibson-Roy, 2018). In this current work, the exotic species cover in the scalping plots declined very effectively at spring 2014 and autumn 2015, but it was seen that this rate increased during spring 2015 to 31% due to regrowth of exotic species in the plots. It is thought that this increase is largely due to wind-blown seeds from outside the plots.

The eighth hypothesis was that the application of sugar with addition of native seeds will be sufficient to catalyse the reduction of exotic weeds and facilitate the recruitment of native species. This addition of a concentrated carbon resource such as sugar (or cold green waste) was expected to restrict the exotic species cover regrowth for a reasonably long-period, thus giving native species time to re-establish. It is noted that this also occurred in the hot, hot + mulch, hot + sugar and cold treatments for the duration of the experiment, which all added extra carbon resource to the soil. It is also thought that the addition of sugar to soil can rapidly stimulate microbial activity (Nottingham *et al*., 2009) which will tend to reduce nutrient levels. On the basis of this notion, sugar applications

have frequently been used to effectively decrease above-ground biomass production and reduce the competitive ability of invasive plants which tend to proliferate in soils with high nutrient levels (Morghan & Seastedt, 1999; Eschen *et al.,* 2007). In the current investigation, the restriction of exotic species cover regrowth was very effective in the hot + sugar, hot + mulch, hot and sugar treatments in spring 2014 assessment (Table 3.2). However, exotic species cover tended to increase over time in all treatments plots due to reestablishment of exotic species through air-borne seeds or from the residual seed bank germination under good environment conditions for germination such as available water, light, space and oxygen.

Importantly, although exotic species abundance was reduced in the current investigation by carbon addition, as indeed was observed in other studies (Prober *et al.,* 2005; Morris & De Barse, 2013; Cole *et al.,* 2016). Prober *et al.'s* (2005) study showed that carbon addition was not effective for controlling exotic annuals for more than 12 months unless *Themeda* was established through seed addition. Paschke *et al*. (2000) and McLendon and Redente (1992) found that sugar addition to two successional shortgrass grassland ecosystems favoured slower growing perennial species over faster growing annual grasses such as *Bromus tectorum* (Cheat grass) and annual forbs. It is noted here that these findings reinforce the complexity of this restoration undertaking, in that a single treatment applied to significantly altered soil conditions is unlikely to be completely effective, and that a series of repeated treatments is likely to be necessary.

Regarding the ninth hypothesis, which was that treatment of the topsoil with Phoslock®, followed by the addition of native seeds will be sufficient to control the exotic weeds and facilitate the recruitment of native species, it was found that this treatment did not have an effective influence on the exotic species cover. As earlier noted, Phoslock® was originally designed to reduce the concentration of phosphorus in lake water (Douglas *et al.,* 1999; Robb *et al.,* 2003; Reitzel *et al.,* 2013). It was used here in an effort to bind the free phosphorus in a bentonite clay matrix which was hoped to prevent the uptake of

phosphorus by plants (Geurts *et al.,* 2011). More comment on the action of Phoslock® will be made during Chapter 5, and reasons for the failure of this approach will be proffered.

It was noted that there was very little establishment of native species in spring 2014 after six months of seeding of mixed native species in the hot + mulch and hot treatments. In addition, the native species cover for the hot, hot + sugar, hot + mulch and scalping plots declined from 30, 25, 23 and 17% at spring 2015 to 10, 11, 5 and 1.3% at autumn 2016. This was found to be due to grazing by kangaroos (*Macropus giganteus*) that were occupying the study site at that period time, but, encouragingly, native species cover increased again by spring 2016 to 19, 17, 12 and 16% respectively (Table 3.2).

As a final note, it was clear that cover of both native and exotic species fluctuated across time, suggesting exogenous factors outside the control of the experiment were at play. For example, the native species cover fluctuated within the control and Phoslock® treatments from low-high-low (Table 3.2), and the trend of native species' cover at the sugar addition treatment plots was increasing slightly till autumn 2016, but declined from 20 to 16% during spring 2016. At the same time, there were fluctuations in the exotic species' cover in the all treatment plots between the seasons. In this latter case, it was thought to be due to the regrowth of exotic species stimulated by wind-borne seeds, especially those of *Avena sativa* (Wild oats), which were found in most treatment plots throughout the period of sampling at spring seasons. Inspection showed that the experiment site area was surrounded by this dominant species.

3.6.1.4 Management Implications of above-ground survey

The results of vegetation assessment clearly indicated the positive effect of most restoration methods such as hot, hot+sugar, hot+mulch, scalping, cold and sugar treatments to reduce the exotic weed species cover and to facilitate recruitment of native species. However, it became clear that there were obstacles to restoration management in the VVP grassland such as: (i) the reintroduction of exotic weed species after the apparently successful application of weed reduction treatments. This was most likely due

to the wind-blown exotic seeds accessed from the untreated areas around the study site. This notion was supported by the observation that *Avena sativa* seedlings, in particular, were found in most treatment plots throughout the period of sampling at spring seasons, and that this species dominated the surrounding untreated site area; (ii) the abundance of free-ranging kangaroos in the study site, where they played a negative role in eating the mature native plants which emerged in most study plots, and therefore significantly reduced the count of native cover throughout the study; and (iii) there was concern about the chosen native mix of seeds, which may not have provided robust enough competition for the exotic species. In addition, the rate of seed distribution may have been too low to raise a critical density of native cover.

Given that these confounding factors are relatively self-evident from the results, it is suggested that future restoration management methods could be applied more confidently on landscape scale levels if these obstacles were to be specifically addressed. For example, steps could be taken to: (i) decrease wind-blown species entering the management area by controlling the source of weed seeds within a reasonable distance around the management area, (ii) exclude kangaroos and any other fauna which consume native plants by installing fencing, and (iii) use an increased rate of seeding with a larger number of native species in the mix to catalyse the seeding process and to establish a more robust native cover community. This seeding addition could be repeated 3-4 times in order to cover a wider area with successfully grown native species.

3.6.2 Soil seed bank germination: Investigation of the effect of hot green waste and scalping on soil seed bank germination

The objective of the study was to determine the survival rate of seeds in the soil seed bank, (i) after composting on the soil surface using hot green waste (hot) created high temperatures in the ground and (ii) after the scalping treatments which physically removed seeds from the seedbank.

Soil samples were collected from plots after application of hot, hot+sugar, hot+mulch and scalping treatments on the 15th August 2014. A composite of five soil samples were collected within each 5 m x 5 m plot. Soil samples were placed in labelled plastic zip lock bags, kept cool, then transferred to the Federation University glasshouse, Mount Helen, Ballarat. The soil preparation and seed bank germination methods were described in detail in Chapter 2. The study commenced on the 14th October 2014. Figure 3.17 shows the experimental design and description of the treatments.

.

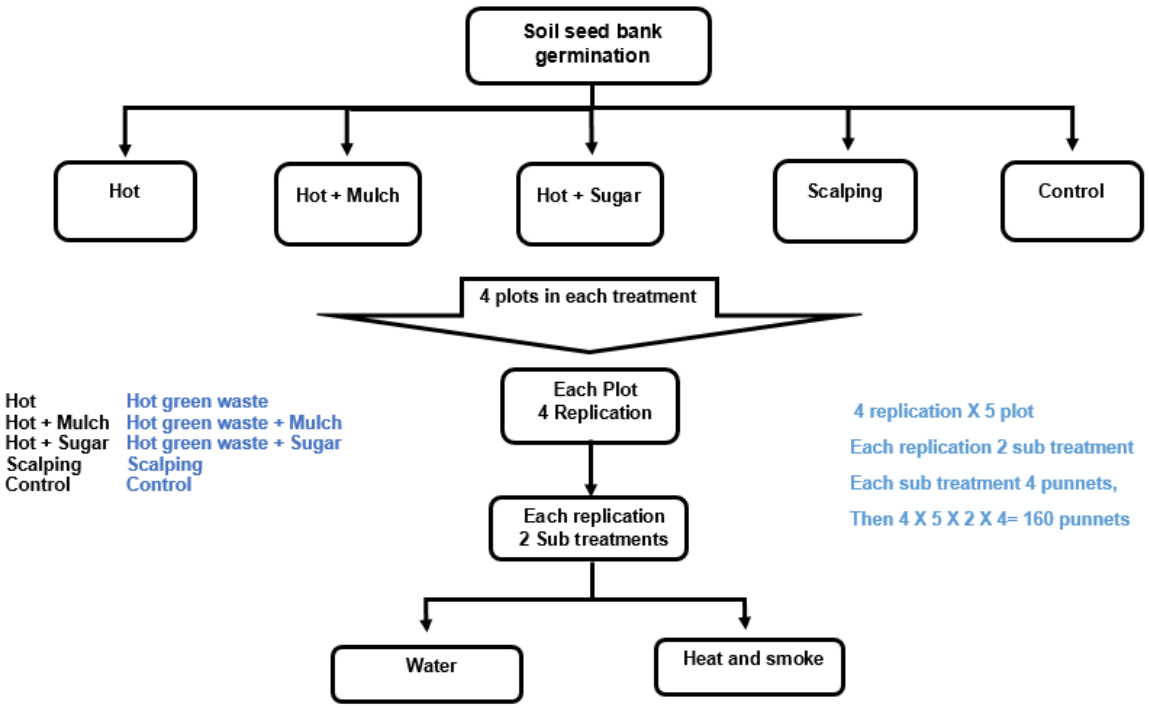

Figure 3.17 Flowchart of soil seed bank germination study design.

3.6.2.1 Statistical analysis of soil seed bank germination

Prior to statistical analysis, the counted number of germinated seeds per square metre in each species category (exotic species, native species) and sub treatments (water, heat and smoke) in each treatment (control, hot, hot+mulch, hot+sugar and scalping) were square-root transformed so that the residual variation in the analysis did not systematically change as the mean changed (McDonald, 2009). Data were analysed with the MINITAB 17 statistical package (Minitab, 2014) using a General Linear Model (GLM). GLM with two

factors (two-way) ANOVA were used to assess the effect of the various factors (treatments and sub treatments) on seed germination per square metre of each category. To find the significant differences between the treatments, Tukey's test was used for all pairwise comparisons of the mean response to the different treatments with a 0.05 level of significance. The mean data were back transformed.

3.6.2.2 Results of soil seed bank germination

A total 1698 seeds from 14 species were found to be present in the soil seedbank germination (Table 3.3.) corresponding to an average seed density of 816 seeds m^{-2}. The mean total seed emergence for the control plot was 235 seed m^{-2}, which was significantly higher than in the hot, hot + mulch, hot + sugar and scalping treatments (54.7, 70.3, 70.9 and 79.3 seeds m^{-2}, respectively; Table 3.4, Figure 3.18). Most germinated seeds were exotics species belonging to seven families. Only one native species germinated in the pots, The emergence of *Einadia nutans* (Climbing saltbush) was 51 seeds m^{-2} in the control plot and 3 seeds m^{-2} hot + mulch plot (Table 3.3, Figure 3.19). The effects of hot, hot + mulch, hot + sugar and scalping treatments were evident on the seed bank germination of species after treatment. Mean seed emergence of *Avena sativa* species was 41.2 seed m^{-2} in the control plot, but this had decreased to 10.5, 4.8, 2.4 and 8.1 seeds m^{-2} respectively at hot, hot + mulch, hot + sugar and scalping treatments (Table 3.3). Similarly, mean seed emergence of *Polygonum aviculare* (Wire weed) species declined dramatically after treatment from 27.6 at control to 4.5, 5.4, 1.5 and zero seeds m^{-2} for the hot, hot + mulch, hot + sugar and scalping treatments respectively (Table 3.2).

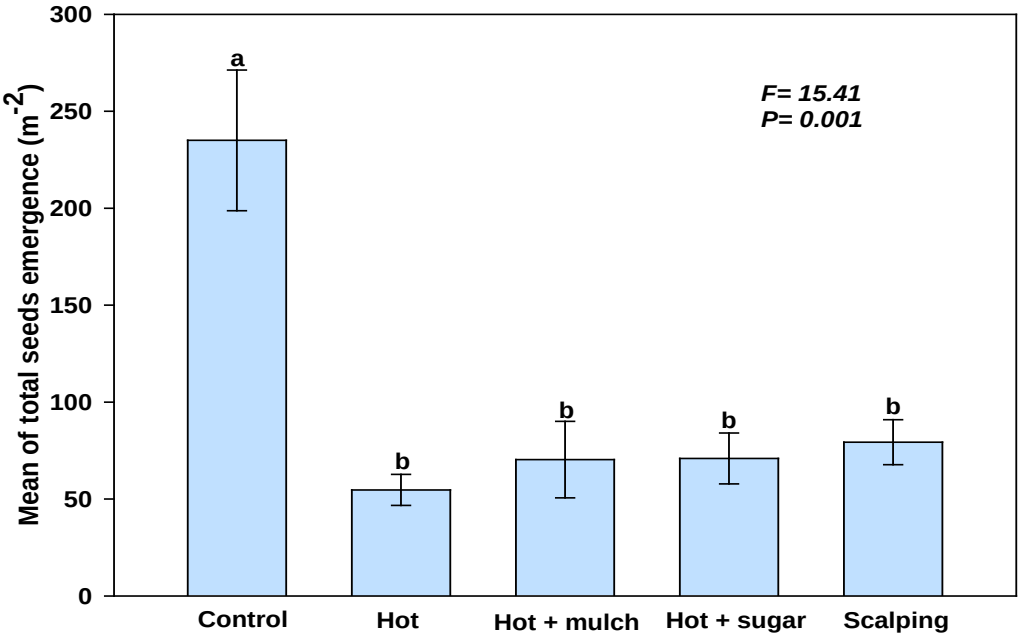

Figure 3.18 Mean total seeds emergence per square metre in control, hot, hot + mulch, hot + sugar and scalping treatments. Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

The mean seed density of *Nassella trichotoma* (Serrated tussock) species was 37.6 and 37.9 m^{-2} in the control and scalping plots, but this number declined dramatically in the hot, hot + mulch and hot + sugar treatments (respectively 5.1, 8.1 and 9.6 seeds m^{-2}). Interestingly, there was some species that were not present at the control plot but were found at other sites. *Medicago truncatula* (Barrel medic) species was found at the hot, hot + mulch, hot + sugar and scalping treatment sites 14.7, 17.7, 37.3 and 3.6 seeds m^{-2} respectively with *Psilocaulon granulicaule* (Wiry noon-flower) species (0.9 seeds m^{-2}) at the hot treatment, *Trifolium resupinatum* (Persian clover) species 4.8 seeds m^{-2} at the scalping plot and some seeds of *Lactuca virosa* (Wild lettuce) species 0.3 seeds m^{-2} at the hot + mulch plot (Table 3.3).

There was strong evidence for the effect of sub-treatments (heat and smoke, and water) on seed bank germination. This was demonstrated clearly in the control plot, which revealed 321 seeds m^{-2} with heat and smoke, compared to 149 seeds m^{-2} with water only

(Figure 3.20). There was no evidence of an effect of sub-treatment (heat and smoke, and water) on seed bank germination across the other four treatments (Figure 3.20).

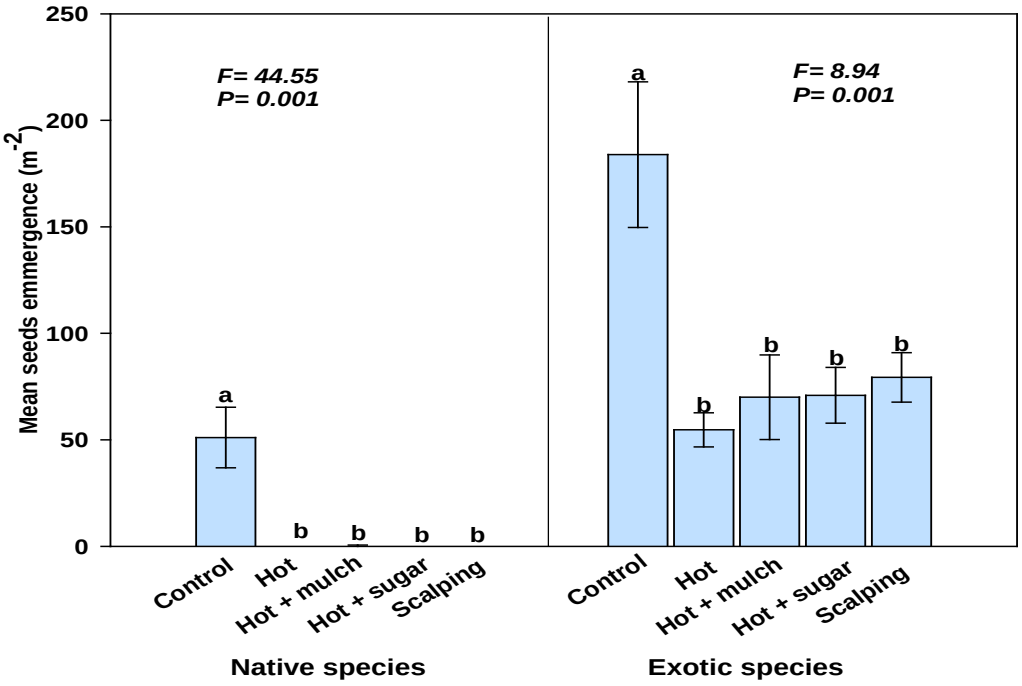

Figure 3.19 Mean native and exotics species seeds emergence per square metre in control, hot, hot + mulch, hot + sugar and scalping treatments. Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

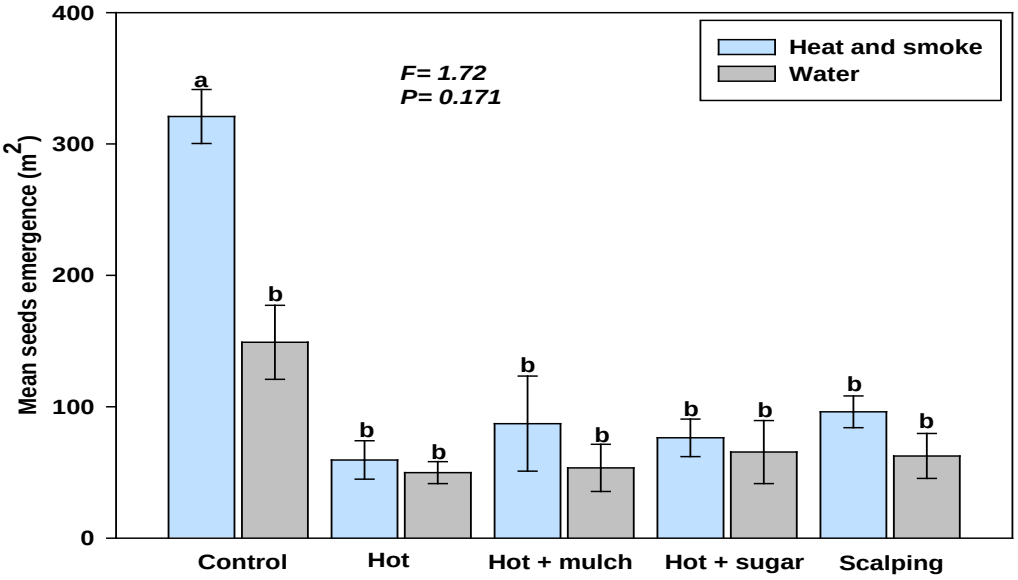

Figure 3.20 Mean total seeds emergence per square metre at different sub-treatment (heat and smoke, water) in control, hot, hot + mulch, hot + sugar and scalping treatments. Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

Table 3.3. Mean number of seeds emergence per square metre of each species in control, hot, hot + mulch, hot + sugar and scalping treatments. The value in the brackets represent ± standard error of the means.

Species	Common names	Families	Control	Hot	Hot+ mulch	Hot+ sugar	Scalping
Avena sativa	Common oat	Poacaciae	41.2 (6.2)	10.5 (2.4)	4.8 (1.4)	2.4 (1.2)	8.1 (1.4)
Conyza bonariensis	Fleabane	Asteraceae.	10.5 (2.3)	1.5 (1.0)	-	-	-
Einadia nutans	Climbing saltbush	Chenopodiaceae	51.1 (14.8)	-	3.0 (3.0)	-	-
Hypochaeris radicata	Flatweed	Asteraceae	0.3 (0.3)	-	-	-	3.9 (1.2)
Lactuca virosa	Wild lettuce	Asteraceae.	-	-	0.3 (0.3)	-	-
Lolium perenne	Perennial ray-grass	Poacaciae	30.3 (7.5)	10.8 (2.9)	30.6 (7.6)	13.8 (5.4)	14.7 (1.9)
Malva parviflora	Mallow	Malvaceae	12.3 (2.9)	-	-	1.2 (0.7)	0.6 (0.4)
Medicago truncatula	Barrel medic	Fabaceae	-	14.7 (2.6)	17.7 (3.1)	37.3 (4.9)	3.6 (1.2)
Nassella trichotoma	Serrated tussock	Poacaciae	37.6 (3.9)	5.1 (1.4)	8.1 (1.9)	9.6 (2.1)	37.9 (4.8)
Polygonum aviculare	Wire weed	Polygonaceae	27.6 (4.8)	4.5 (1.7)	5.4 (1.6)	1.5 (0.9)	-
Psilocaulon granulicaule	Wiry noon-flower	Aizoaceae	-	0.9 (0.5)	-	-	-
Sonchus oleraceus	Milk thistle	Asteraceae	0.9 (0.5)	3.0 (1.1)	2.1 (0.8)	2.1 (0.7)	5.7 (1.3)
Taraxacum officinale	Dandelion	Asteraceae	23.1 (2.8)	3.6 (1.1)	1.2 (0.6)	3.0 (1.3)	-
Trifolium resupinatum	Persian clover	Fabaceae	-	-	-	-	4.8 (1.4)

Table 3.4. Summary of the mean abundance of emergent seeds per square metre of categories (native and exotics species) and number of all seeds in control, hot, hot + mulch,(hot + sugar and scalping treatments. The value in the brackets represent ± standard error of the mean.

Categories	Control	Hot	Hot + mulch	Hot + sugar	Scalping
Native species	51.1 (14.8)	0.0	3.0 (3.0)	0.0	0.0
Exotic species	183.9 (17.3)	54.7 (5.1)	70.0 (10.1)	70.9 (7.9)	79.3 (6.7)
All species	235.0 (21.9)	54.7 (5.1)	70.3 (10.1)	70.9 (7.9)	79.3 (6.7)

3.6.2.3 Discussion of soil seed bank germination

The restoration methods used in this study were (i) control area with no treatment (control), (ii) application of hot green waste (hot), (iii) application of hot green waste plus mulch (hot + mulch), (iv) application of hot green waste plus added sugar (hot + sugar), and (v) scalping of topsoil (scalping).

For the investigation, five hypotheses were tested. These hypotheses were:

I. The first hypothesis was that no treatment (control) will not affect the survival rate of exotic seeds in soil seed bank.

II. The second hypothesis was that the high temperature created from the hot green waste treatment will be sufficient to kill the exotic weed seeds in the soil seed bank.

III. The third hypothesis was that the high temperatures created from the application of hot green waste followed by the addition of mulch will be sufficient to kill the exotic weeds in the soil seed bank.

IV. The fourth hypothesis was that the high temperature created from the hot green waste treatment in combination with added sugar as a carbon source would be sufficient to kill exotic weed seeds in the soil seed bank.

V. The fifth hypothesis was that scalping the topsoil is an effective treatment to control and remove all exotic weeds and prevent their re-emergence.

The first hypothesis, that no treatment of the control will not affect the survival rate of exotic weeds in the soil seed bank, was sustained. The results clearly showed that the exotic weed cover was higher in untreated plots than all the other treated areas. The possibility that, in the absence of invasive agricultural practice, native species would be aggressive enough to re-establish control in the area, was not evidenced. This supports the claim that sufficient weed control is an important factor in the successful restoration of highly degraded sites to their original grassland condition (Cole & Lunt, 2005; Kiss *et al.,* 2018). I thus concur with previous research that stated that the use of effective methods to control the exotic weeds in their seed bank is a significant, though not sufficient step, to the restoration of degraded grassland.

Regarding the second, third and fourth hypotheses, the notion that the high temperature created from the hot green waste treatment alone and in its combinations with mulch and added sugar as a carbon source would be sufficient to kill exotic weed seeds in the soil seed bank, was tested. It was found that hot treatments had a significant effect in reducing the exotic weed concentration in the soil seed bank, thought to be due to a raise in the temperature of the treated plots above $60^{O}C$ to a level 10 cm below the surface of the soil for as long as 15 days. It was clear that this temperature was sufficient to kill most of the seeds of weed species under the hot, hot + mulch and hot + sugar treatments. The hot treatment and the combinations with mulch and sugar had measureable and positive effects in reducing the infestation of exotic weeds and inhibited seedling emergence. This mulch treatment generally left a considerable layer of organic material above the ground which inhibited airborne or dropped seeds in reaching the surface (Coleman *et al.,* 2015; Khan *et al.,* 2016).

The fifth hypothesis was that scalping the topsoil alone is an effective treatment to control and remove all exotic weeds and to prevent their re-emergence. It has been previously reported that the scalping treatment is an effective tool for the restoration of degraded grassland on the Victorian Volcanic Plains (Gibson-Roy, 2010a; Morris & Gibson-Roy, 2018), where their results clearly showed that the physical removal of topsoil for 10 cm is sufficient to remove all unwanted exotic seeds and excessive inorganic nutrients.

The mean total seed emergence for the control plot was 235 seed m^{-2}, which was significantly higher than in the hot, hot + mulch, hot + sugar and scalping treatments (54.7, 70.3, 70.9 and 79.3 seeds m^2, respectively (Table 3.4, Figure 3.18). The relevance of the observations summarised in Table 3.4 will be discussed in detail in Chapter 6, but it is immediately clear that the re-establishment of native plants is a problematic exercise, and that the lessening of intervention of exotic seeds is not a straightforward task.

3.6.2.4 Management Implications of soil seed bank germination

The results of soil seed bank germination study clearly showed the significant effect of all restoration methods such as hot, hot + mulch, hot+ sugar and scalping treatments to control the exotic weed species seed bank. It was found that the hot green waste and its combination with mulch and sugar treatments had positive impact in reducing the exotic weed infestation and inhibiting seedling emergence. Whilst the scalping treatment also had positive effect in reducing the exotic weed seed bank germination, it also removes nutrient and microbial organisms which are important for native species' growth, and as indicated earlier, accumulates a large amount of excess soil on the site. In the treatments of hot green waste and its combinations with mulch and sugar, adding carbon will arise from the sugar and the wood chips, which will increase the microbial activity in the soil and thus provide a suitable environment for native species regrowth.

3.6.3 Effects of hot green waste temperature treatment on viability of seeds

As previously noted, the existence of a soil seedbank is a significant problem for restoration of native grassland, because it is loaded with exotic seeds which often have long residence times. This work with hot green waste was carried out to determine the effect of the high temperatures generated from the decomposing waste piles on the viability of *Themeda triandra* (Kangaroo grass), *Nassella trichotoma* (Serrated tussock) and *Galenia pubescens* (Galenia) seeds. The research hypothesis for this investigation was that high temperatures, which are produced from the decomposition of the hot green waste piles, will be sufficient to kill exotic seeds to a reasonable depth in the soil, and thus prevent their germination.

The trial was commenced during June 2014, and seeds of *Themeda triandra*, *Nassella trichotoma* and *Galenia pubescens* were buried in the study area south east of Werribee River, Victoria. Seeds of each species were placed in 3 cm x 3 cm nylon mesh bags (50 seeds per bag), then buried at three different depths; at the surface, 5 cm and 10 cm under both the control and hot green waste pile treatments, with four replicate seed bags.

To measure the temperatures generated from the decomposition processes, in parallel with the buried seeds, 15 temperature data logger buttons (DS1922L Thermochrom iButton high resolution data logger) were placed under the piles at three different depths: 0, 5 and 10 cm respectively for each of the four hot green waste treatments and the control site. The buried seed bags and temperature data loggers were collected after 62 days when the green waste pile were removed on 15th August 2014.

The viability test for all seeds was conducted at Federation University laboratories with 2, 3, 5-triphenyl tetrazolium chloride solution by using the procedure recommended for Solanaceae species (Peters, 2000). The seeds were soaked in water for 24 hours, then cut transversely with a scalpel to expose the embryo. This was then exposed to 1% TTC solution for 24 hours at 25^{O}C. The seeds were removed from the tetrazolium solution, then washed with distilled water and the red-coloration of embryos was evaluated. Seeds in which the embryos exhibited a reddish colour were scored as viable, while seeds where the embryo showed no overall carmine red staining were counted as non-viable (Kambizi *et al.,* 2006).

3.6.3.1 Statistical analysis of seed viability

Seed viability data of each species were analysed separately with the MINITAB 17 statistical package (Minitab, 2014) for two treatments (hot green waste and control) under three different burial depths (0, 5 and 10 cm) using a General Linear Model (GLM). Seed viability value as a percentage were tested for normality using the Ryan-Joiner test, which is similar to the Shapiro–Wilk test (a = 0.05). Non-normal distributed data were angularly transformed. GLM with two factors (two-way) ANOVA were used to assess the effect of the various factors (two treatments and three depths) on seed viability of each species separately. Tukey's test with a significance of 0.05 were applied to test for significance between the all factor means and their combinations.

3.6.3.2 Results of seed viability

The composting green waste mulch reached temperatures above 60^{O}C for as long as 15 days in some treatments. The maximum temperature recorded was 63^{O}C at 5 cm depth while, as expected, the temperatures in the control plot remained below 20^{O}C in the surface, 5 cm and 10 cm soil depths (Figure 3.21). The heat generated from the hot green waste pile has dramatically reduced the viability of buried seeds at different soil depths.

The mean viability percentage of *Themeda triandra* seeds buried at surface, 5 and 10 cm soil depth under hot green waste pile were 0.3, 0.3 and 2% compared to control 76, 75 and 86% respectively (Figure 3.22 A,. Table 3.5).

Similar results were observed for *Nassella trichotoma* and *Galenia pubescens* seeds;buried at surface, 5 and 10 cm soil depth under hot green waste pile were 2, 2.75 and 6.75% compared to control 82, 71 and 82% respectively (Figure 3.22 B,. Table 3.5) and were 2.75, 5 and 13% compared to control plot 80, 73 and 82% respectively (Figure 3.22 C, .Table 3.5).

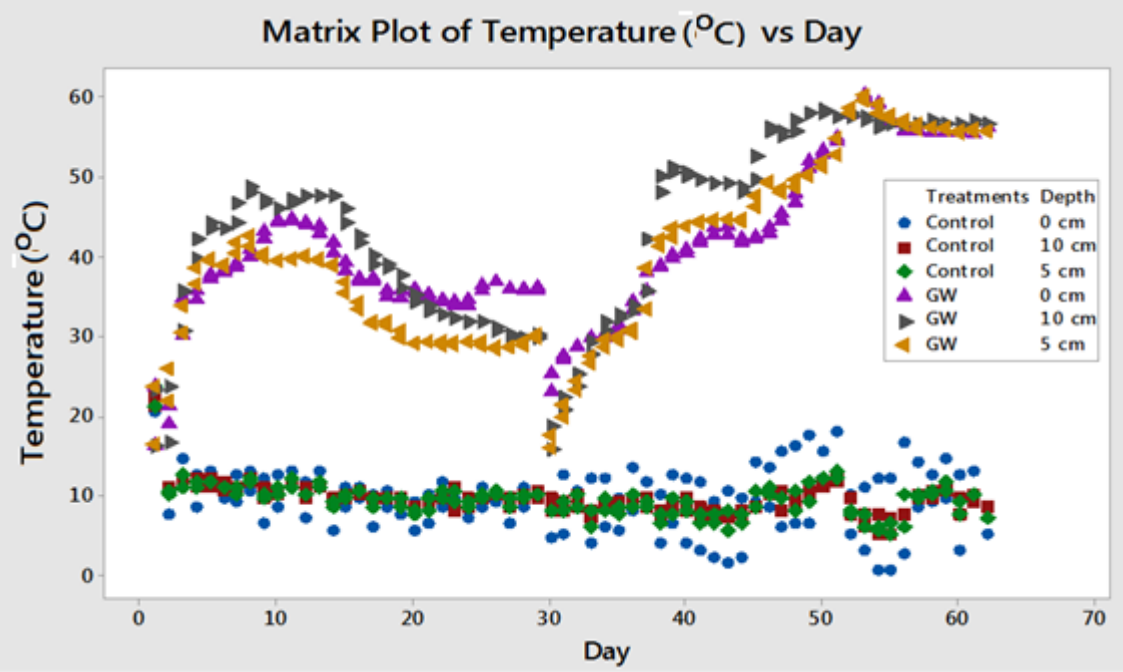

Figure 3.21 Matrix plot of temperature vs day, showing the value of temperature degree of data loggers at hot green waste and control under three different depths (0, 5 and 10 cm), created with MINITAB 17 statistical package (Minitab, 2014).

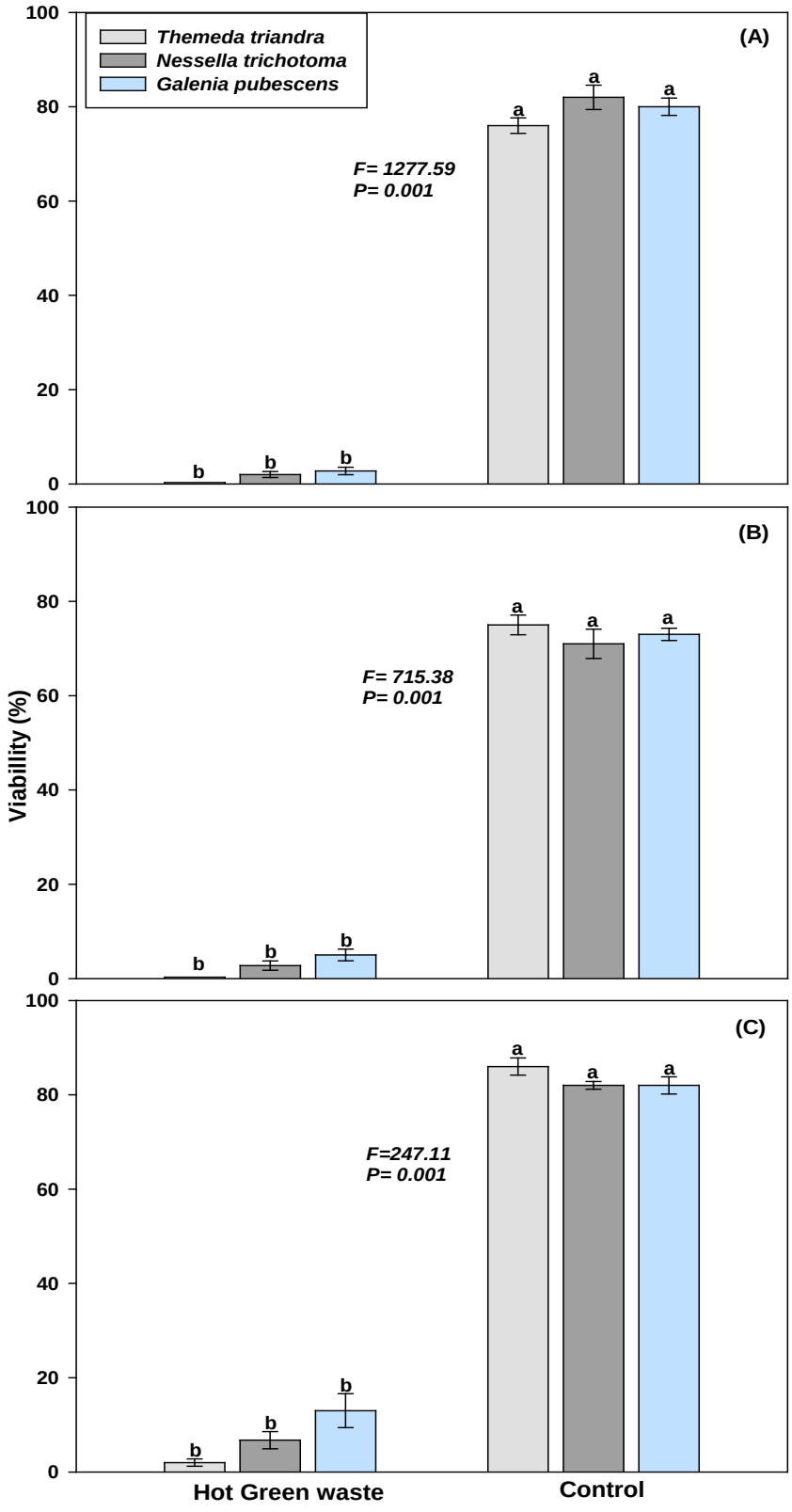

Figure 3.22 The effect of hot green waste on seed viability of (A) *Themeda triandra,* (B) *Nassella trichotoma* and (C) *Galenia pubescens* under three different depths 0, 5 and 10 cm. Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

Table 3.5. The effect of hot green waste on seed viability of *Themeda triandra, Nassella trichotoma* and *Galenia pubescens* under three different depths (0, 5 and 10 cm). The data were angularly transformed.

		Themeda triandra			*Nassella trichotoma*			*Galenia pubescens*		
Source	**DF**	**SS**	**F-Value**	**P-Value**	**SS**	**F-Value**	**P-Value**	**SS**	**F-Value**	**P-Value**
Treatments	1	11.0057	2569.75	**0.001**	8.8469	457.52	**0.001**	7.83516	239.15	**0.001**
Depth	2	0.1041	12.16	**0.001**	0.0708	1.83	0.170	0.03798	1.16	0.321
Treatments* Depth	2	0.0057	0.66	0.521	0.0330	0.85	0.432	0.03828	0.58	0.561
Error	54	0.2313			1.0442			1.76918		
Total	59	11.3635			10.0139			9.81148		

3.6.3.3 Discussion of seed viability

The research hypothesis for this investigation was that high temperatures, which are produced from the decomposition of the hot green waste piles, will be sufficient to kill exotic seeds to a reasonable depth in the soil, and thus prevent their germination. Clearly, this has occurred at three different depths (0, 5 and 10 cm) which suggests that I can be confident that the seed bank will not be a source of exotic (or native) seeds. Larney and Blackshaw, (2003) study showed that beef feedlot manure composts reached temperatures as high as 68^{O}C and that the composting process drastically reduced the viability of a range of weed seeds. They also showed that the temperatures required to achieve complete elimination of viability was weed species dependent as was the duration of the temperature. In this study I identified oversize green waste from kerbside collection as a potential source of recycled carbon that could be re-used for weed management and to possibly alter soil nutrients to benefit native grassland restoration. Mathur (1998) argues the importance of composting for preserving carbon and mineral nutrients in an ecosystem. In conclusion, this trial has shown that large volume oversize green waste compost can heat soils down to 10 cm deep to above 50-60^{O}C temperatures for several days killing mature *G. pubescens* and *N. trichotoma* plants while also destroying 95% of their seedbanks. This has enabled direct seeding of native seed mix with little competition from a resident seedbank and early assessments of its establishment are promising.

3.6.3.4 Management Implications of seed viability

The management implications are also clear. Treatment with hot green waste, which is plentiful and cheap, will give a blank slate to begin the restoration of the grassland in terms of the seed problem. However, there are three issues to be mentioned. The first is new ingress of exotic seeds from surrounding areas of land; the second is the possibility that seeds still reside >10cm under the surface and could be exposed by tilling or other ground disturbance; and the third is that the soil characteristics need to be brought back to their original levels. A final thought is that the application of waste could also acts as a vector for ingress of exotic seeds.

3.6.4 Soil nutrients study

The previous sections have concentrated on the change in vegetation cover and the change in the soil seedbank composition after various interventions. However, because effects of cropping have also altered the physical and chemical properties of the soil, any amelioration of these effects by the interventions need to be carefully examined, and this work is presented here.

A total of five soil samples were collected from each plot at two times: on the 15th August 2014 after removal of hot green waste piles, and on the 20th April 2016, to determine the effect of the treatments on the condition of soil nutrients at the study site, as compared to a control. The soil collection, transferring and preparation methods were as described in Chapter 2 ‘Current soil nutrient conditions’.

3.6.4.1 Statistical analysis of soil nutrients

Prior to statistical analysis, with the exception of pH, results of chemical analyses of soil were logarithmically transformed ($\log_{10}$) so that the residual variation in the analyses did not systematically change as the mean changed (McDonald, 2009). Chemical measurements for ammonium nitrogen, nitrate nitrogen, phosphorus, organic carbon, pH and potassium, were analysed with the MINITAB 17 statistical package (Minitab, 2014) using a General Linear Model (GLM). Tukey’s test with a significance level of 0.05 was

applied to test for significance differences between the means of the measured soil factors. The mean data were back transformed.

3.6.4.2 Results of soil nutrients

The results of chemical measurements on the study site soil, across two years of monitoring assessment, are presented in Table 3.6 and Figures 3.23, 3.24, 3.25, 3.26, 3.27 and 3.28. Generally, it can be seen that the application of hot and cold green-waste treatments and their various combinations caused an increase of most nutrient levels, especially nitrogen and phosphorus. The scalping treatment led to a decrease of all nutrient levels, but unexpectedly raised the pH level, which was consequently higher than that seen in the control and other treatments.

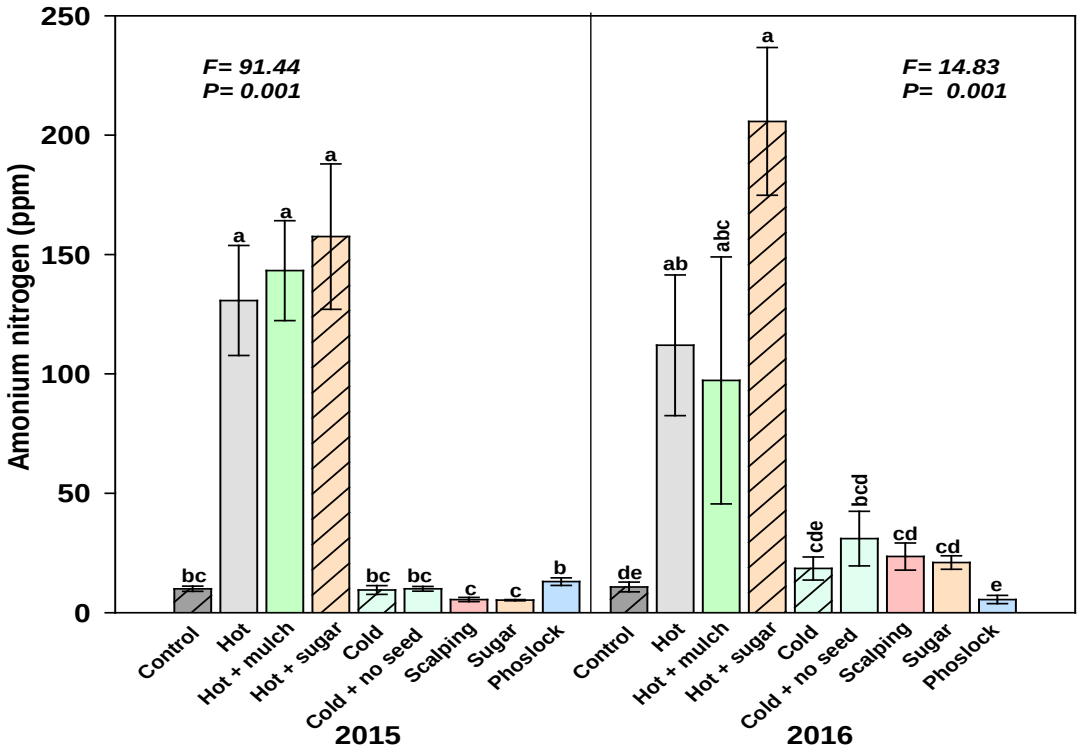

Figure 3.23 Mean value of ammonium nitrogen (ppm) during 2015 and 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

The ammonium nitrogen content in the various treatments across the two years, ranged from 5.2–146.9 and 10.1–198.6 ppm in 2015 and 2016 respectively (Figure 3.23, Table

3.6), and it can be seen that the amount of ammonium nitrogen was significantly higher at hot, hot + mulch and hot + sugar treatments, compared to the controls, for both sampling periods. The lowest ammonium nitrogen readings were recorded for sugar only and scalping treatment, which were 5.2 and 5.3 ppm respectively, but these were not significantly lower than the control (9.8 ppm).

In 2016, the amount of ammonium nitrogen increased at the hot + sugar treatment site to 198.6 ppm compared to 2015, but it declined at the hot and hot + mulch treatments to 101.2 and 63.4 ppm, respectively. These values were still significantly higher than control, sugar and Phoslock® treatments in 2016. The amount of ammonium nitrogen increased slightly in 2016 the cold and cold + no seed. The amount of ammonium nitrogen declined at the Phoslock® treatment site from 12.7 ppm in 2015 to its lowest value of 4.7 ppm in 2016 (Table 3.6).

Figure 3.24 shows the levels of nitrate nitrogen (ppm) across the two year assessment periods (2015 and 2016). In 2015, the highest nitrate nitrogen amounts were recorded at cold + no seed, cold and Phoslock® treatments (92.6, 83.8 and 53.3 ppm respectively) and these were significantly higher than control ($p= 0.040$), scalping ($p= 0.001$), sugar ($p=0.000$) and hot + sugar ($p= 0.011$) treatments (Table 3.6). The lowest nitrate nitrogen amount was recorded at the scalping treatment site (5.6 ppm) which was significantly different to the control value (19.1 ppm).

In 2016, the trend shows an increase in the amount of nitrate nitrogen at most treatments except cold green waste and Phoslock® treatment plots. After two years of treatment, hot green waste and its combination hot + sugar and hot + mulch led to an increase in the amount of nitrate nitrogen. The amount of nitrate nitrogen at the hot + sugar ($p= 0.001$) and hot green waste treatments ($p= 0.001$) were significantly higher than at the control plot. Sugar and scalping treatments had lower nitrate nitrogen value (24 and 29 ppm) respectively than control (41 ppm), but these differences were not significant. The

amounts of nitrate nitrogen at Phoslock® and cold green waste treatments were 34.1 and 47.5 ppm correspondingly (Table 3.6).

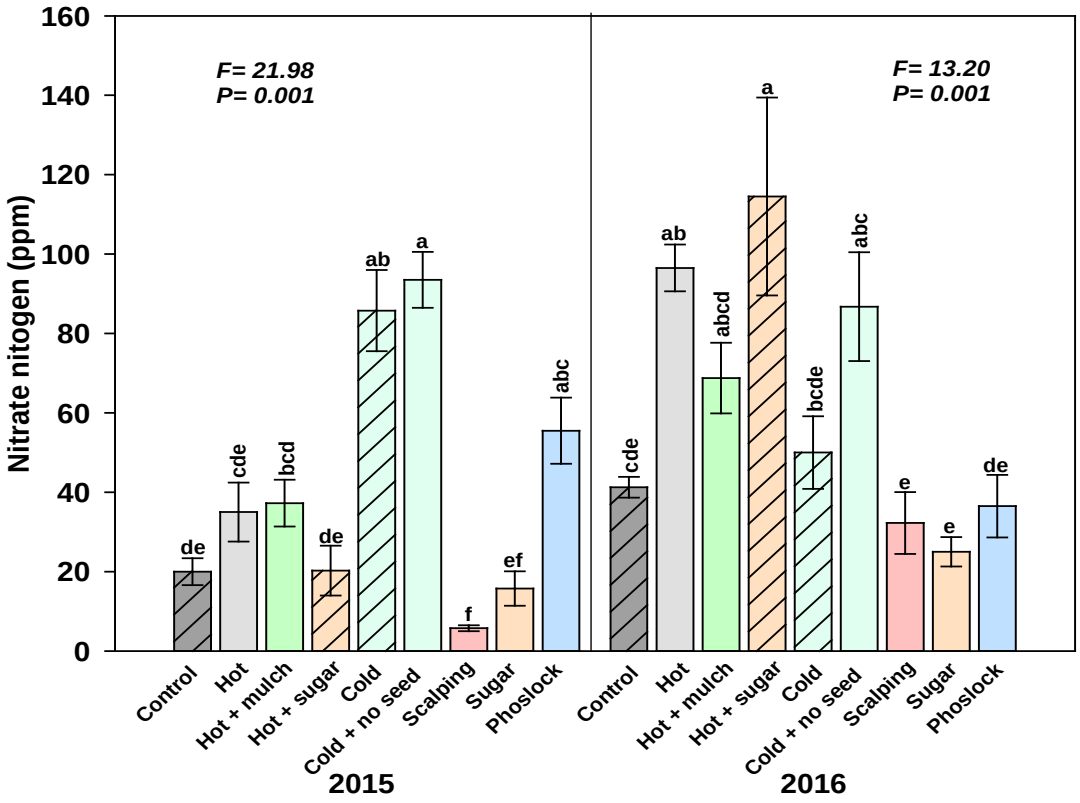

Figure 3.24 Mean value of nitrate nitrogen (ppm) measured during 2015 and 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

Figure 3.25 shows the amount of phosphorus (ppm) at different treatment sites across the two years of assessment (2015 and 2016). In 2015, the phosphorus level in scalping plot 17.3 ppm (p= 0.001) was significantly lower than the control and other treatments plots. There was an increase of phosphorus amount at all other treatments, especially the cold and cold + no seed treatments which were 162.8 ppm and 156.8 ppm (p= 0.001), and these values were significantly higher than at the control site at 87.4 ppm (Table 3.6).

In 2016, the amounts of phosphorus at the cold and the cold + no seed treatments were 204.4 and 218.6 ppm which suggests that the concentration of phosphorus will increase over time with the use of cold green waste. There was no change between 2015 and 2016

for phosphorus levels at the scalping treatment sites, which remained significantly lower than control and other treatments. Phoslock® and sugar treatments had lower amounts of phosphorus 78.1 and 79.9 ppm than control 82.8 ppm (p= 0.209; Table 3.6), but these differences were not significant.

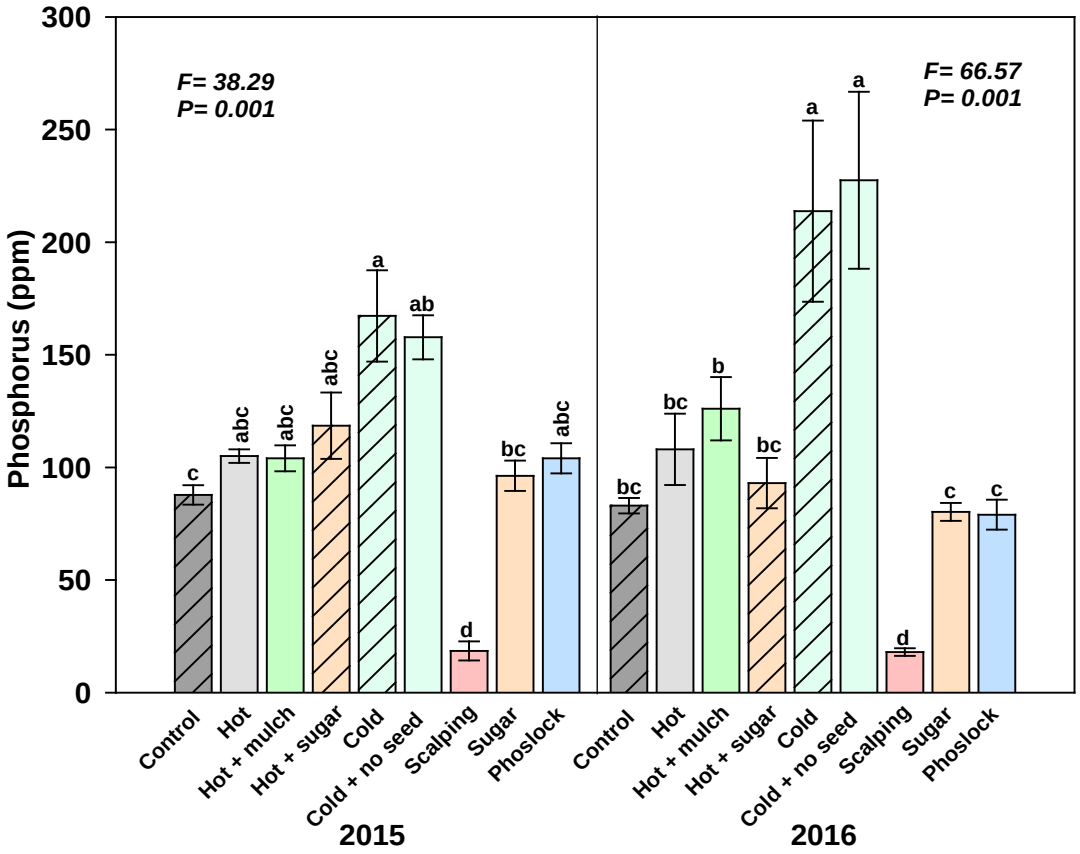

Figure 3.25 Mean value of phosphorus (ppm) measured during 2015 and 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

The mean percentages of organic carbon of different treatments across two assessment years 2015 and 2016 are presented in Figure 3.26. In 2015, the scalping treatment had a significantly lower percentage organic carbon 1.5 % (p= 0.001) than the control site (p=0.658), as well as for all other treatments. The highest organic carbon was recorded at the cold + no seed treatment 3.5 % as shown in Table 3.6.

In 2016, the organic carbon (%) remained the same at the scalping treatment site, but there was a slightly increased organic carbon level at the control, hot green waste and hot

+ mulch treatments. There were significant differences of organic carbon at cold and cold + no seed treatments (p= 0.001) after two years of application (4.1 and 4%) respectively compared to the control, and also with the hot, hot + sugar, Phoslock® and scalping treatments (Table 3.6).

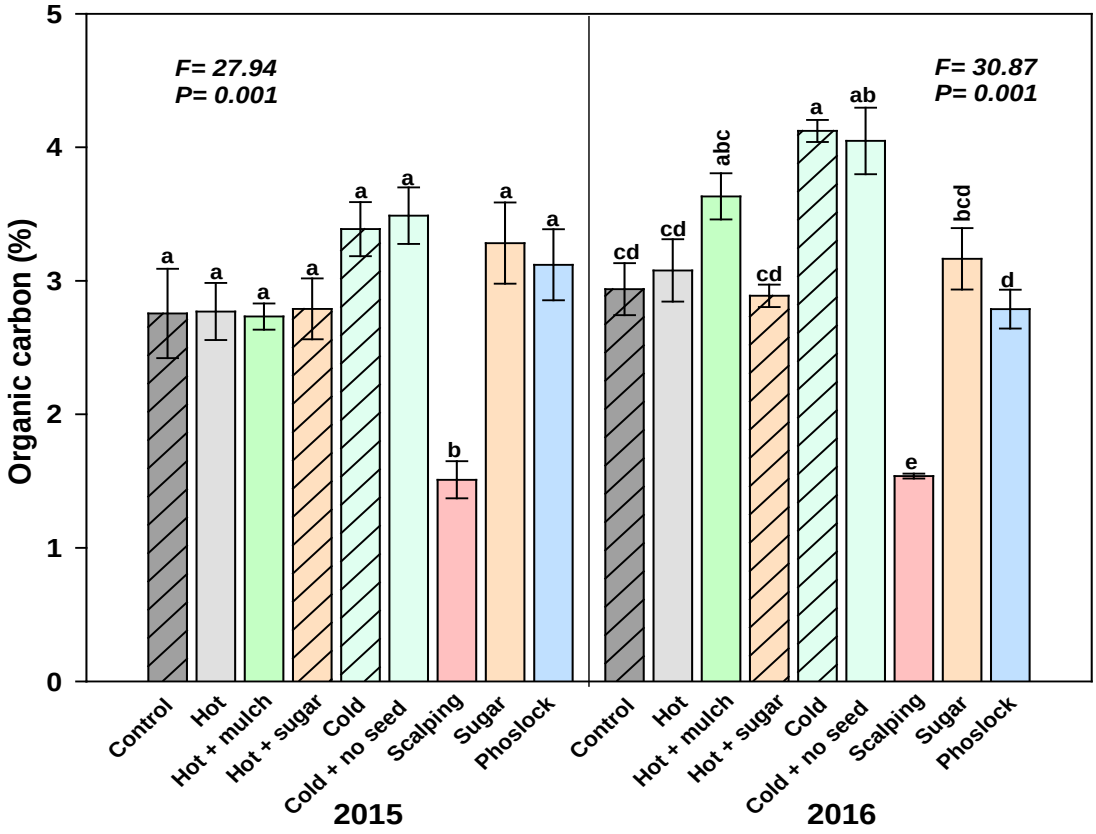

Figure 3.26 Mean value of organic carbon (%) during 2015 and 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

Figure 3.27 records the pH levels of different treatments across the two years of assessment (2015 and 2016). In 2015, the pH level at the scalping treatment site was 7.1 (neutral) which was significantly higher than the control site (p= 0.001) and other treatments. The pH level of cold + no seed and cold green waste treatments were moderately acid (5.8 and 5.9 respectively; Table 3.6). The pH level in the control plot and the other treatments was slightly acid.

In 2016, the pH level of all treatments were slightly increased except for the control site which remained the same at 6.1. Although this overall increase did not change the status of the neutral and slightly acid pH soil values, it has changed the status of cold and cold + no seed treatments from moderate acid to slightly acid soils 6.6 and 6.3 (Table 3.6).

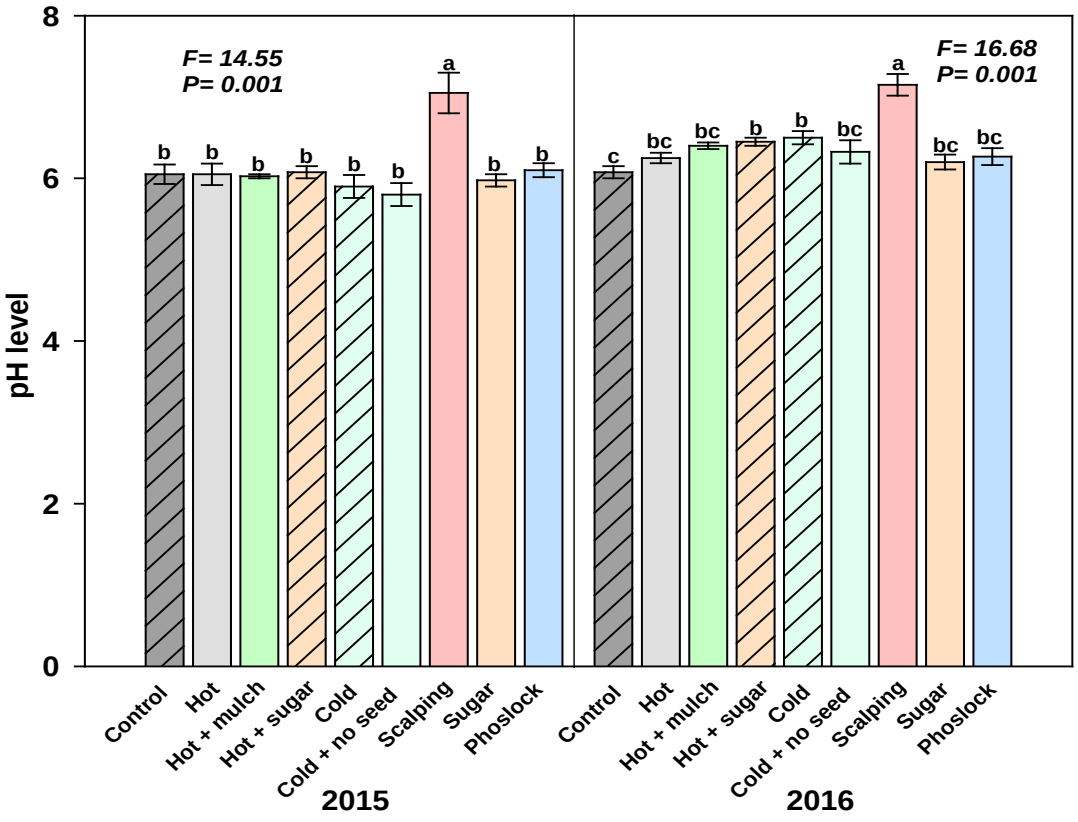

Figure 3.27 Mean value of pH level at 2015 during 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

The potassium content in the different treatments across the two years 2015 and 2016, ranged from 526.4 to 1197.2 ppm during 2015 and from 527 to 1616.7 ppm in 2016 (Figure 3.28). The application of the treatments all caused an increase of the amount of potassium especially in the case of the use of hot and cold green waste alone and their combinations (Table 3.6).

In 2015, the amount of potassium at hot, cold and cold + no seed treatments (1231.9, 1197.2 and 1146.2 ppm) were significantly higher than control, scalping, sugar and

Phoslock® treatments (526.4, 612.6, 631.8 and 778.7 ppm respectively) as shown in Table 3.6.

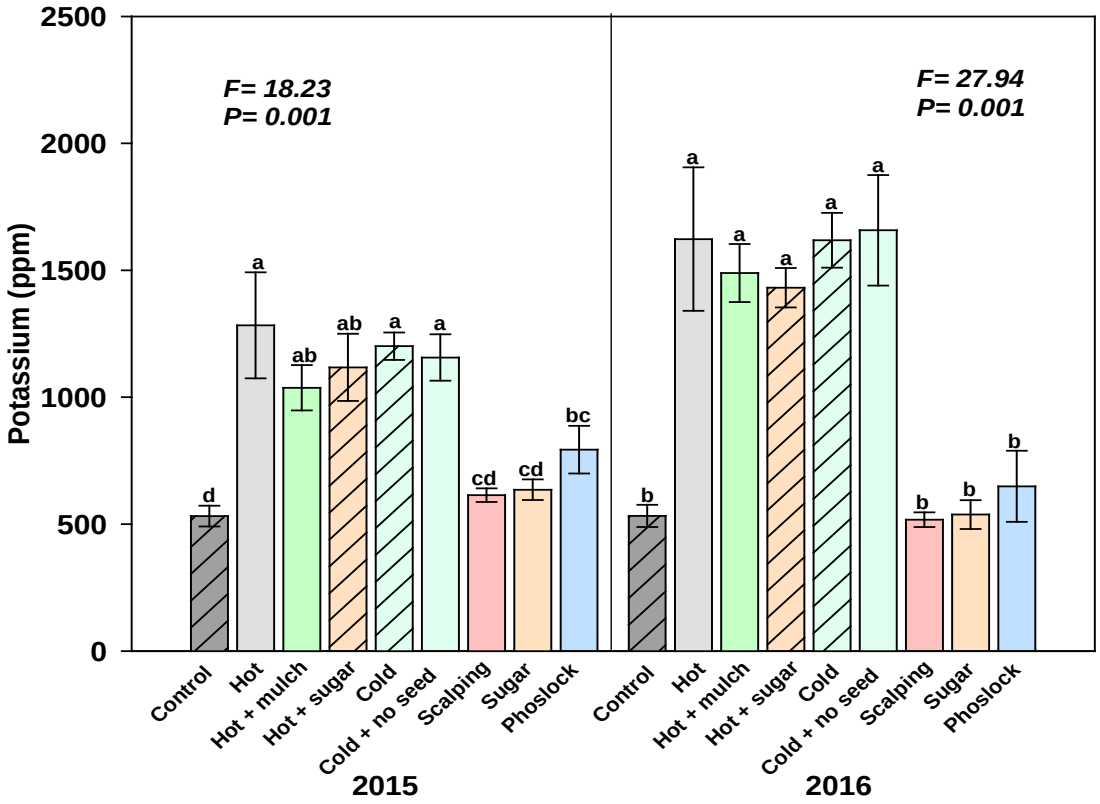

Figure 3.28 Mean value of potassium (ppm) during 2015 and 2016, after application of treatments. Vertical bars represent ± standard error of the means; the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

Similarly, in 2016, the amounts of potassium were significantly higher at cold + no seed, cold, hot, hot + mulch and hot + sugar treatments (1616.7, 1607.5, 1560.6, 1476 and 1424.8 ppm respectively) as recorded in Table 3.6. In contrast, the amounts of potassium declined slightly at scalping, sugar and Phoslock® treatments (514.9, 528.1 and 611.7ppm). There were significant differences between hot and cold green waste and their combinations with control, scalping, sugar and Phoslock® treatments.

3.6.4.3 Discussion of soil nutrients

To aid systematic evaluation of these treatments, seven hypotheses were suggested and tested using field experiments or laboratory investigations, namely:

I. The first hypothesis was that the hot green waste treatment, will lead to an increase in the carbon content and a decrease in the nitrogen level in the soil;

II. The second hypothesis was that the application of hot green waste, followed by the addition of mulch, will lead to an increase in the carbon content as well as a decrease in the nitrogen level in the soil;

III. The third hypothesis was that the hot green waste treatment, in combination with added sugar as a carbon source, will lead to an increase in the carbon content as well as a decrease in the nitrogen level in the soil;

IV. The fourth hypothesis was that the application of cold green waste, will lead to an increase in the carbon content as well as a decrease in the nitrogen level in the soil;

V. The fifth hypothesis was that scalping the topsoil, will lead to a decrease in the nitrogen and phosphorus levels, and the organic carbon content, in the soil;

VI. The sixth hypothesis was that the application of sugar will lead to an increase in the carbon content as well as a decrease in the nitrogen level in the soil; and

VII. The seventh hypothesis was that treatment of the topsoil with Phoslock®, will lead to a decrease in the phosphorus level in the soil. This latter hypothesis will be further investigated in laboratory conditions.

Regarding the first three hypotheses, that application of hot green waste alone and in combination with sugar and mulch, would lead to an increase in the carbon content as well as a decrease in the nitrogen level in the soil, were rejected because the results of soil nutrients analysis was opposite to our expectations. The levels of ammonium nitrogen increased dramatically during the 2015 sampling years directly after the application of hot green waste, which may have been due to mobilisation of N from the green waste material through decomposition. Total nitrogen amount of hot green waste was 0.85% before the application at 2014. Water content and temperature of the green waste pile are known to influence the mobilization of nitrogen. In the field trials, all green waste piles were watered to increase the microbial activity (see section 3.5.7 Hot green waste). This led to a rise in

the pile temperature to 62^{O}C (see section 3.6.3 Effects of hot green waste temperature treatment on viability of seeds). The increase in the nitrate nitrogen, phosphorus and potassium levels may be a result of the high temperatures created from hot green waste, or, alternatively, it might be due to the waste containing large amounts of residual N-P-K fertilizer which has moved to soil during the treatment. Duong *et al.* (2013) established that nutrients released during the decomposition of compost move into the adjacent soil resulting in increased N and P availabilities as well as increased microbial biomass and activity. Similarly, Gu *et al*. (2009) established that green waste compost application can increase soil total nitrogen and available phosphorus contents. Scotti *et al.* (2016) examined the impact of commercial compost from organic fraction of municipal solid waste and compost of plant residues (green waste) on soil quality and found that the total nitrogen was increased by 60 and 40%, respectively, compared to the untreated soil. Similarly, Keeling *et al.* (2003) found that the use of mature green waste compost can reduce reliance on conventional fertilisers. However, losses of N through leaching may be possible, and hence increases in N may be a short-term effect.

The fourth hypotheses was rejected because the level of nitrate nitrogen was significantly higher in the cold green waste treatment compared to the control. Similarly, Miller and Seastedt (2009) showed that woodchips did not reduce plant-available soil nitrogen at ambient levels of fertility. However, the organic carbon levels were significantly higher than control and other treatments at second sampling year. Indeed, several studies have shown that addition of biologically available carbon (for example as sugar or sawdust) can increase microbial activity which helps to draw down the nitrogen level (Morghan & Seastedt, 1999; Prober *et al.,* 2005; Faithfull *et al.,* 2010). In another study, Blumenthal *et al*. (2003) outlined how carbon additions can promote native species cover. However, in my study, any promotion of microbial activity through carbon addition has not been able to offset the large addition of N introduced by the green waste itself. The increase of phosphorus and potassium levels also suggests that these have been introduced in the

urban green waste at high levels, and mobilized sufficiently so that they are available to plants.

The fifth hypothesis was partially accepted because the scalping treatment showed significantly lower levels of nitrate nitrogen, 5.6 ppm, phosphorus 17.3 ppm and organic carbon 1.5% than the control at 2015, and this trend was similar for phosphorus and organic carbon levels in 2016, but the amount of nitrate nitrogen was not significantly lower than control in 2016. There was no significant differences in the amount of ammonium nitrogen and potassium at both sampling years 2015 and 2016. The pH level was significantly higher than control and all other treatments in both sampling years 2015 and 2016, as scalping removed the acidic sand at the top of the soil profile, exposing the more calcareous clay below it. The pH level in the current study concur with the results of other studies (Allison & Ausden, 2004; Jaunatre *et al.,* 2014). These results clearly showed that the scalping treatment is sufficient to remove all unwanted exotic species and excessive inorganic nutrients. This physical removal of the soil matrix is designed to reduce soil nitrate nitrogen, phosphorus and the exotic seed bank (Gibson-Roy, 2000; Allison & Ausden, 2004; Gibson-Roy *et al.,* 2010a; Jaunatre *et al.,* 2014), and thus it has been previously reported that the scalping treatment is an effective tool for the restoration of degraded grassland on the Victorian Volcanic Plains (Gibson-Roy, 2010a; Brown, *et al.*, 2017; Morris & Gibson-Roy, 2018).

The sixth hypothesis was rejected – the sugar treatment did not significantly reduce soil nitrogen or increase soil carbon. The ammonium nitrogen and nitrate nitrogen levels were decreased at first sampling year 2015 but these differences were not significant compared to the control. Whilst, Eschen *et al*. (2007) showed that carbon addition during grassland restoration is a useful management technique to reduce nitrogen availability on ex-cropping land, and it has been shown that addition of carbon can also reduce inorganic nitrogen and exotic plant biomass, other studies have shown that addition of sugar with an application rate of 5 t/ha every 3 months, is unlikely to be practical or affordable,

restricting its use to small remnants or high-value conservation areas (Perry *et al.,* 2010; Morris & De Barse 2013; Brown, *et al.*, 2017).

Finally, the seventh hypothesis was rejected – the Phoslock® did not significantly reduced the phosphorus level in the soil. The level of phosphorus was higher than the control in 2015, but this level decreased slightly in the second sampling year (2016). It was predicted that introducing Phoslock® into the ex-cropland soil would bind the available phosphorous in an insoluble form, thus making it unavailable to plants (Geurts *et al.,* 2011), but this was not found to be a practical or economic approach.

3.6.4.4 Management Implications of soil nutrient

The results of soil nutrient level analysis clearly indicated the positive effect of some of the restoration methods regarding the reduction of nitrogen and phosphorus levels and increasing the organic carbon content. However, it became clear that addressing the various barriers to restoration management in the VVP grassland brought a number of complications. For example, the scalping treatment removed the exotic weed bank and nitrogen and phosphorous accumulations, but decreased the organic carbon and increased the soil pH level. The hot, hot+sugar, hot+mulch, cold and cold+no seed treatments were sufficient to reduce the exotic weed species cover and to facilitate recruitment of native species with an increase the organic carbon content, but they led to an increase in nitrogen, phosphorus and potassium levels. The Phoslock® treatment was not an effective tool in regard to the reduction of available phosphorus levels. However, the use of Phoslock® will be investigated further in a controlled glasshouse experiment in Chapter 5.

It was thus suggested that successful restoration management must include more than one restoration method, step by step or in combination, to overcome the barriers that accrue throughout the management process. It was also evident that the hot and cold green waste treatment was not an ideal restoration method in Australia due to the significant increase in available nitrogen, phosphorus and potassium levels in the soil.

This will favour the spread of exotic weeds which will outgrow any native species. That said, the hot and cold green waste material might be a good restoration tool in some African and East European countries which have poor soil nutrient levels, or possibly in agricultural lands as a method to improve the soil nutrients and improve the production yield in crops.

Table 3.6. Summary of mean values of ammonium nitrogen (ppm), nitrate nitrogen (ppm), phosphorus (ppm), organic carbon (%), pH and potassium (ppm) resulting from experimental treatments assessed in 2015 and 2016.

Treatments	Ammonium Nitrogen (ppm)		Nitrate Nitrogen (ppm)		Phosphorus (ppm)		Organic Carbon (%)		pH Level		Potassium (ppm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	9.8	10.1	19.1	41.0	87.4	82.8	2.7	2.9	6.1	6.1	526.4	527.0
Hot	124.1	101.2	32.1	95.9	104.9	104.8	2.7	3.0	6.1	6.3	1231.9	1560.6
Hot + mulch	139.1	63.4	35.8	66.7	103.5	123.9	2.7	3.6	6.0	6.4	1025.0	1476.0
Hot + sugar	146.9	198.6	17.2	105.5	115.6	91.1	2.8	2.9	6.1	6.5	1093.1	1424.8
Cold	8.9	16.4	83.8	47.5	162.8	204.4	3.4	4.1	5.9	6.5	1197.2	1607.5
Cold + no seed	9.9	24.8	92.6	83.5	156.8	218.6	3.5	4.0	5.8	6.3	1146.2	1616.7
Scalping	5.3	21.6	5.6	29.0	17.3	17.8	1.5	1.5	7.1	7.2	612.6	514.9
Sugar	5.2	20.4	13.7	24.0	95.5	79.9	3.2	3.1	6.0	6.2	631.8	528.1
Phoslock®	12.7	4.7	53.3	34.1	103.4	78.1	3.1	2.8	6.2	6.3	778.7	611.7

Whilst the findings derived from Table 3.6 will be discussed in detail in Chapter 6. This summary Table and the previous discussion of results has been presented to allow a first appreciation of the effects of the separate treatment regimes on nutrient levels by simple comparison with the 'Control' values. As can be clearly seen from the spread of values, management of the nutrient and mineral content of soils in ex-cropland is a significant and complex problem, and it will require careful interpretation of the outcomes of various treatment methods before sensible management strategies can be offered.

3.6.5 Soil bulk density

To determine whether there were any significant effects of applied treatments on soil bulk density of the study sites, soil samples were collected on 2nd May 2016 using a cylindrical steel core cutter (7.5 cm long and 7.5 cm diameter). This period of two years (since application of treatments) was thought sufficient to indicate whether these non-invasive treatments would make any significant alteration to the soil bulk density. Three soil cores were randomly collected from each 5 m x 5 m plot and bulk density was calculated. The soil collections, transferral and preparation methods with the bulk density procedure were previously detailed in Chapter 2 'Soil bulk density'.

3.6.5.1 Statistical analysis of soil bulk density

One-way ANOVA was used to assess the effect of treatments on soil bulk density. Data were tested for normality using the Ryan-Joiner test (similar to the Shapiro–Wilkes test) ($a = 0.05$) before the ANOVA, then logarithmically transformed ($\log_{10}$) so that the residual variation in the analyses did not systematically change as the mean changed (McDonald, 2009). Data were analysed with the MINITAB 17 statistical package (Minitab, 2014). Tukey's significance level of 0.05 was applied in all cases to determine if there were significance differences between the means. The mean data were back transformed.

Table 3.7. One way ANOVA of effects of the treatments on the soil bulk density. The presented data were logarithmically (log_{10}) transformed.

Source	DF	SS	F-Value	P-Value
Treatments	8	0.043	3.63	**0.001**
Error	99	0.147		
Total	107	0.191		

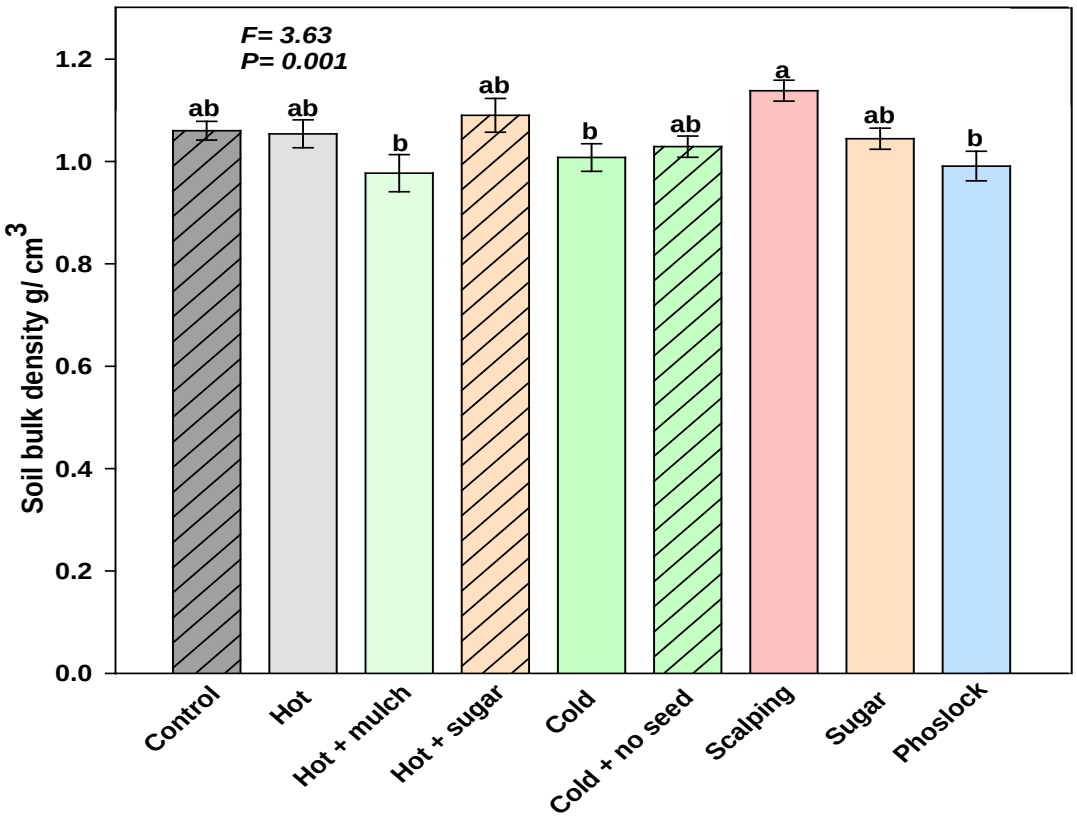

Figure 3. 29 The effect of treatments on soil bulk density of experiment site. Vertical bars represent ± standard error of the means, the different letter on the vertical bars represent the significant differences between the treatments using Tukey's test.

3.6.5.2 Results of soil bulk density

Results indicated that there were significant differences in mean soil bulk density between treatments as shown in (Table 3.6; Figure 3.29). The highest soil bulk density recorded was for the scalping treatment 1.14 g/cm^3 , and this was significantly higher than hot + mulch and Phoslock® treatments at 0.970 and 0.986 g/cm^3 respectively. There was no significant differences between all applied treatments compared to the control plot (no treatment). The mean soil bulk density of the control plot was 1.058 g/cm^3 (Figure 3.29).

3.6.5.3 Discussion of soil bulk density

Compaction of soil presents a problem for restoration because it presents a barrier to water and nutrient permeability around the roots of developing plants. Soil compaction could occur as a result of physical stress from machinery (Soane & Van Ouwerkerk, 1994) or possibly from some forms of soil composition change (Chaudhari *et al.,* 2013). The treatments were non-invasive, and whilst it was hoped that alleviation of soil compaction might be achieved by alteration in soil composition, it does not appear that this has occurred.

3.6.5.4 Management Implications of soil bulk density

These investigation into changes of soil compaction as a result of non-invasive treatments would suggest that further physical manipulation of the soil may be needed. This could lead later to a discussion of seed-bank results and soil compaction needs – there will be a problem if soil is disturbed below 10 cm because exotic seeds may be exposed. This again demonstrates the complexity of the situation, and may suggest that if soil compaction is a problem, tilling may need to be done first, before application of hot green waste, cold green waste and sugar addition treatments.

3.6.6 Microbial activity measurement with cotton strips

Soil micro-organisms play an important role in degradation of organic matter and in nutrient cycling in the soil (Bing-Ru *et al.,* 2006). Cotton strips were used to determine the presence of microbial activity in the soil (Correll *et al.* 1997). The degradation of cotton is widely used as a cheap and simple index of microbial activity in a variety of environments (Lategan *et al.,* 2010), and is based on the premise that the breakdown of cellulose, such as is found in cotton, is almost exclusively carried out by microbiological processes.

This trial investigates the relationships between cotton tensile strength loss, abundance of cellulose-degrading organisms and general microbial activity. It considers the suitability of cotton tensile strength loss to be a convenient and reliable surrogate for general microbial activity in the degraded native grassland.

Investigations were conducted at the experimental site on 10th February 2016. Four 5 cm x10 cm cotton strips were buried to a 10 cm soil depth in each plot. The subsequent collection of strips was undertaken after 60 days on 11th April 2016. The collected cotton strips were placed in nylon bags, labelled, and then transported to Federation University laboratories. They were washed with deionized water and dried. When dry, the tensile strength was measured by a Stable Micro Systems Texture Analyser (Model TA-XT Plus).

Approximately 1 cm long portions of the ends of each strip were placed in the holds of the apparatus, and were adjusted to be sufficiently tight to ensure that the strips did not slip during the pulling process, but not so tight as to tear the strips at their point of contact. They were lined with cotton-based tape to prevent slippage. Strips were pulled and maximum tensile strength was recorded for each strip.

Tensile loss was expressed as percentage of the initial tensile-strength lost per day of incubation, and determined using the following formula (Tiegs *et al.,* 2013):

$$\textbf{\textit{Tensile Loss}} = 1 - \left[\left(\frac{\textit{Tensile strength treatment strips}}{\textit{Tensile strength reference strips}}\ x\ 100\right)\right] / \textit{Incubation time}$$

where 'tensile strength treatment strips' is the maximum tensile strength recorded for each of the strips incubated in the field, and 'tensile strength reference strips' is the mean tensile strength of eight strips that were not buried in the field but were stored in a desiccator.

The main objective of this trial was to assess the effect of each treatment on the degrading rate of the buried cotton strips, and to relate this to the level of microbial activity.

3.6.6.1 Statistical analysis of microbial activity

Data related to the cotton strips' tensile strength loss percentage were analysed with MINITAB 17 statistical package (Minitab, 2014) using a one way ANOVA. All data were tested for normality using the Ryan-Joiner test (a = 0.05). Fisher LSD a significance level of 0.05 were applied to determine the significance differences between the means.

Table 3.8. One way ANOVA of effects of the treatments on the cotton strips' tensile loss.

Source	DF	SS	F-Value	P-Value
Treatments	8	1.674	2.88	**0.019**
Error	27	1.962		
Total	35	3.638		

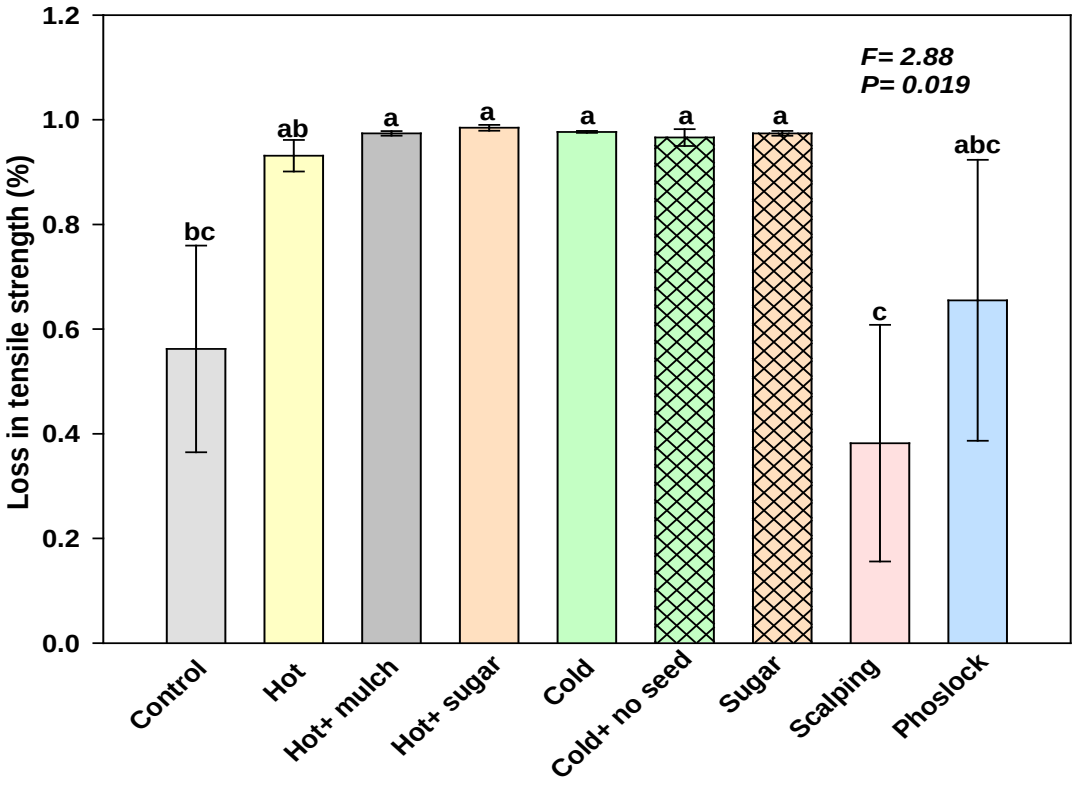

Figure 3.30 The effect of treatments on the cotton strips tensile strength loss rate. Vertical bars represent ± standard error of the means, the different letter on the vertical bars represent the significant differences between the treatments using Fisher LSD test.

3.6.6.2 Results of microbial activity

As indicated, this work is predicated on the assumption that there is a positive relationship between tensile strength loss of the cotton strips and the level of microbial activity in the soil. The results of tensile strength loss of cotton strips that were buried in the treated plots soil and collected after 60 days, are presented in Figure 3.30. It can be seen that there were significant differences in mean loss in tensile strength of cotton strips (p=0.019; Table 3.8) between the soil treatments. The results clearly show the effect of various treatments, especially green waste and sugar, on the tensile strength loss of cotton strips. The mean tensile strength losses were 0.985, 0.977, 0.974, 0.974 and 0.966 % recorded

at hot+ sugar, cold, sugar, hot+ mulch and cold + no seed treatments respectively and these were significantly different (Figure 3.30) from scalping and control treatments respectively (0.382 and 0.562%). In contrast, the mean tensile strength loss of hot and Phoslock® treatments were 0.931 and 0.655%, but these differences were not significant (Figure 3.30) compared to the control treatment.

3.6.6.3 Discussion of microbial activity

As discussed earlier, it is understood that soil microbes can play a significant role in grassland restoration of former agricultural land through (i) their effects on soil porosity, (ii) organic material storage through decomposition, (iii) increased water holding capacity, and (iv) natural nutrient cycling (Jastrow *et al.,* 1998), all of which favour the establishment of native plant species. In addition, microbes play a crucial role in the development and maintenance of soil structure by binding soil elements and organic matter and creating healthy aggregates (Jastrow *et al.,* 1998; Six *et al*., 2000).

In this current study, the effect of different restoration methods on cotton strip degradation (tensile strength loss) as well as soil microbial activity were investigated and tested in all treatments.

It was anticipated that the application of different carbon sources such as hot green waste (Hot), cold green waste (Cold) and sugar would increase the soil microbial activity and produce nitrogen drawdown that should improve the soil for direct seeding of native grasses. Smith *et al*. (2010) showed that by adding biochar to the soil, microbial community structure and function might be shifted by changing physicochemical properties of the soil. Similarly, Steinbeiss *et al.* (2009) asserted that the addition of glucose-derived biochar rarely changed the composition of the soil microbial community. Other studies have shown that the soil organic matter and soil organic carbon are strongly associated with increased soil microbial biomass (Allison *et al.,* 2005; Williamson *et al.,* 2005). However, changes in soil conditions such as pH, soil moisture, temperature, oxygen supply, and available substrate affect the fluidity of bacterial cell membranes,

which is important for molecular transport of substrate and waste between a bacterium and the environment and ultimately, cell growth. Also, ecological stress may reduce overall microbial activity and affect nutrient cycling and energy flow for an entire ecosystem (Schimel *et al.,* 2007).

3.6.6.4 Management Implications of microbial activity

The results of soil microbial activity clearly showed the significant effect of most carbon sources hot+ sugar, hot+ mulch, cold, cold+ no seed and sugar on percentage of tensile strength loss of cotton strips as well as the increase in the soil microbial activity. It was found that the scalping of topsoil has the lowest effect on the cotton strips' tensile strength, and this is thought to be due to the removal of most of the microorganisms from the soil. It is implied that an additional carbon source will arise from the sugar and wood chips, which will increase microbial activity in the soil and thus provide a suitable environment for native species regrowth.

3.7 Conclusion

In an attempt to contribute to a more detailed understanding of the biotic and abiotic barriers to restoration of grasslands in the VVP, in addition to evaluation of traditional techniques such as adding sugar and scalping, some novel restoration approaches such as the loading of hot and cold green waste onto the site, and the introduction of the commercially available phosphorus sequestering material Phoslock® into the soil, have been used. A native species seed mix has been broadcast with most restoration techniques in order to stimulate the establishment of native cover and assisting in the competition with invasive exotic weeds. The results of each of the management techniques on the above-ground vegetation cover, the composition of the soil seed bank, the soil bulk density and the level of microbial activity have been presented. It was found that the use of hot green waste alone, a combination of hot green waste plus mulch), and a mixture of hot green waste plus sugar, all led to a reestablishment of native species: *Austrostipa semibarbata, Bothriochloa macra, Rytidosperma* spp. and *Themeda triandra,*

when the topsoil was deliberately seeded. There was also a significant increase in the level of organic matter in the soil, and this was especially so for the cold green waste treatment which was increased in the second assessment year (2016). This approach has had a positive effect on the microbial activity within the soil, and has also reduced the amount of ammonium nitrogen in the first assessment year (2015).

There was little effect of Phoslock treatment on reduction of the amount of phosphorus amount in the soil, but it did appear to reduce ammonium nitrogen levels during the second assessment year (2016). Scalping has a positive effect in removing all the exotic weeds in the soil seed bank, thus preventing their re-emergence; whilst this treatment is an effective tool to reduce or remove the unwanted high levels of nitrogen and phosphorus levels required for native species reestablishment in the soil. A detailed discussion on the findings and outcomes of this work, and the implications for restoration management, will be given in Chapter 6.

Prior to this discussion, in the next chapter an examination of techniques to break the seed dormancy of *Ptilotus macrocephalus* and *Ptilotus polystachyus* species will be presented. The practical problem of seed dormancy, which needs to be addressed before using these species for glasshouse experiments, will involve a determination of their response to a number of seed pre-treatments: (i) smoke water, (ii) heating shock, (iii) cold stratification and (iv) gibberellic acid. These results will be required in order to use these species in a study aimed to reduce phosphorus level in the soil using plant species. In this latter study, the effect of phosphorous diminution in soil by the use of Phoslock® will be compared with the effect of harvesting plant species such as *Ptilotus* and *Lupin*.

Chapter 4. Overcoming seed dormancy in *Ptilotus macrocephalus* and *Ptilotus polystachyus*: an investigation of treatments to stimulate germination rates

4.1 Introduction

The aim of this thesis is to investigate and test approaches to the restoration of old fields. This thesis has systematically introduced a range of strategies to help understand the best way to overcome abiotic and biotic challenges that prevent native seeds from re-establishing in an ex-cropping area.

To restore old fields, successful reseeding with native seeds is essential. However, the germination requirements of species targeted for restoration are often unknown, and as a result, restoration efforts using native seed have often had poor success rates (Carr *et al.,* 1992). Native seeds in quantities required for restoration are expensive, and as such high germination rates are important to the success and economic viability of restoration efforts. Native seeds exhibit significant dormancy properties that require specific conditions for stimulating germination (Dixon *et al.,* 1995; Plummer *et al.,* 1995; Roche *et al.,* 1997a, b; Morris, 2000; Merritt *et al.,* 2007). The high level of seed dormancy in the Australian flora is related to hostile and arid environments in which many of these plants have evolved; seed dormancy helps prevent germination until a time at which the environmental conditions are suitable for seedling establishment and survival (Commander *et al.,* 2009).

Another barrier to restoration of old fields is high residual phosphorus content in the soil, which is a severe abiotic barrier to native species re-establishment (Ryan *et al.,* 2009). One possible method to address this barrier is to use phosphorus-accumulating plants to help decrease soil phosphorus levels. Two species known to concentrate phosphorous in their tissues as they grow are *Ptilotus macrocephalus* and *Ptilotus polystachyus* (Islam *et al*., 1999; Ryan *et al.,* 2009). Including these species in early-stage restoration efforts in high-phosphorus soils has potential to be a convenient and economical way of reducing

phosphorus levels and preparing the way for further native plant restoration possibilities. The phosphorus-accumulating properties of *Ptilotus* spp. will be further introduced and tested in Chapter 5.

This chapter addresses aspects of both the barriers to restoration discussed above: unknown germination requirements of native species and high residual phosphorus in old field's soil. This chapter describes experimental work to determine the optimum conditions for seed germination of *P. macrocephalus* and *P. polystachyus*. The chapter firstly provides a brief review of seed dormancy and then describes the trials and results. The chapter ends with a recommendation for the most effective germination treatment, which was then used in the glasshouse trials of Chapter 5.

4.2 A review of the nature of seed dormancy

Seed dormancy is an innate seed survival feature that determines the most suitable environmental conditions in which a seed is able to germinate and flourish (Baskin & Baskin, 2004). It can be usefully defined as a mechanism to prevent seed germination during unsuitable ecological conditions when the probability of seedling survival is low, thus conserving the available seed bank resources for more suitable conditions (Bewley *et al.,* 2006). There are three types of dormancy categorised on the basis of their mode of action: these are physical, physiological and morphological dormancy (Baskin & Baskin, 2004; 2014). Physical dormancy is dormancy that is facilitated by the protection of the embryo by an impermeable seed coat, which is an impenetratable layer or layers of material that develops during the maturation and drying of the seed or fruit (Offord & Meagher, 2009). Seeds showing physiological dormancy are, in contrast, water-permeable, but to ensure dormancy they have an internal physiological germination-inhibiting mechanism within the embryo that prevents radicle emergence during unsuitable conditions (Nikolaeva,1977; Baskin & Baskin, 2014). Finally, morphological dormancy prevents germination when the embryo is still underdeveloped, and where seeds need to mature further before germination. If seeds germinate too early, the embryo

tissues will not be differentiated at the time of fruit ripening (Nikolaeva, 1977; Baskin & Baskin, 2004; 2014).

There are several environmental and physiological factors which play a key role in seed dormancy mechanisms, including temperature, light, moisture, fire (both temperature and smoke effects), and the nature of the seed coat (Eira & Caldas, 2000; Geneve, 2005; Martinkova *et al.,* 2006; Sweedman & Merritt, 2006). In this respect, seed dormancy is a complex phenomenon, and may be caused by one or more dormancy type. For example, some seed coats limit movement of moisture and or oxygen into the seed (Offord & Meagher, 2009), having inhibitors that specifically block germination because of external environmental cues (Baskin & Baskin, 2014). Similarly, 'after-ripening' refers to further development of immature seeds once they have left the parent plant, and dormancy can serve to delay germination until after this has happened (Baskin & Baskin, 2004; Erickson *et al.,* 2016). The specific nature of the cues that either prevent or facilitate germination are often difficult to determine.

The wide variety of dormancy mechanisms have been developed because there are many environmental situations encountered by a seed which are not conducive to both germination and seedling health; (i) the seed needs to survive passage through the digestive tract of animals (Copeland & McDonald, 2012), (ii) in colder areas the seed needs to survive for long periods (over a severe winter, or for several years when conditions are not suitable for germination and growth (Copeland & McDonald, 2012), or (iii) seeds in temperate areas need to be exposed to the effects of fire before germination, which is important because the plant needs to have competing species cleared to facilitate development of the seedlings (Copeland & McDonald, 2012). Further, there is an adaptive advantage in delaying germination for at least a portion of the seeds in a population, even when conditions are conducive to growth, as it prevents the commitment of the total seedbank and spreads the risk of germination failure across multiple sets of environmental conditions (Finch-Savage & Leubner-Metzger, 2006). This adaptation is known as 'bet-

hedging'. Despite these insights, the germination cues of many species are unknown, and this is the focus of this current work with the *Ptilotus* genus.

The genus *Ptilotus* (family Amaranthaceae) includes approximately 100 species that are mostly endemic to Australia (Benl, 1971; Commander *et al.,* 2009). Of these 100 species, a significant number occur in arid and semi-arid regions. They range in growth from prostrate to erect herbs, while some may form small, woody shrubs (Commander *et al.,* 2009). *Ptilotus polystachyus* has long been thought a promising candidate for domestication as a short-lived perennial forage herb for acid soils in the cropping zones of southern Australia (Gardner, 1934). In the context of this thesis, it is thought that some members of the *Ptilotus* family may be suitable as native species to assist in the reclamation of former cropland. As such, this study seeks to understand the optimum germination characteristics of *Ptilotus* seeds to provide the best opportunity for reestablishment of these native species during the reseeding experiments detailed in the previous chapter.

There are some generally accepted observations rising from this previous work: (i) temperature and light both play a part in the control of dormancy and germination, whilst it appears that light alone regulates the rate of germination (Benech-Arnold *et al.,* 2000; Batlla *et al.,* 2004), and (ii) temperature plays a major role in determining the periodicity of seed germination and the distribution of species (Baskin & Baskin, 2014). There are exceptions to these general observations – for example, some species are insensitive to changes in light levels such as *Aristida contorta* (Bunched kerosene grass) and *Helipterum craspedioides* (Yellow billy buttons), while others are inhibited by any exposure to light during germination such as *Helichrysum cassinianum* (Pink everlasting) (Mott, 1974a; Carta *et al.,* 2014). Furthermore, the response of seeds to light also can control the timing of germination (Pons, 2000), and previous studies have demonstrated that combined light and gibberellic acid can both break physical dormancy and promote

germination (Sanchez & Mella, 2004; Kucera *et al.,* 2005; Finch-Savage & Leubner-Metzger, 2006).

The labyrinthine nature of seed dormancy is further illustrated by the following observations: (i) the seed dormancy of many seeds can be released by low temperature and hormonal treatments (Schneider & Gifford, 1994; Clarke *et al.,* 2000); (ii) seeds of many species such as *Lupinus sulphureus* (Sulphur lupin)*, Marianthus bicolor* (Painted marianthus) and *Xyris lanata* (Yellow eyed grass)*,* require cold treatment before they are able to germinate (Taylor *et al.,* 1993; Nicolas *et al.,* 1996; Walck *et al.*, 2000; Kaye & Kuykendall, 2001; Merritt *et al.,* 2007), (iii) high temperatures (up to 80^{O}C) have been used to release seed dormancy of many species from arid zones such as *Actinotus leucocephalus* (Flannel flower)*, Anigozanthos manglesii* (Red and green kangaroo paw)*, Gompholobium knightianum* (Glory peas) and *Stylidium affine* (Queen trigger plant) (Mott, 1974b; Hagon, 1976; Tieu *et al.,* 2001); (iv) heat shock (up to 80^{O}C) was found to encourage germination of some legume shrub species such as *Acacia pulchella* (Western prickly moses) (Portlock *et al.,* 1990); and (v) smoke water is widely agreed to be a key agent for the release of seed dormancy in a range of native Australian species (Dixon *et al.,* 1995; Roche *et al.,* 1997a, b; Smith *et al.,* 1999).

There is a paucity of information on the seed ecology of the *Ptilotus* genus. However, Williams *et al.* (1989) studied methods to overcome the seed dormancy of *Ptilotus exaltatus* (Pink mula mula), and showed that scarification of the seed husk and application of gibberellic acid will stimulate effective germination. The study of Roche *et al.* (1997a) on the effect of seed ageing and smoke on seed germination of 75 Australian native plant species has also included information on eight species of *Ptilotus* include *P. polystachyus* and demonstrate that the smoke treatments was not effective to increase the seed viability of *Ptilotus* species.

The purpose of this study is to examine the response of *P. macrocephalus* and *P. polystachyus* seeds to the following seed pre-treatments: (a) smoke water, (b) shock

heating, (c) cold stratification and (d) gibberellic acid. The study also investigates the interaction of these three pre-treatments with different temperature and light exposure periods to help determine the optimum germination conditions for these species.

4.3 The Target Species Biology

Ptilotus macrocephalus (R. Br.) Poir (family Amaranthaceae), commonly known as Featherheads or Tall mulla mulla, is an annual or perennial herb and that can reach a height of up to 50 cm (Walsh & Entwisle, 1996). It is found across all Australian mainland states, but mainly in the drier inland areas, though it is heavily depleted through much of its former range due to its primary occurrence on land suitable for agriculture (Walsh & Entwisle, 1996). Leaves are narrowly obovate to linear, crowded toward the base. The plant flowers throughout the year, especially between July and December, and usually commences seven to eight weeks after germination (Walsh & Entwisle, 1996). The fruit is globular to cylindrical, with a head containing numerous long papery and hairy fruits, each containing one seed. The seeds are light brown, 2 mm long and 1.5 mm wide, and the embryo type is peripheral (Walsh & Entwisle, 1996).

Ptilotus polystachyus (Gaudich) F.Muell (family Amaranthaceae), is commonly known as Long tails, and is an annual or short-lived perennial herb. This species can grow up to one metre in height (Walsh & Entwisle, 1996). The stems are finely pubescent in the upper part, and are sparsely to densely hairy, while leaves are linear to oblanceolate, sparsely or densely hairy with crisp, nodose or verticillate hairs. Flowering usually commences four to six weeks after germination, mainly between May and November (Walsh & Entwisle, 1996). Fruit is white to pale brown, with a cylindrical head containing numerous long papery and hairy fruits, each containing one seed. The seeds are generally brown reinform seeds, 2 mm long and 1.2 mm wide, and the embryo type is peripheral. Ryan *et al.* (2003) reported the approximate temperature range required for growing for *Ptilotus* species has been reported to be 27/19^{O}C (Ryan *et al,.* 2003).

4.4 Materials and Methods

4.4.1 Seed collection and storage

Seeds of *P. macrocephalus* and *P. polystachyus* were purchased from *Nindethana Australia Seeds Company.* It was confirmed by the company that the seeds were collected from Newbridge, Victoria (36°46'02.7"S, 143°54'25.8"E) and Payne's Find, Western Australia (29°08'54.4"S, 117°30'30.1"E) respectively, during December 2009. The cleaned seeds, which were prepared by removing any residual seed husks or other extraneous material, were stored in a labelled air tight container and kept in a dark room at approximately 10^{O}C and 50% humidity at Federation University Australia, Mount Helen Campus, Seed Ecology Lab, until use.

4.4.2 General seed germination protocol pertaining to all treatments

Seeds were surface-sterilized by rinsing in 1% sodium hypochlorite for two minutes to destroy or remove surface fungi and bacteria, then washed clean with double-distilled water before the start of each germination trial. Three replicates of 20 seeds each were used in all treatments. Seeds were consistently positioned in 9-cm diameter Petri dishes lined with Whatman® No. 11 filter paper, and moistened with approximately 8 mL sterile distilled water or the appropriate treatment solution to ensure adequate moisture for the seeds. The Petri dishes containing the seeds were then placed in incubators (Thermoline Scientific and Humidity Cabinet, TRISLH- 495-1-SD, Vol. 240, Australia) fitted with cool-white fluorescent lamps (Philips 18 W/840 at 60 µ mol m^{-2}) that provided a photosynthetic photon flux of 40 µ mol m^{-2} s^{-1} to provide the 12 hr light/12 hr dark conditions. To simulate 24 hr of dark conditions, Petri dishes were wrapped with double layer of aluminium foil. Seeds were monitored regularly under green safe light for a period of 50 days from the date they were placed into the incubator, and the matrix was moistened when necessary. Seeds were considered germinated when the radicle was approximately 2 mm long and cotyledons had emerged from the seed coat (Ferrari & Parera, 2015). At the conclusion of the germination test, non-germinated seeds were checked for viability using the 2, 3,

5-triphenyltetrazolium chloride (TTC) test (Van Waes & Debergh, 1986; Saatkamp *et al*., 2011) to estimate the Viability Adjusted Germination (VAG), which gives the proportion of germinated seeds, expressed as a percentage of the total viable seeds, as follows:

$$\mathbf{VAG} = \frac{\boldsymbol{N}_{\mathbf{seed\_germ}}}{\boldsymbol{N}_{\mathbf{seed\_germ}} + \boldsymbol{N}_{\mathbf{viable\_non\_germ}}}$$

Where $N_{\text{seed\_germ}}$ = number of seeds germinated, and $N_{\text{viable\_seed\_germ}}$ = number of seeds that did not germinate, but were viable. VAG corrects for the number of seeds used in the experiment that were non-viable, to provide a better comparison of treatment effects.

4.4.3 Treatments and specific experiments

Seeds of each species were subjected to five general treatments, the experimental conditions of which are detailed below and summarised in Table 4.1. The five treatments and experiments were as follows:

Treatment 1 (Control)

To establish control conditions, the seeds were not treated with any pre-germination external temperature or any added chemical stimuli, and only RO water was used to begin the germination process.

Treatment 2 (Smoke water)

The smoke water treatment was conducted using commercially available aqueous smoke water (Regen 2000, SMOKEWATER) by adding 1 mL of 1% smoke water at three-week periods to the germinating seeds.

Treatment 3 (Heat shock)

To examine the effects of pre-heat on seed germination, groups of seeds were exposed to two sub-treatment levels of heat-shock; (a) 60^{O}C and (b) 80^{O}C. To apply heat shock, dry seeds were placed in an aluminium foil punnet (19 cm x 8 cm x 5 cm) then placed in a preheated convection oven (Memmert, Model. ULE 500) for two minutes (Scott, 2006) at which point the temperature reached the required 60^{O}C or 80^{O}C.

Treatment 4 (Cold)

For the cold stratification treatment, seeds were placed in separately labelled containers at 5^{O}C in a refrigerator for eight days (Kaye & Kuykendall, 2001; Walck *et al.*, 2000) before germination trials. There were two sub-treatments within treatment 4: (a) seeds with floral bracts, and (b) seed with floral bracts removed.

Treatment 5 (GA)

In the GA treatment, the effect of three sub-treatment concentrations of gibberellic acid (gibberellic acid 99%, ACROS ORGANICS) on the germination of the seeds were investigated. Cleaned seeds were soaked in 100 mL of the following concentrations: (a) 350 ppm, (b) 500 ppm and (c) 750 ppm gibberellic acid for 24 hr (Commander *et al.,* 2009; Kirmizi *et al.*, 2010).

As detailed in Figure 4.1, all treatments (and sub-treatments) were investigated under two diurnal temperature ranges (a) 25/10^{O}C and (b) 20/18^{O}C for at least 50 days, since these were the most likely temperature ranges to be experienced in the VVP area. Treatments 1, 2 and 3 were tested under two different photoperiods, where seeds were held (a) in the dark for 24 hr and (b) in a 12 hr light/12 hr dark regime, to test for light-induced reactions on germination. Treatments 4 and 5 were tested under the 12 hr light/12 hr dark photoperiod only, as the previous treatments had shown no significant difference between the photoperiods.

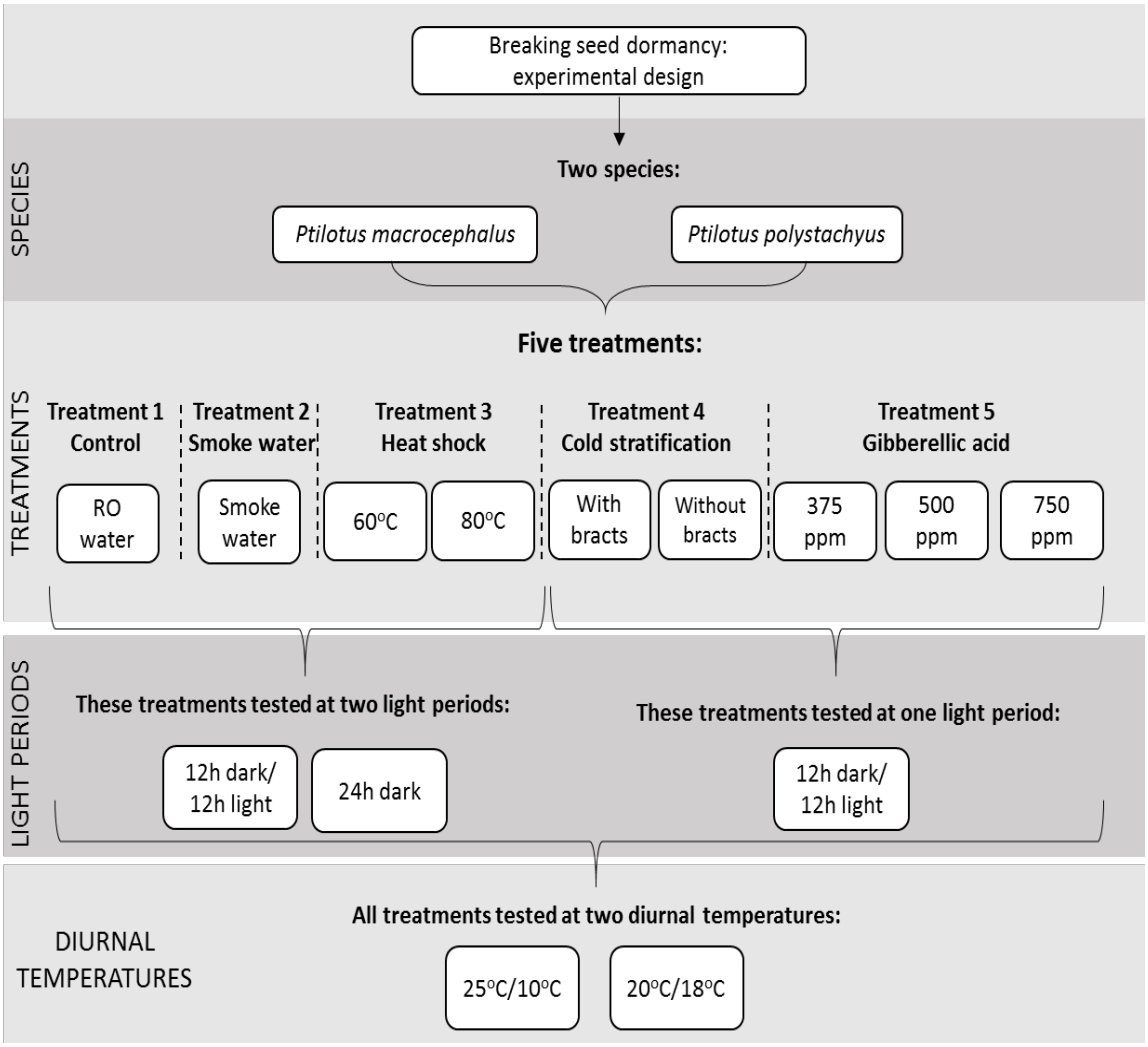

Figure 4.1 Flow chart of breaking seed dormancy study of both species, RO water, smoke water and heating (60^{O}C and 80^{O}C) treatments under two temperature ranges (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark /12h light).

4.4.4 Statistical analysis

Data were analysed with MINITAB 17 statistical package (Minitab, 2014) using a General Linear Model (GLM). VAG of *P. macrocephalus* and *P. polystachyus* were tested for normality using the Ryan-Joiner similar to Shapiro–Wilk test (a = 0.05). To assist in the statistical treatments, non-normally distributed data were square root transformed, to normalise the data.

For the control experiment, I needed to establish whether there was any significant difference in the germination rates of the two species under two light conditions in the dark for 24h and in a 12h light/ 12hdark regime, and under two temperature ranges (25/10^{O}C

and 20/18^{O}C). For this purpose, a GLM (General linear model) with two fixed factors (i) temperature (25/10^{O}C and 20/18^{O}C) and (ii) light periods (24h dark and 12h dark/12h light periods, was used to test the VAG of both species for RO water (control) treatment.

To test the effect of smoke water and heat treatment, each required a GLM with three fixed factors (i) water treatments (RO water and smoke water), (ii) temperature (25/10^{O}C and 20/18^{O}C) and (iii) light periods (24h dark and 12h dark/12h light periods).These GLMs tested the effect of each factor and their combination on VAG of both species.

To test the effect of cold stratification and gibberellic acid, each required a GLM with two fixed factors (i) water treatments (RO water, smoke water), heat shock (60^{O}C and 80^{O}C) and cold stratification treatments (with bracts and without bracts) and (ii) temperature (25/10^{O}C and 20/18^{O}C). The GLMs were used to examine the effect of each of the factors and their combination on VAG of both species at one light period (12h dark/12h light).

Tukey's a significance level of 0.05 were applied to test for significance between the all factor means and their combinations. Back-transformed data are presented in the results section.

4.5 Results and discussion

The VAG achieved for all experiments are presented in Table 4.1. The research questions posed were: (i) do untreated seeds, under various light and temperature conditions, provide a reasonable germination? (ii) will prior smoke water treatment increase the VAG% compared to the best control result? (iii) can heat shock treatment give a better VAG% than the best control or smoke water treatment? (iv) can a period of cold treatment stimulate germination to a greater extent than control, smoke water or heat shock treatments?, and (v) finally, does the use of pre-treatment with gibberellic acid provide a better return than the control, smoke water, heat shock or cold treatments?

To help to systematise the investigation, five hypotheses were tested.

I. There is no significant difference between the four variants of light and temperature on the VAG of both species of seeds when treated with RO water only.

II. There is no difference between the four variants of light and temperature on the VAG of both species of seeds when treated with RO water and smoke water.

III. There is no difference between the four variants of light and temperature on the VAG of both species of seeds when treated with RO water, smoke water and heating (60^{O}C and 80^{O}C).

IV. There is no difference between the all variants of treatments and temperature on the VAG of both species of seeds when treated with RO water, smoke water, heating (60^{O}C and 80^{O}C) and cold stratification treatments (with bracts and without bracts).

V. There is no difference between the all variants of treatments and temperature on the VAG of both species of seeds when treated with RO water, smoke water, heating (60^{O}C and 80^{O}C),cold stratification treatments (with bracts and without bracts) and gibberellic acid treatments (GA375, GA500 and GA750).

Table 4.1. A complete listing of all experiments, with recorded raw mean ± standard error VAG%, for *P. microcephalus* and *P. polystachyus*. The highlighted results indicated the highest germination% for each species within a treatment.

Experiments	Mean VAG (%)	
	P. macrocephalus	*P. polystachyus*
Treatment 1 ('Control')		
Experiment 1a: 24h dark; 25/10^{O}C	3.33 ± 3.33	12.50 ± 12.50
Experiment 1b: 24h dark; 20/18^{O}C	10.83 ± 5.83	6.94 ± 3.67
Experiment 1c: 12h dark/12h light; 25/10^{O}C	9.52 ± 9.52	0
Experiment 1d: 12h dark/12h light; 20/18^{O}C	0	2.78 ± 2.78
Treatment 2 ('Smoke water')		
Experiment 2a: 24h dark; 25/10^{O}C	10.07 ± 0.58	0
Experiment 2b: 24h dark; 20/18^{O}C	19.39 ± 0.61	0
Experiment 2c: 12h dark/12h light; 25/10^{O}C	45.92 ± 0.23	0
Experiment 2d: 12h dark/12h light; 20/18^{O}C	19.63 ± 1.61	0
Treatment 3 (Heat shock')		
Experiment 3a: 60^{O}C; 24h dark; 25/10^{O}C	0	0
Experiment 3b: 60^{O}C; 24h dark; 20/18^{O}C	0	0
Experiment 3c: 60^{O}C; 12h dark/12h light; 25/10^{O}C	8.19 ± 0.57	0
Experiment 3d: 60^{O}C; 12h dark/12h light; 20/18^{O}C	3.03 ± 3.03	0
Experiment 3e: 80^{O}C; 24h dark; 25/10^{O}C	0	0
Experiment 3f: 80^{O}C; 24h dark; 20/18^{O}C	0	0
Experiment 3g: 80^{O}C; 12h dark/12h light; 25/10^{O}C	0	0
Experiment 3h: 80^{O}C; 12h dark/12h light; 20/18^{O}C	0	0
Treatment 4 ('Cold')		
Experiment 4a: with bracts; 12h dark/12h light; 25/10^{O}C	5.81± 2.91	0
Experiment 4b: with bracts; 12h dark/12h light; 20/18^{O}C	0	15.93 ± 2.59
Experiment 4c: without bracts; 12h dark/12h light; 25/10^{O}C	0	0
Experiment 4d: without bracts; 12h dark/12h light; 20/18^{O}C	0	32.22 ± 1.11
Treatment 5 ('GA')		
Experiment 5a: 350 ppm; 12h dark/12h light; 25/10^{O}C	10.32 ± 5.20	16.67 ± 16.67
Experiment 5b: 350 ppm; 12h dark/12h light; 20/18^{O}C	30.00 ± 15.28	35.35 ± 21.02
Experiment 5c: 500 ppm; 12h dark/12h light; 25/10^{O}C	24.54 ± 7.62	23.50 ± 15.92
Experiment 5d: 500 ppm; 12h dark/12h light; 20/18^{O}C	64.68 ± 15.98	39.44 ± 10.56
Experiment 5e: 750 ppm; 12h dark/12h light; 25/10^{O}C	36.90 ± 25.45	14.76 ± 8.66
Experiment 5f: 750 ppm; 12h dark/12h light; 20/18^{O}C	44.44 ± 24.22	34.43 ± 17.51

Treatment 1

Table 4.2 presents the ANOVA table of VAG of each species separately treated with RO water only under two temperature ranges (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark/12h light).

Table 4.2. ANOVA results showing the effects of temperature, light periods, and their interaction on VAG. These tests included treatment with RO water only under two temperature (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark/12h light).

		P. macrocephalus			***P. polystachyus***		
Source	**DF**	**SS**	**F**	**P**	**SS**	**F**	**P**
Temperature	1	0.021	0	0.948	0.85	0.18	0.683
Light periods	1	2.83	0.61	0.456	7.78	1.65	0.235
Temperature* light periods	1	8.65	1.88	0.208	0.56	0.12	0.739
Error	8	36.84			37.64		
Total	11	48.34			46.82		

Ho: there is no significant difference between the four variants of light and temperature on the VAG of both species of seeds when treated with RO water only.

Treating the seeds with water only, under both conditions of light stimulus, was not sufficient to break the seed dormancy significantly in either species (Table 4.4). As a consequence, this null hypothesis was accepted. The highest VAG for *P. macrocephalus* was 10.83% and 9.52% at 25/10^{O}C temperature under 24h dark and 25/10^{O}C under 12h dark/12h light period respectively, while, the highest VAG for *P. polystachyus* was 12.50% at the 25/10^{O}C temperature range under 24h dark (Table 4.1). Other studies have also found low germination of *Ptilotus* species (Williams *et al.,* 1989; Roche *et al.,* 1997a, b; Peters, 2000; Commander *et al.,* 2009).

This experiment implies that the use of untreated seeds of *P. macrocephalus* and *P. po*lystachyus in restoration will achieve low germination percentages; thus a suitable pre-treatment of seeds is recommended prior to reseeding attempts.

Treatment 2

Table 4.3 shows the ANOVA table of VAG of each species, separately treated with RO water and smoke water under two temperature ranges (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark/12h light).

Table 4.3. ANOVA results showing effects of smoke water (compared to RO water only), temperature and light periods, and the interactions among them. These tests include treatments with RO water and smoke water under two temperature (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark/12h light).

		P. macrocephalus			*P. polystachyus*		
Source	**DF**	**SS**	**F**	**P**	**SS**	**F**	**P**
Smoke	1	66.09	28.45	**0.000**	9.92	4.22	0.057
Temperature	1	0.62	0.27	0.612	0.42	0.18	0.677
Light periods	1	1.06	0.46	0.508	3.89	1.65	0.217
Smoke * Temperature	1	0.34	0.15	0.706	0.42	0.18	0.677
Smoke * Light periods	1	11.63	5.01	**0.040**	3.89	1.65	0.217
Temperature* Light periods	1	18.29	7.88	**0.013**	0.28	0.12	0.735
Smoke* Temperature* Light periods	1	0.013	0.01	0.940	0.28	0.12	0.735
Error	16	37.12			37.64		
Total	23	135.09			56.74		

Ho: there is no difference between the four variants of light and temperature on the VAG% of both species of seeds when treated with RO water and smoke water.

This hypothesis was rejected, because there were significant differences (p= 0.001) between the VAG of *P. macrocephalus* measured for each treatment; the VAG was 22.05% for smoke water and 1.83% for RO water (Figure 4.2). However, it was found that there were no significant differences between the temperature, light periods, interaction of treatment-temperature and interaction of treatment-temperature-light periods. There were significant differences on VAG in term of interaction of treatment-light periods, where the highest VAG was 31.35% for smoke water at 12h dark/12h light period, 14.33% smoke water at 24h dark, 3.46% RO water at 24h dark and 0.79% for RO water 12h dark/12h light period, respectively (Figure 4.3). However, the study of Roche *et al.* (1997b) showed no positive effect of smoke treatment on germination of seven *Ptilotus* species. In contrast, our study showed significant effect of smoke treatment on *P. macrocephalus* seed

germination. This may be related to an evolutionary history of burning in the primary habitat of *P. macrocephalus* – i.e. regular burning in the VVP may have resulted in an adaptation to germinate in response to smoke.

In regard to VAG% of *P. polystachyus* species, there were no significant differences, between treatments, temperature, light periods and their interactions because all results were zero. This means that treatment with smoke water did not have any effect on the germination rate of the *P. polystachyus* species. This was similar to Roche e*t al.* (1997b).

The highest measured VAG for *P. macrocephalus* was 45.92% at a temperature range of 25/10^{O}C under 12h dark/12h light periods, while the VAG of *P. polystachyus*, as noted above, was zero (Table 4.1).

This experiment shows that the VAG of *P. macrocephalus* species seeds was increased significantly when treated with smoke water treatment, while the *P. polystachyus* seeds VAG percentage remained at zero. However, notwithstanding the positive effect in the first instance, it is clear that more influential treatments are needed to increase the germination rate of both species if an economical solution is to be found.

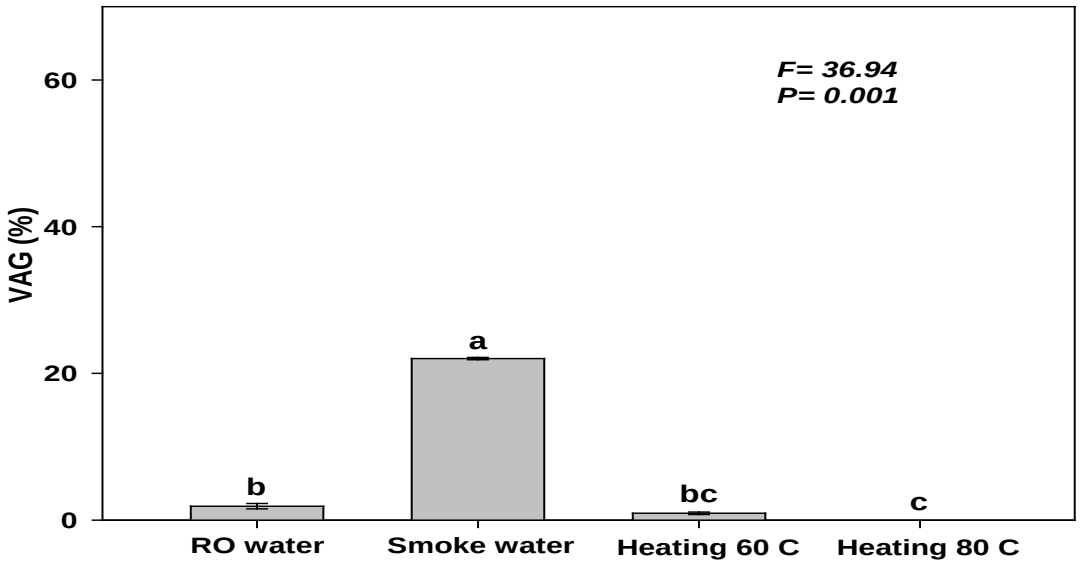

Figure 4.2 Effects of RO water, smoke water, and heating 60^{O}C and 80^{O}C treatments on VAG% of *P. macrocephalus*. Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

Treatment 3

Table 4.4 shows the ANOVA table of VAG of each species separately treated with RO water, smoke water and heat shock (60^{O}C and 80^{O}C) under two temperature ranges (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark/12h dark/12h light).

Table 4.4. The ANOVA table of VAG of each species separately treated with RO water, smoke water and heat shock (60^{O}C and 80^{O}C) under two temperature (25/10^{O}C and 20/18^{O}C) at two light periods (24h dark and 12h dark/12h light).

		P. macrocephalus			***P. polystachyus***		
Source	**DF**	**SS**	**F**	**P**	**SS**	**F**	**P**
Treatments	3	149.74	36.94	**0.000**	14.89	4.22	**0.013**
Temperature	1	1.85	1.37	0.251	0.21	0.18	0.675
Light periods	1	5.77	4.27	**0.047**	1.94	1.65	0.208
Treatments* Temperature	3	1.69	0.42	0.742	0.63	0.18	0.909
Treatments *Light periods	3	18.11	4.47	**0.010**	5.83	1.65	0.197
Temperature* Light periods	1	14.64	10.83	**0.002**	0.14	0.12	0.733
Treatments* Temperature* Light periods	3	6.24	1.54	0.224	0.42	0.12	0.948
Error	32	43.25			37.64		
Total	47	241.29			61.70		

Ho: there is no difference between the four variants of light and temperature on the VAG% of both species of seeds when treated with RO water, smoke water and heating (60^{O}C and 80^{O}C).

This hypothesis was rejected, since there were significant differences on VAG of *P. macrocephalus* between treatments; the VAG was 22.05, 1.83, 0.93 and 0% for smoke water, RO water, heat 60^{O}C and heat 80^{O}C, respectively (Figure 4.3). There were significant differences on VAG between different light periods, where the VAG was 4.43% and 1.99% (p= 0.047) at 12h dark/12h light and 24h dark.

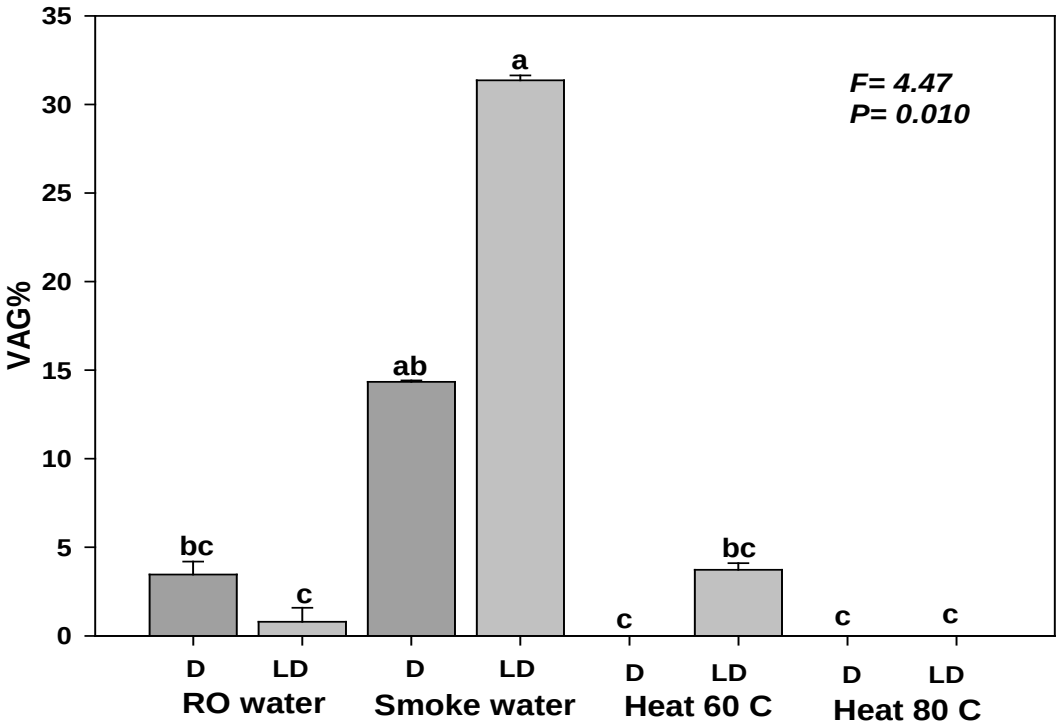

Figure 4.3 The effect of interaction between RO water, smoke water, and heating 60^{O}C and 80^{O}C treatments and different light periods on VAG% of *P. macrocephalus*. The letter **D** represent 24h dark and **LD** represents the 12h dark/12h light period, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

There were significant differences in VAG between the interaction of treatments and light periods; the VAG was 31.35, 14.33, 3.73, 3.46 and 0.79% for smoke water 12h dark/12h light, smoke water 24h dark, heat 60^{O}C 12h dark/12h light, RO water 24h dark, and RO water 12h dark/12h light, respectively (Figure 4.3). There were also significant differences between the interactions of temperature and light periods, the highest VAGs being recorded were 8.14% at 25/10^{O}C under 12h dark/12h light, 3.12% at 20/18 ^{O}C under 24h dark, 1.84% at 20/18^{O}C under 12h dark/12h light and 1.11% at 25/10^{O}C under a 24h dark period (Figure 4.4).

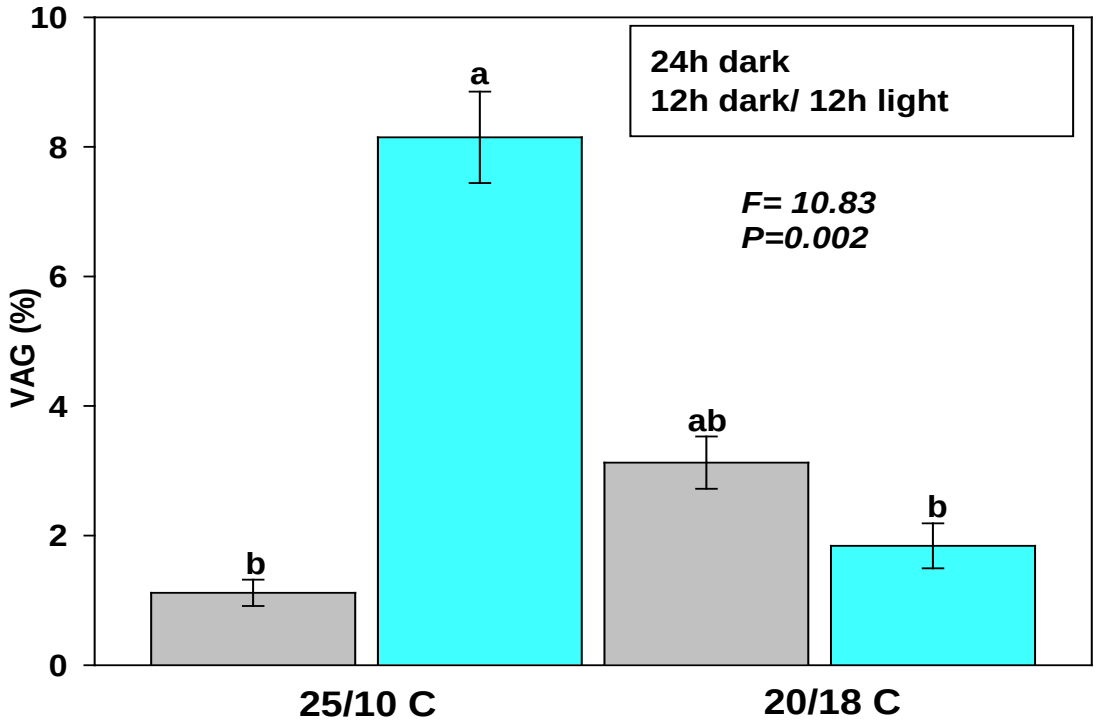

Figure 4.4 The effect of interaction between different light periods and different temperature 25/10^{O}C and 20/18^{O}C on VAG% of *P. macrocephalus*. The different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

In regards to VAG of *P. polystachyus* species, there were no significant differences, between treatments, temperature, light periods and their interactions because the germination rate was still zero. This means that a combination of smoke water and heat shocks (60^{O}C and 80^{O}C) also did not increase the VAG% of *P. polystachyus*.

The best VAG for *P. macrocephalus* was 8.19% after heating at 60^{O}C and germination at a temperature range of 25/10^{O}C under a 12h dark/ 12h light period; the VAG of *P. polystachyus* was zero (Table 4.1).

This experiment indicates that the VAG of *P. macrocephalus* seeds were only slightly increased when treated with (60^{O}C) heat shock treatment with a 12h dark/12h light photo period. This was not a useful increase in germination rate. In addition, the *P. polystachyus* seeds VAG percentage germination was zero under all temperature and both light periods. The VAG percentage was very low to zero for both species under 24h dark light

period. In a study of 65 Australian native understorey species from grasslands and grassy woodlands, Clarke *et al.* (2000) also found that very few species had a germination requirement for darkness, and most showed either no effect of darkness, or an inhibitory effect. As such, the 24h dark/ light photoperiod option was omitted in the subsequent experiments.

Treatment 4

Table 4.5 show the ANOVA table of VAG of each species separately treated with RO water, smoke water, heating (60^{O}C and 80^{O}C) and cold stratification treatments (with bracts and without bracts) under two temperature (25/10^{O}C and 20/18^{O}C) at 12h dark/ 12h light periods.

Table 4.5. The ANOVA table of VAG of each species separately treated with RO water, smoke water, heating (60^{O}C and 80^{O}C) and cold stratification treatments (with bracts and without bracts) under two temperature (25/10^{O}C and 20/18^{O}C) at 12h dark/ 12h light periods

		P. macrocephalus			***P. polystachyus***		
Source	**DF**	**SS**	**F**	**P**	**SS**	**F**	**P**
Treatments	5	132.62	20.41	**0.001**	45.16	34.49	**0.001**
Temperature	1	15.82	12.18	**0.002**	28.09	107.27	**0.001**
Treatments* Temperature	5	8.20	1.26	0.312	45.16	34.49	**0.001**
Error	24	31.19			6.28		
Total	35	187.84			124.69		

Ho: there is no difference between all variants of treatments and temperature on the VAG% of both species of seeds when treated with RO water, smoke water, heating (60^{O}C and 80^{O}C) and cold stratification treatments (with bracts and without bracts).

For this combination of treatments, this hypothesis was rejected since there were differences between the VAG of both species. There were significant differences (p=0.001) on VAG of *P. macrocephalus* between the treatments; the VAG was 31.35, 3.73, 0.96 and 0.79% with smoke water, heat 60^{O}C, cold stratification with bracts treatments and RO water respectively, and zero for heat 80^{O}C and cold stratification without bracts (Figure 4.5). There were significant differences between the temperature

ranges (p= 0.002), where the highest VAG was 4.97% recorded at 20/18^{O}C temperature. There was no significant difference between the interaction of treatments-temperature (p=0.312).

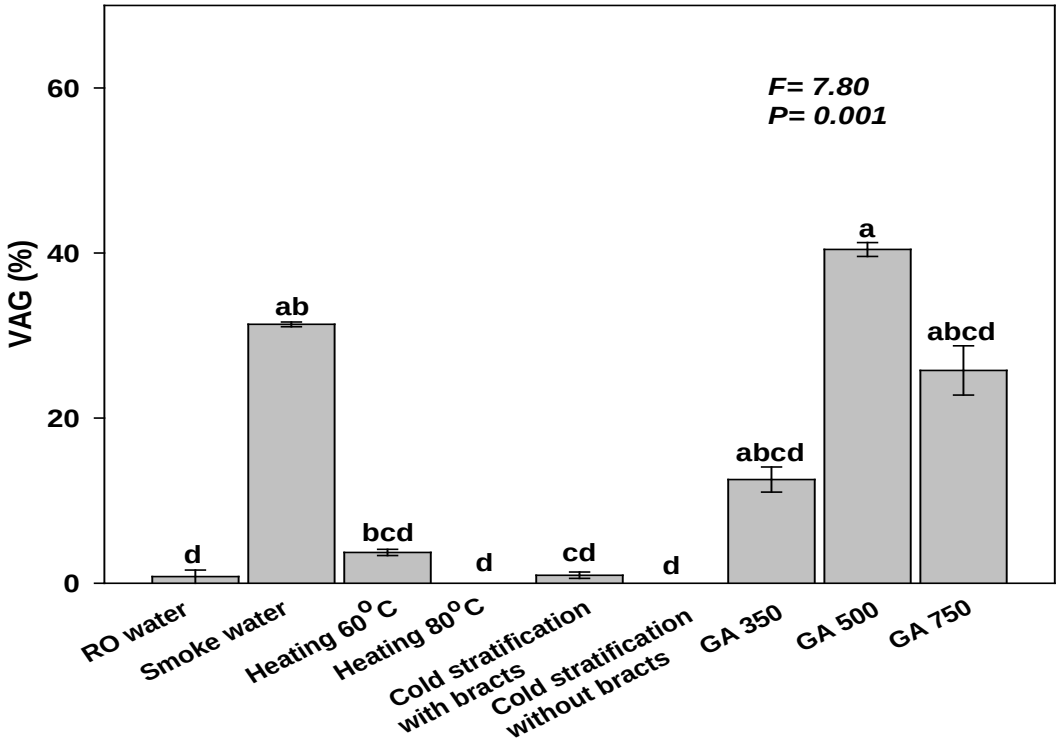

Figure 4.5 Effects of various treatments on VAG% of *P. macrocephalus.* Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

In regard to VAG of *P. polystachyus* species, there were significant differences between treatments, temperature and interaction of treatments-temperature. The VAG recorded was 8.05, 3.09, 0.23 and 0% at cold stratification without bracts, cold stratification with bracts, RO water and heat shock 60^{O}C and 80^{O}C respectively (Figure 4.6). There were significant differences between the temperature ranges, the highest VAG was 3.12% recorded at 20/18^{O}C. There were no significant differences between the interactions of treatments-temperature.

The best VAG for *P. macrocephalus* was 5.8% for cold stratification with bracts at temperature 25/10^{O}C, while the best VAG of *P. polystachyus* was 32.22% for cold

stratification without bracts at 20/18^{O}C (Table 4.1 & 4.7). Removing the bracts increased VAG for *P. polystachyus* in our study, and this result was similar to studies of other *Ptilotus* species; Williams *et al.* (1989) study of *P. exaltatus* found that removing the bracts increased germination rate dramatically. Cold stratification also had a small positive influence on VAG for each species, a result that was also found by Tsuyuzaki & Miyoshi (2009), who demonstrated that the cold stratification for 1 month was effective for increasing seed germination for 11 species of the non-deep physiological dormancy group.

Overall, cold stratification with or without bracts was zero to very low in both temperature regimes. Thus, treatments to increase the germination rate of both species of seeds are needed to achieve an economical and practical management strategy rate.

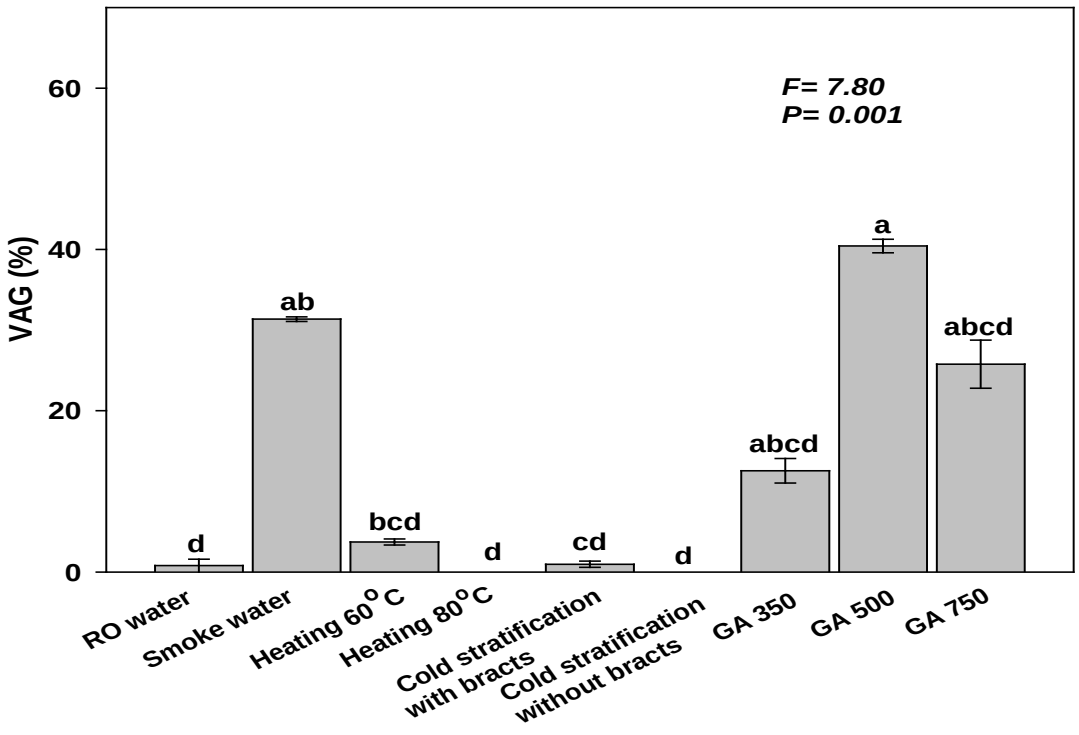

Figure 4.6 Effects of various treatments on VAG% of *P. polystachyus.* Vertical bars represent ± standard error of the means, the different letters on the vertical bars represent the significant differences between the treatments using Tukey's test.

Treatment 5

Table 4.6 shows the ANOVA table of VAG of each species separately treated with RO water, smoke water, heat shock (60^{O}C and 80^{O}C), cold stratification treatments (with bracts and without bracts) and gibberellic acid treatments (GA375, GA500 and GA750) under two temperature ranges (25/10^{O}C and 20/18^{O}C) at 12h dark/ 12h light periods.

Table 4.6. The ANOVA table of VAG of each species separately treated with RO water, smoke water, heat shock (60^{O}C and 80^{O}C), cold stratification treatments (with bracts and without bracts) and gibberellic acid treatments (GA375, GA500 and GA750) under two temperature (25/10^{O}C and 20/18^{O}C) at 12h dark/ 12h light periods.

		P. macrocephalus			***P. polystachyus***		
Source	**DF**	**SS**	**F**	**P**	**SS**	**F**	**P**
Treatments	8	297.22	7.80	**0.001**	180.77	5.12	**0.001**
Temperature	1	0.95	0.20	0.658	48.59	11.01	**0.002**
Treatments* Temperature	8	43.02	1.13	0.368	46.10	1.31	0.272
Error	36	171.41			158.90		
Total	53	512.60			434.35		

Ho: there is no difference between the all variants of treatments and temperature on the VAG% of both species of seeds when treated with RO water, smoke water, heating (60^{O}C and 80^{O}C), cold stratification treatments (with bracts and without bracts) and gibberellic acid treatments (GA375, GA500 and GA750).

This hypothesis was rejected, since it was clear that there were differences between the VAG of both species. There were significant differences on VAG of *P. macrocephalus* between the treatments, where the VAG was 40.42, 31.4, 25.76, 12.55, 3.73, 0.96 and 0.79% for GA500, smoke water, GA750, GA375, heat 60^{O}C, cold stratification with bracts treatments and RO water respectively, while the VAG was zero at heat 80^{O}C and cold stratification without bracts treatments (Figure 4.5). There was no significant difference between the temperature and interaction of treatments- temperature.

In regards to VAG of *P. polystachyus* species, there were significant differences between treatments and temperature while there were no significant differences between the interactions of treatments- temperature. The VAG was 24.90, 15.48, 12.68, 8.05, 3.92 and 0.23 at GA500, GA375, GA750, cold stratification without bracts, cold stratification with

bracts and RO water respectively, while the VAG was zero at heat 60^{O}C and 80^{O}C (Figure 4.6). There were significant differences between the temperatures, with the best temperature range being 20/18^{O}C where the VAG was 8.55%. There was no significant difference between the interactions of treatments-temperature.

Gibberellic acid was very effective at overcoming dormancy in both *P. macrocephalus* and *P. polystachyus*, and for both, the most effective concentration was GA500 at 20/18^{O}C. The highest VAG for *P. macrocephalus* was 64.68% at GA500, and the highest VAG of *P. polystachyus* was 39.44% at GA500 at 20/18^{O}C (Table 4.1). My results are similar to those of Williams *et al.* (1989), who found that the use of gibberellic acid treatment under a low rate will overcome the seed dormancy of *P. exaltatus*.

Table 4.7. Showing back transformed VAG mean ± standard error of *P. macrocephalus* and *P. polystachyus* at two different temperature (25/10^{O}C and 20/18^{O}C) at one light period 12h dark/12h light.

Treatments	*P. macrocephalus*		*P. polystachyus*	
	25/10^{O}C	**20/18^{O}C**	**25/10^{O}C**	**20/18^{O}C**
RO water	3.17 ± 3.17	0	0	0.93 ± 0.93
Smoke water	45.92 ± 0.001	19.56 ± 0.03	0	0
Heating 60^{O}C	8.17 ± 0.01	1.01 ± 1.01		0
Heating 80^{O}C	0	0	0	0
Cold stratification with bracts	3.87 ± 0.97	0	0	15.70 ± 0.11
Cold stratification without bracts	0	0	0	32.20 ± 0.01
GA 375	6.87 ± 1.72	19.94 ± 5.03	5.56 ±5.56	22.73 ± 6.31
GA 500	23.22 ± 0.66	62.37 ± 1.16	14.49 ± 4.51	38.11 ± 0.67
GA 750	22.59 ± 7.16	29.16 ± 7.64	9.52± 2.62	22.89 ± 5.77

4.6 Conclusion

This current chapter has tested for the optimum germination conditions of *P. macrocephalus* and *P. polystachyus*. The practical problems of seed dormancy, which needs to be addressed before using these species for glasshouse experiments, involved a determination of their response to a number of seed pre-treatments. These were the use of (i) smoke water, (ii) heating shock, (iii) cold stratification and (iv) gibberellic acid,

and results were compared with those observed with RO water (no pre- treatment). These two species of seeds have been chosen for this investigation because it is known that one of the outcomes of use of land for crops is that there is a high residual phosphorus content in the soil, and this is a severe abiotic barrier to native species re-establishment. It is known that both these species (*P. macrocephalus* and *P. polystachyus*) have the ability to concentrate phosphorous as they grow (Ryan *et al.,* 2009; Islam *et al*., 1999), and this suggests that use of these plants may provide a convenient and economical way of reducing phosphorus levels and preparing the way for further native plant restoration possibilities.

This study noted that the use of untreated seeds of both *P. macrocephalus* and *P. po*lystachyus in restoration programs will achieve low germination percentages. In summary, the use of smoke water and heat shock (60^{O}C) did increase the germination rate of *P. macrocephalus* species while the germination rate of *P. polystachyus* seeds remained at zero (Table 4.7). The cold stratification without bracts increased the germination rate of *P. polystachyus* significantly, but the germination rate of *P. macrocephalus* was still very low. However, the use of gibberellic acid with a concentration of 500 ppm was sufficient to increase the germination rate of both species significantly (Table 4.7).

The highest germination values were observed when the seeds of *P. macrocephalus* and *P. polystachyus* were exposed to temperatures (20/18^{O}C) and 12h dark/ 12h light regimes. From these results, it is recommended that the best pre-treatment, optimum temperature and light period for both species is GA 500 (20/18^{O}C) and 12h dark/ 12h light regimes. As a consequence, this system will be used in the work described in Chapter 5, which will investigate the use of these species to reduce the phosphorus level in the soil using plant species. In the next chapter, details of this investigation of effective methods to reduce the proportion of phosphorus in ex-cropping land to facilitate native grassland restoration will be carried out.

Chapter 5. Investigation of effective methods to reduce the proportion of phosphorus in ex-cropping land to facilitate native grassland restoration

5.1 Introduction

Intense agricultural activity on previous grassland sites, including the addition of considerable amounts of inorganic fertiliser, has significantly effected the original soil's chemical structure (Dorrough *et al.,* 2004). Superphosphate, for example, has been a common agricultural fertilizer, and has been used to increase both phosphorus and nitrogen levels in the soil to stimulate the production of exotic crops. As a consequence, efforts to restore native grassland have failed due to exotic species regenerating better than native species in the high nutrient status soils, and this investigation is designed to redress this situation. The high phosphorus availability in post-agricultural soil has been widely recognized as a barrier to restoration of semi-natural vegetation (Pywell *et al.,* 2007).

Ii has been observed that, in general, Australian native species prefer low nutrient levels, and thus the substantial and regular historical application of phosphorus fertilizers to cropland soil in temperate Australia will have had significant negative effects on the germination and growth of native plant species (Yates & Hobbs, 2000; Kirkpatrick *et al.,* 2005). A number of studies have shown that the species richness of native forbs declines with increasing phosphorus levels (Huenneke *et al.,* 1990. McIntyre & Lavorel, 1994; Dorrough *et al.,* 2006), and this implies that any management strategy which aims to re-establish native grassland must address the problem of high phosphorous soil content.

One remedy to this problem is topsoil removal (scalping), which physically removes soil with elevated nutrient status, as well as removing the exotic seed bank. This exposes a relatively poor under-layer of soil which is more suited to the establishment of native species. However, this technique is often not economically viable, or indeed not possible in some areas where natural obstacles may occur. In particular, in the VVP grasslands, a

considerable area of the soil is dominated by rocks, making scalping challenging, so alternative methods for mineral reduction should be investigated.

There are a number of innovative studies that have investigated the use of plants to reduce soil phosphorus concentration (Trinick, 1977; Bates, 1990; Saito & Kato, 1994; Brennan *et al.,* 2000; Veneklaas *et al.,* 2003; Watt & Evans, 2003; Le Bayon *et al.,* 2006; Gilbert *et al*,. 2009; Lelei & Onwonga, 2014a;). The approach used in this investigation is partly based on the known biology of legume species, which indicates that they are adaptable to a wide range of phosphorus levels, where a special root structure, called cluster (proteoid) roots, are formed in response to phosphorus deficiency in the plant (Shane *et al.,* 2005). This allows the plant to concentrate phosphorus into the plant's structure. In this respect, *Lupinus albus* (Fabaceae) has been studied widely with respect to cluster-root growth and carboxylate release (Gardner *et al.,* 1983; Dinkelaker *et al.,* 1989; Neumann *et al.,* 2000; Shane *et al.,* 2003), and has showed significant ability to absorb phosphorous from the soil. A number of plant species native to Australia possess a range of adaptations to maintain adequate phosphorus nutrition when growing in soil with low availability of labile inorganic phosphorus (Handreck, 1997) – for example, species that are adapted to soils with very low P-status in south-west Western Australia. These root-system adaptations enhance phosphorus uptake, and their function includes symbiosis with mycorrhizal fungi (Johnston & Ryan, 2000).

In this respect, Islam *et al*. (1999) examined the phosphorus uptake by *Ptilotus exaltatus*, *P. microcephalus, P. aerovoides* (Amaranthaceae), *Dysphania kalpari* (Chenopodiaceae) and *Abutilon oxycarpum* (Malvaceae) on soil with low phosphorus concentration in subtropical, semi-arid Australian grasslands. That study found that the concentration of phosphorus in shoots was greatest in the *Ptilotus* species, ranging up to 1.75 mg/ plant. Similarly, Ryan *et al.* (2009) investigated the growth response of *P. polystachyus* and *Cichorium intybus* (Asteraceae) to phosphorus and nitrogen addition in a sandy soil of extremely low bicarbonate-extractable phosphorus and mineral nitrogen. It was shown

that the *Ptilotus* species grew much better, and produce more biomass, than the chicory in soil with low bicarbonate-extractable phosphorus or mineral nitrogen. In addition, the concentration of phosphorus in plant shoots kept steadily increasing in *Ptilotus* when there was an increase in the concentration of phosphorus in the soil. These observations suggest that phosphorous levels in ex-agricultural soil might be managed by sowing and subsequently harvesting these phosphorous-tolerant and phosphorous-concentrating plants over the course of a number of seasons.

Another possible strategy for the lowering of available phosphorous in ex-arable cropland soil is by the addition of a chelating agent. Such a compound is 'Phoslock', a natural product produced from modified bentonite clay which was initially developed by the Land and Water Division of Australia's CSIRO (Commonwealth Scientific and Industrial Research Organisation). It was formulated in an attempt to reduce the amount of Filterable Reactive Phosphorus (FRP) present in the water column and in the sediment pore water of a water body. FRP was seen to be an important growth factor for blue green algae and other algae (Douglas, 2002) which was a particular problem in phosphorous-effected water bodies. It was also thought at the time that the addition of Phoslock® could be expected to be especially effective in flooded soils, because it can form an active layer on top of the soil and thereby reduce phosphate mobilization into the mobile water layer (Geurts *et al.,* 2011).

In this current study, the aim is to lower the level of available phosphorous in the soil in order to give a range of native plants a better chance of germinating and competing with the exotic flora. It was postulated that introducing Phoslock® into the ex-cropland soil would bind the available phosphorous in an insoluble form, thus making it unavailable to plants.

This work investigates the effectiveness of reducing available soil phosphorus by either binding it within the soil as an insoluble matrix, or by biologically extracting the phosphorus using suitable native plants. These two approaches have been evaluated in a glasshouse

trial which was designed to investigate the following research question: *How does Phoslock® compare to a range of high-phosphorus binding plant species in lowering the level of available phosphorous in ex-arable grassland soil?*

Two null hypotheses were tested. The first (null) hypothesis is that the addition of Phoslock® to soils high in P will not appreciably lower the available P, and thus not facilitate native plant establishment. The second (null) hypothesis is that seeding and harvesting areas high in phosphorous will not appreciable lower the available P content in the soil, and thus not facilitate native plant establishment.

5.2 Material and methods

5.2.1 Glasshouse experimental design

Approximately 200 kg of soil from the top 10 cm of ex-cropping land was collected randomly from two different locations, Half of this amount was collected from the field experiment site northwest of Werribee, Victoria (37° 49' 20" S, 144° 34' 23" E) and the other half was taken from adjacent fence line (37° 49' 21.5" S, 144° 34' 42.3" E). In the field, soil samples were placed in labelled plastic bags and transported to Federation University Australia and stored in the glasshouse (27^{O}C) until use. Air-dried soil was sieved through a 4 mm diameter sieve, and mixed thoroughly, and four sub-samples from each site were chosen randomly from the collection. These sub-samples were sent to CSBP laboratories (Soil and Plant Analysis Laboratory; NATA accredited) for chemical analysis. In addition, samples from each site were placed in labelled polyethylene bags and sterilized in an autoclave (Atherton Cyber Series Jerboa 2011, Melbourne). The experiment was carried out in a temperature-controlled glasshouse; the temperature range during the day was 22–30^{O}C and during the night the range was 16–22^{O}C.

Pre-chemical analysis of soil subsamples showed that the experimental site soil was 21 ppm and at the fence line was 8 ppm (Olsen, 1954). For this experiment, I required three different levels of phosphorus. The fence line soil was thus accepted as ‘low P level’ and the experiment site soil as ‘medium P level’. To prepare ‘high level soil P’, phosphorus

was applied as finely powdered Ca $(H_2PO_4)_2.H_2O$ to the experiment site soil to give a level of 40 ppm (Brennan *et al.,* 2000).

The experimental design used was randomised block design with six treatments and a control. Three of the treatments were plant species (*Lupinus albus, Ptilotus polystachyus* and *Ptilotus macrocephalus*), and three treatments were only soil subjected to different levels of Phoslock® addition (100, 500 and 1500 gm/m^2) (see Figure 5.1). Each treatment was applied at each of three levels of phosphorus-containing soil (low, medium and high), and there was six replicates of each treatment at each P level, resulting in a total of 126 pots

The experiment was commenced on the 18th December 2015, in the Federation University Australia glasshouse. Pots with 16 cm height and 13.5 cm diameter were used. These pots were lined with plastic bags to prevent nutrient leaching, and filled with 5 cm of river sand soil (~ 550-650 g) and completed with 10 cm of soil which was about 1350 g of air-dried soil. To prepare three different concentrations of Phoslock® treatments (14.5, 71.5 and 214.5 gm/pot) were mixed with soil top layer of each Phoslock® treatment, respectively. The replicate pots were placed randomly on the glasshouse benches. Seeds of *Lupinus albus* were directly placed in the pots, but *P. polystachyus* and *P. macrocephalus* seeds were germinated in the seed germination cabinets with 500 ppm gibberellic acid at 20/18°C prior to sewing into pots, which were the ideal conditions for germination determined in Chapter 4. On the 4th January 2016, the transplants were thinned to two plants per pot. All the pots were watered automatically for three minutes, once a day using a top sprayer to maintain soil moisture at approximately field capacity throughout the growing period. After approximately 40 days post planting, aphids were noticed on some plants, hence all plants were sprayed with a soil pesticide (active concentration 20g/L) to control aphids and insects.

5.2.2 Sampling and chemical analysis

Forty-five days after the experiment commenced, plant heights were measured using a 100-mm ruler from soil surface to the apical meristem of the plants, and the numbers of leaves and branches were counted for each plant. Sixty three days after planting, plants were harvested by cutting at the root collar with a pair of secateurs. Roots were carefully removed from the soil and washed on top of a mesh sieve to avoid the loss of fine roots. The fresh weight of shoots and roots was then recorded separately. All the leaves were removed and leaf areas were measured with a PATON Electronic Plano-meter. All shoots and roots were then placed in separate labelled bags and placed in an oven at 70^{O}C for 48 hrs and shoots and roots dry weights were then taken. The dried shoot and root samples were subsequently ground to 450 µm, subsampled, then placed in labelled 5 mL plastic vials. These were sent to the University of Melbourne Creswick Campus laboratories for chemical analysis by inductively-coupled plasma weight spectrometry (ICP).

The whole soil profile in pots sampled for P analysis was placed separately in labelled plastic bags, then transferred to another glasshouse (temperature 17-22^{O}C) to be air-dried after opening the bag seals. The dried samples were then ground through a 2 mm sieve and sent to CSBP Laboratories to determine the phosphorus content using the Olsen analysis method (Olsen, 1954).

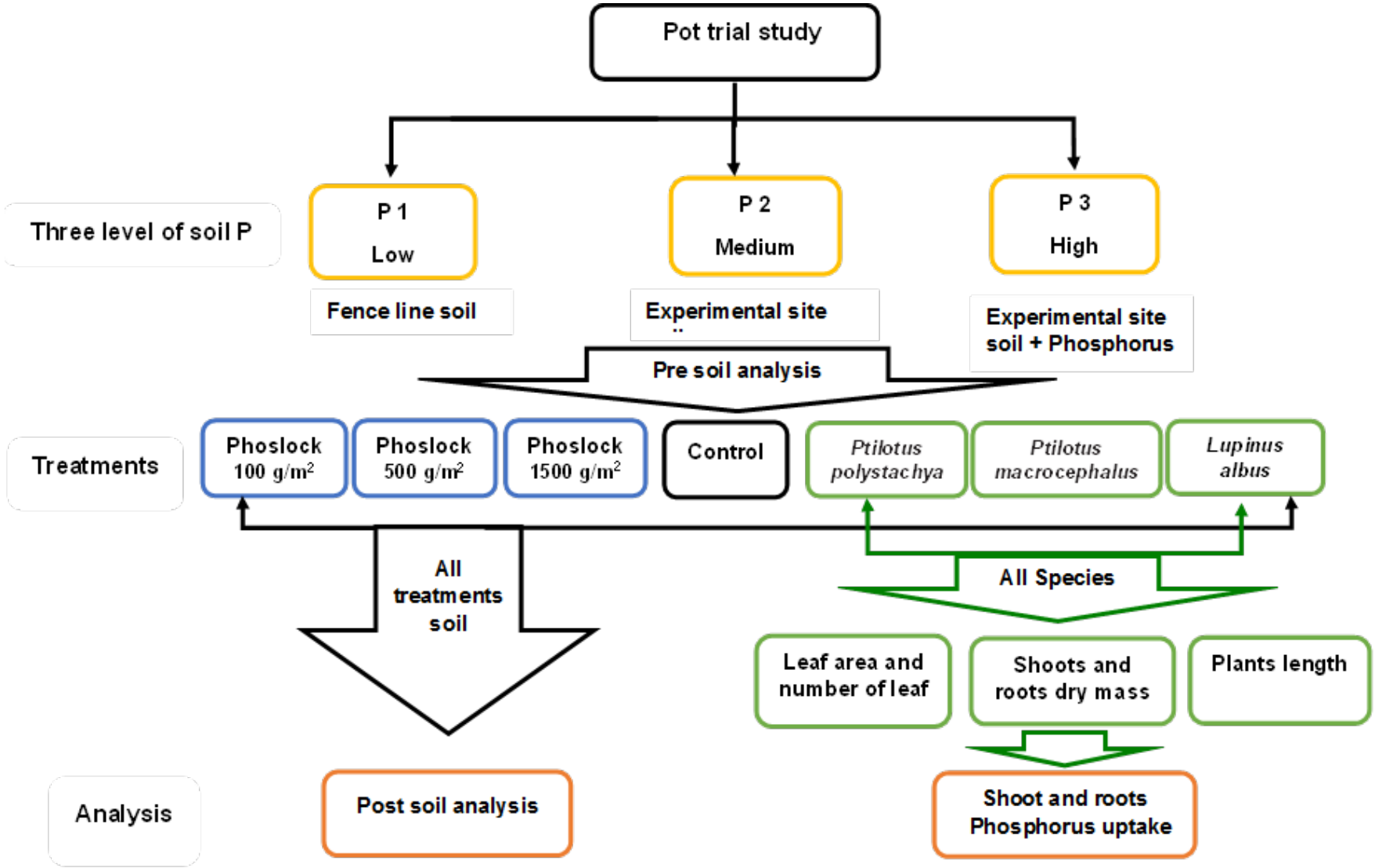

Figure 5.1 Flow chart of glasshouse study, showing three level of soil (low, medium and high). The treatments are three concentration of Phoslock® (100, 500 and 1500 g/m^2), control and planting with three species (*L. albus, P. polyptychs* and *P. macrocephalus*).

5.3 Statistical analysis

Data were analysed with the MINITAB 17 statistical package (Minitab, 2014). All data were tested for normality using the Ryan-Joiner test which is similar to the Shapiro–Wilk test (at a significance level of 0.05). Non-normal distributed data were square root transformed by a Box-Cox transformation.

In regards to determining the effect of different soil phosphorus level (three) and different planted species (three) on plant growth measurements (Shoots dry weight, roots dry weight, leaf area, plant heights and shoot-root), a GLM analysis of variance with fixed factors (species) three levels, and three levels of soil phosphorous concentration was used for analysis of each measurement separately.

To determine the effect of different soil phosphorus level (three) and different planted species (three) on plant shoot and root phosphorus uptake, another GLM analysis of variance with fixed factors (species) three levels, and three levels of soil phosphorous concentration was used for analysis.

Final GLM analysis of variance with fixed factors (treatments) of seven levels, and three levels of soil phosphorous concentration, was used for analysis. The GLMs were used to determine the effect of the treatments (three concentrations of Phoslock®, three planted species and the control) by determining the change of soil phosphorus value (ppm).

Tukey's test, using a significance level of 0.05, was applied to test the significance between the all the factor means (species, soil P level and treatments) and their combinations (species- soil P level and Treatments-soil P level). Back-transformed data are presented in the results section.

5.4 Results

5.4.1 Plant measurements

Shoot dry weight of both *Ptilotus* species and *L. albus* were recorded in different soil P levels (Figure 5.2; Table 5.1). *P. polystachyus* had greater shoot dry weight than *L. albus* and *P. macrocephalus* in the medium and high P level of soils. *P. polystachyus* and *L. albus* shoot dry weight increased with increasing soil P level. However, the shoot dry weight of *P. macrocephalus* was lower at high soil P level than at low and medium soil P level, suggesting P-inhibition in *P. macrocephalus*.

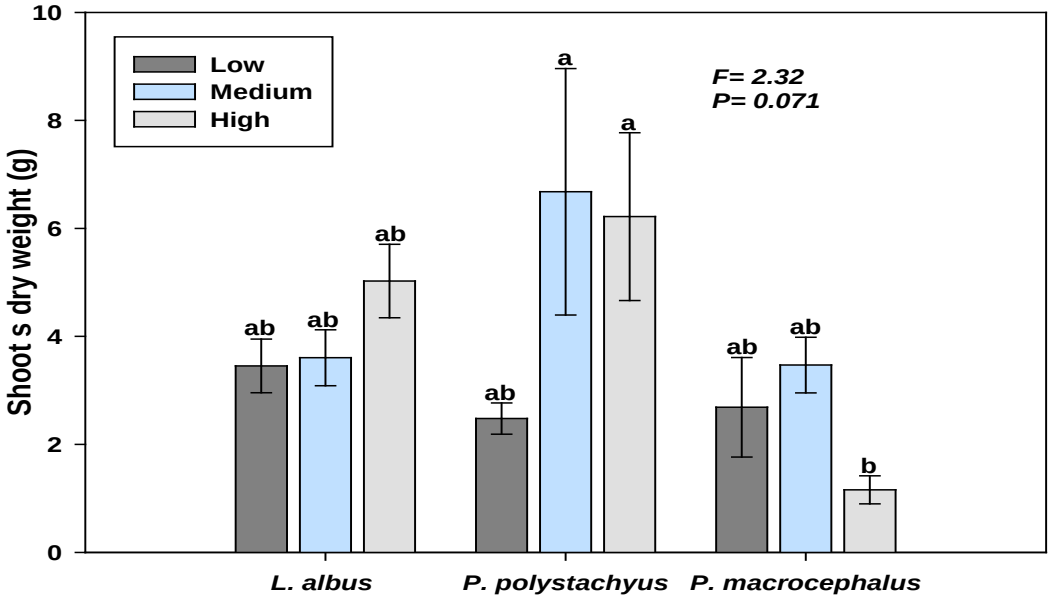

Figure 5.2 Mean shoot dry weght of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

The root dry weight in *L. albus* roots at high P level soil was 0.85 g (p= 0.013), which was significantly higher than *P. macrocephalus* 0.31 g and *P. polystachyus* 0.35 g (Figure 5.3; Table 5.1). Indeed, *L. albus* had approximately three times the root dry weight compared to both *Ptilotus* species at high soil P. While the root dry weight of *P. macrocephalus* in the low and medium level of soil P was higher than *L. albus* and *P. polystachyus* species, these differences were not significant (Figure 5.3; Table 5.1).

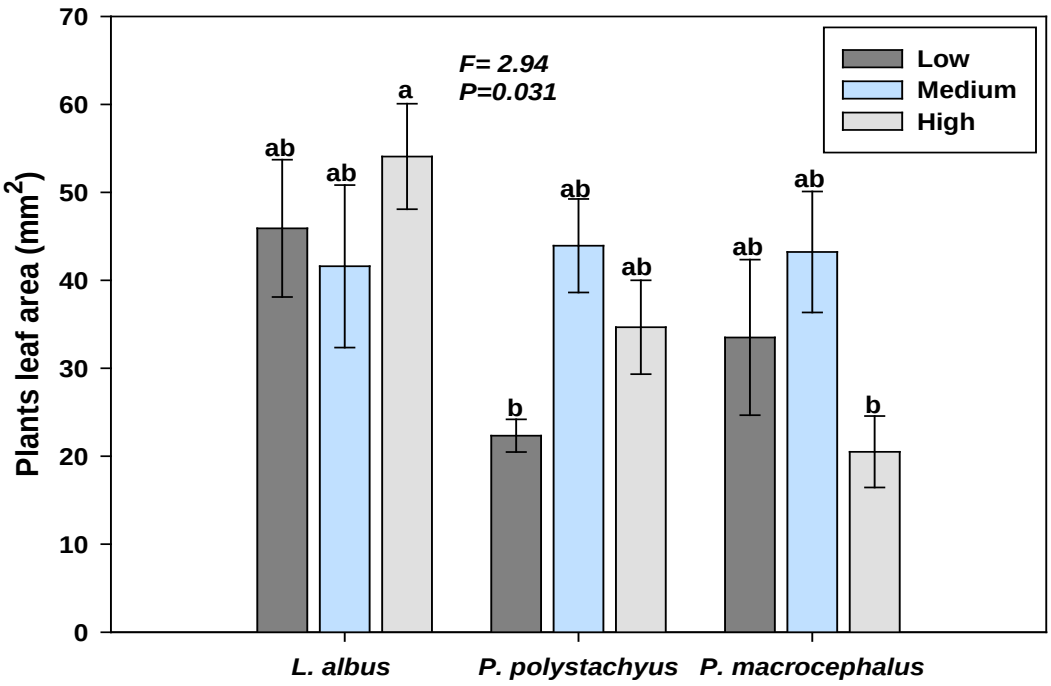

Figure 5.3 Mean root dry weight of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

The highest shoot-root ratio was recorded in *P. polystachyus* (Figure 5.4; Table 5.1). The shoot-root ratio of *L. albus* increased with increasing of soil P level, while the shoot-root ratio of *P. polystachyus* and *P. macrocephalus* was highest in medium P level soils.

The leaf area of *L. albus* was significantly higher than *P. polystachyus* and *P. macrocephalus* at high and low soil P levels (Figure 5.5; Table 5.1). However, the leaf area of *P. polystachyus* and *P. macrocephalus was* slightly higher than *L. albus* at medium soil P.

Table 5.1. Mean ± standard error and p value of plants growth measurements of three species *L. albus, P. polystachyus* and *P. macrocephalus* that planted at three different phosphorous level soils (low, medium and high).

	L. albus			***P. polystachyus***			***P. macrocephalus***		
Plant measurements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Shoots dry weight (g)	3.45 ± 0.50	3.61 ± 0.52	5.03 ± 0.68	2.48 ± 0.29	**6.68 ± 2.28**	**6.22 ± 1.55**	2.69 ± 0.92	3.47 ± 0.52	1.16 ± 0.26
Roots dry weight (g)	0.49 ± 0.14	0.49 ± 0.05	**0.85 ± 0.14**	0.21 ± 0.05	0.30 ± 0.08	0.31 ± 0.09	0.62 ± 0.13	0.63 ± 0.18	0.35 ± 0.09
Leaf area (mm^2)	**45.90 ± 7.80**	41.59 ± 9.23	**54.07 ± 5.99**	22.33 ± 1.85	43.93 ± 5.31	34.66 ± 5.34	33.51 ± 8.84	43.22 ± 6.88	20.51 ± 4.05
Plant heights (cm)	39.08 ± 3.58	42.00 ± 2.78	47.17 ± 2.19	26.25 ± 4.64	33.17 ± 4.92	**48.67 ± 6.62**	19.25 ± 7.38	19.33 ± 5.52	6.42 ± 1.13
Shoot-Root (%)	8.23 ± 0.90	7.43 ± 0.70	6.60 ± 1.28	17.95 ± 7.31	**31.91 ± 16.40**	22.92 ± 4.63	5.82 ± 2.26	7.23 ± 1.34	4.94 ± 2.29

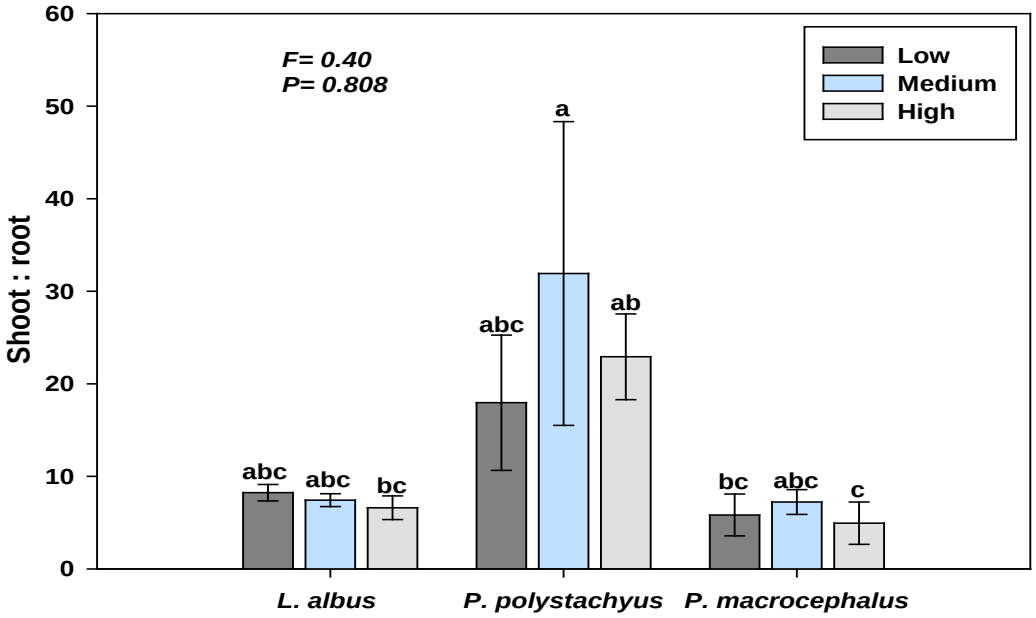

Figure 5.4 Mean shoot-root ratio of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

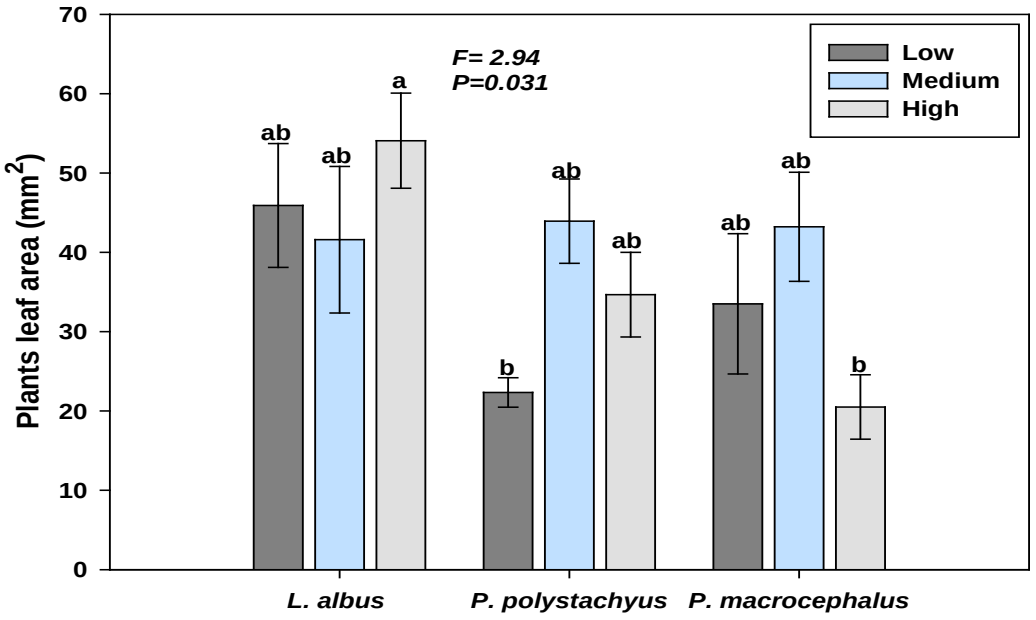

Figure 5.5 Mean plant leaf area of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

Plant heights followed a similar trend of shoot dry weights (Figure 5.6; Table 5.1). *P. polystachyus* had greater plant height than *L. albus* and *P. macrocephalus* at the high soil P level. The plant heights were increased by increasing in soil P level for *P. polystachyus* and *L. albus*. However the opposite trend was shown for *P. macrocephalus*.

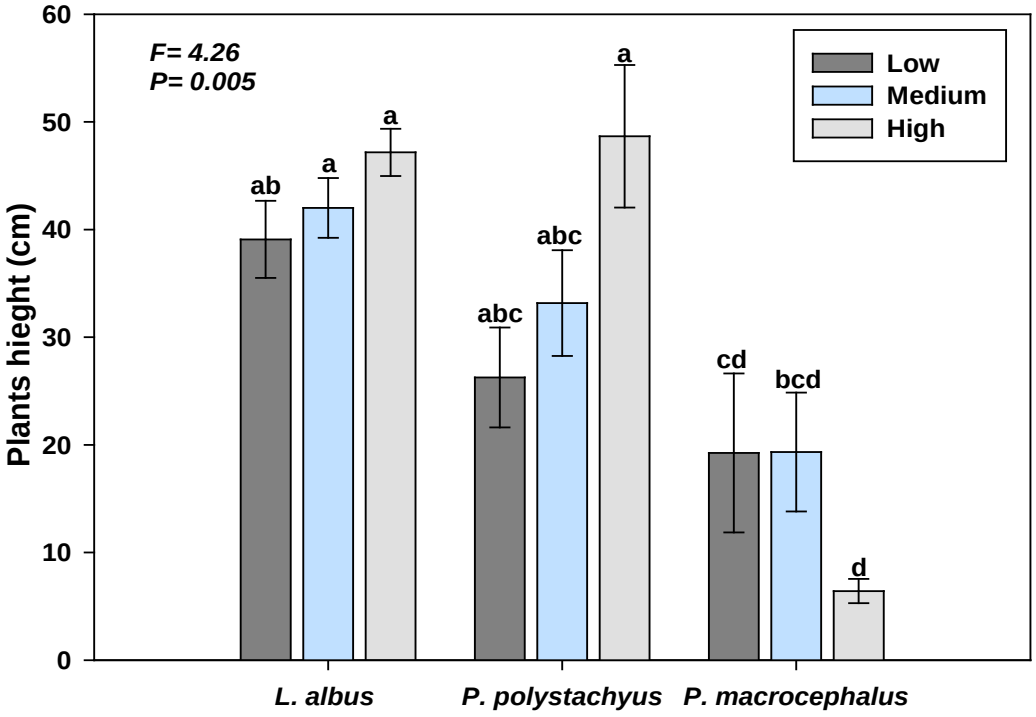

Figure 5.6 Mean plant height of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

5.4.2 Phosphorous concentrations in plant matter

Shoots and root P uptake of all three species were effected by different soil P levels. The concentration of phosphorous within shoots of *P. macrocephalus* at high soil P levels was significantly higher than *P. polystachyus* and *L. albus* (Figure 5.7; Table 5.2). There were no significant differences in shoot P concentration at low P level soils.

Table 5.2. Results of General Linear Model (GLM) to test the effects of soil phosphorus level and plant species on shoot phosphorus uptake.

Source	*DF*	*SS*	*F-value*	*P- Value*
Soil phosphorus level	2	20127	34.41	**0.001**
Species	2	27062	46.26	**0.001**
Soil phosphorus level Species*	4	11004	9.41	**0.001**
Error	44	12869		
Total	52	68744		

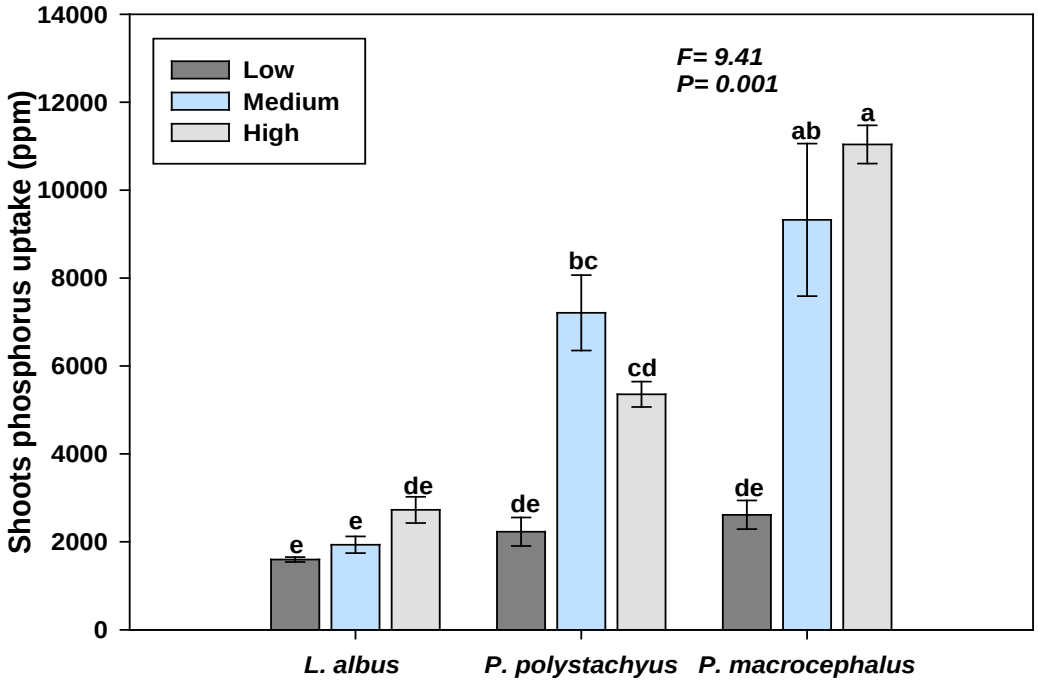

Figure 5.7 Mean phosphorus shoot-uptake of *L. albus, P. polystachyus and P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

The highest root P concentration was recorded at high soil P level for *P. macrocephalus,* whilst the lowest root concentration was 1201 ppm for *L. albus* at the medium soil P level (Figure 5.8; Table 5.3).

Table 5.3. Results of General Linear Model (GLM) to test the effects of soil phosphorus level and plant species on root phosphorus uptake.

Source	*DF*	*SS*	*F-value*	*P- Value*
Soil phosphorus level	2	1552	6.26	**0.020**
Species	2	5289	21.33	**0.001**
Phosphorus level Species*	4	1822	3.67	**0.049**
Error	9	1116		
Total	17	9779		

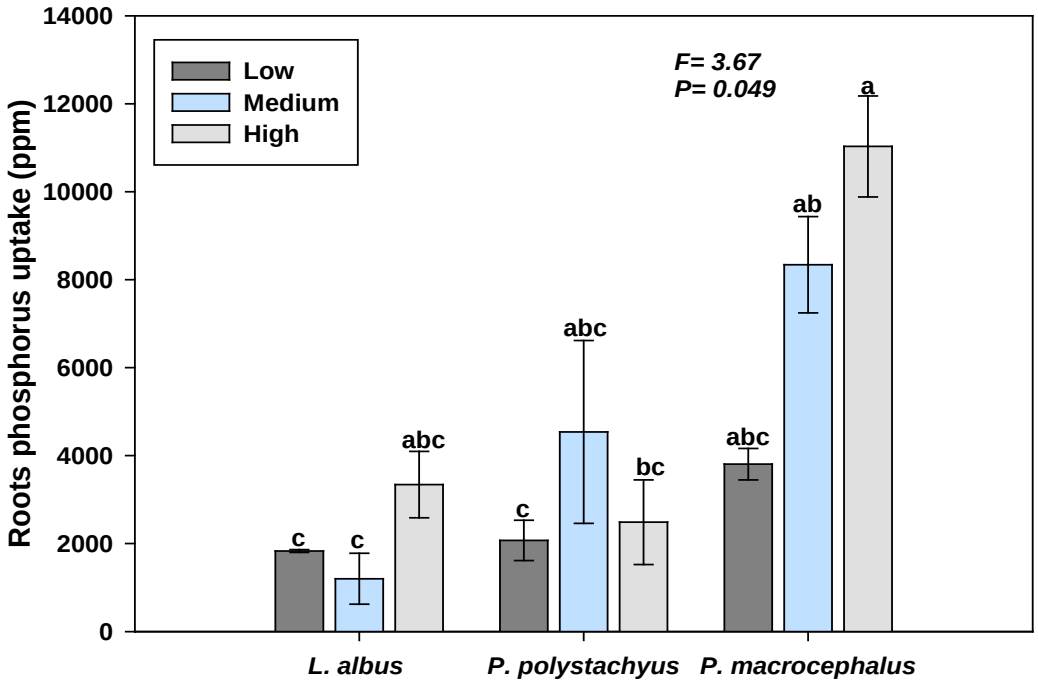

Figure 5.8 Mean phosphorus root-uptake of *L. albus, P. polystachyus* and *P. macrocephalus* grown at three different P levels (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

5.4.3 Concentrations of phosphorous within the soil

Figure 5.9 shows the change in mean soil-available phosphorous levels between the beginning and end of the experiments. It shows that the greatest reduction in soil P was achieved by the two *Ptilotus* species at each of the three soil P levels. At low P levels, the highest P reduction recorded was -2.58 and -2.55 ppm for *P. macrocephalus* and *P. polystachyus* species respectively, and these differences were significant different compared to the control and other treatments (p= 0.001). There were similar trends at medium and high P level soils; for instance, the P reduction of *P. macrocephalus* and *P. polystachyus* was -4.75 and -4.10 ppm at medium soil P, respectively, and -5.20 and -9.13 ppm at high level P soils, respectively.

The two Phoslock® treatments (Phoslock 100 and Phoslock 500) and *L. albus* did not result in significant reductions in soil P level at either low, medium or high P levels.

However, the P reduction of Phoslock 1500 treatments was -4.15 ppm at high P level soil and was significantly different (p=0.001) from the control and other treatments (*L. albus*, Phoslock 100 and Phoslock 500).

Table 5.4. Results of General Linear Model (GLM) to test the effects of soil phosphorus level and all treatments on phosphorus reduction rate in the soil.

Source	*DF*	*SS*	*F-value*	*P- Value*
Soil phosphorus level	2	210.3	63.77	**0.001**
Treatments	6	433.3	43.79	**0.001**
Phosphorus level Treatments*	12	103.1	5.21	**0.001**
Error	105	173.2		
Total	125	920.0		

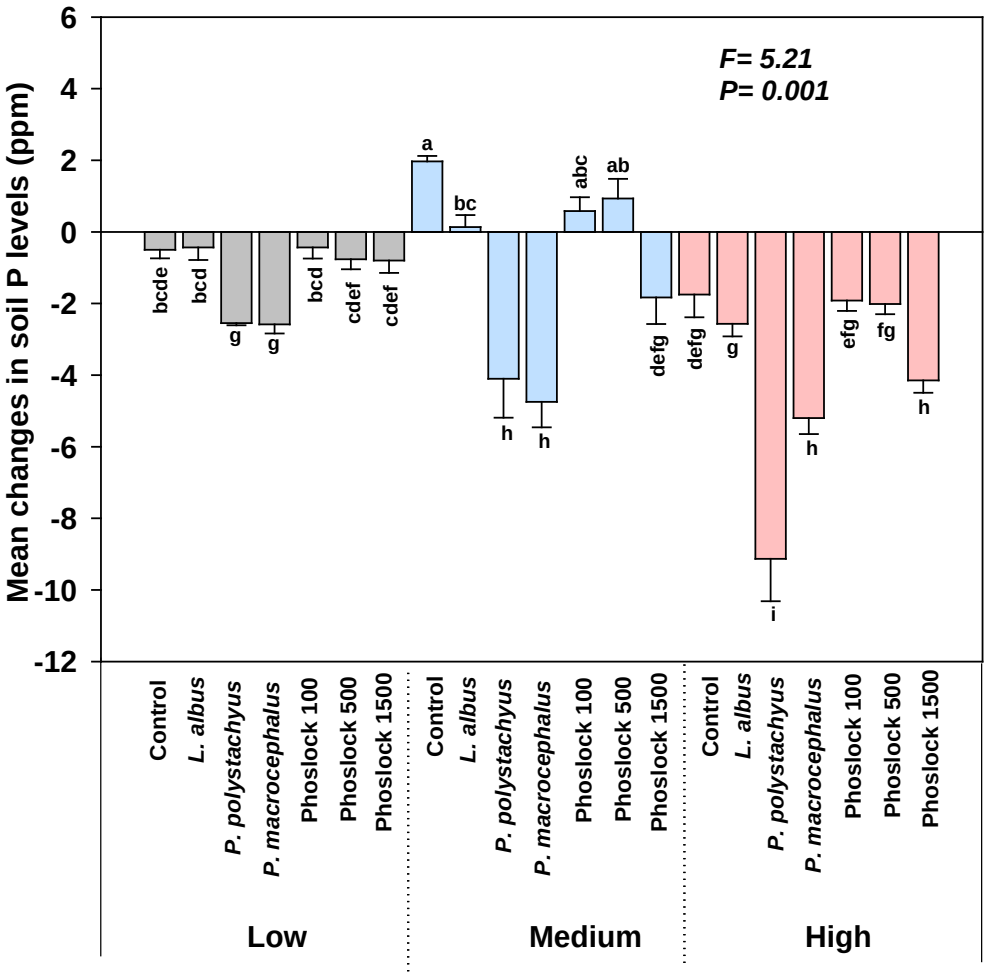

Figure 5.9 Mean change in soil phosphorus levels that were treated with three concentration of Phoslock® (100, 500 and 1500g/m^2) and planted with three species (*L. albus, P. polystachyus* and *P. macrocephalus)* at three different P level (low, medium and high). Vertical bars represent ± standard error of the means. The different letters on the vertical bars represent the results of Tukey's test; treatments that do not share a letter are significantly different from one another.

5.5 Discussion

As expected, the shoot dry weights increased in P among the three species at all soil P levels. The lowest shoot dry weight was found in *P. macrocephalus* which occurred at high soil P levels. This was because native species require significantly less N, P, and K fertiliser than agricultural crops and pastures (Figure 5.2; Table 5.1). This reflects the observation that the greater the dry weight of the shoots that a species produced, the greater is the amount of P taken up from the soil (Ryan *et al.,* 2009; Pang *et al.,* 2010b). The highest shoot dry weight amongst the species was 6.68 g and 6.22 g for *P. polystachyus* at medium and high soil P levels. The study by Ryan *et al.* (2009) shows that the *P. polystachyus* species grew much better and produced more biomass than chicory in soil with low bicarbonate-extractable phosphorus or mineral nitrogen. The study of Lelei and Onwonga, (2014b) showed that the *L. albus* shoot dry weight and seed yield were significantly higher in the high-available phosphorus soil.

In regard to root dry weight, *L. albus* had approximately three times the root dry weight than both *Ptilotus* species at high soil P. That is because the root system of *L. albus* consists of a prominent tap root, and at irregular intervals along the lateral roots there are dense clusters of rootlets of limited growth (Gardner *et al.,*1983). The higher root dry weight obtained at higher P level soil can be attributed to higher P uptake due to higher available P in soil. Kisetu and Teveli (2013), in a screen house pot study designed to assess the response of *Vigna radiata* L. (Fabaceae) to application of phosphorus fertilizer on a neutral Olasiti soil, showed that the number of pods and seeds increased from 3-6 and 7-9, respectively, in treatments 40 to 160 mg per 4 kg soil of phosphorus fertilizer applied. Overall, *Ptilotus* species have low root dry weight at the high P level while the root dry weight of *P. macrocephalus* in the low and medium level of soil P was higher than *L. albus* and *P. polystachyus* species (Figure 5.3; Table 5.1; $p<0.05$). The Ryan *et al.* (2009) study showed that *P. polystachyus* had a greater root dry weight than chicory at the lowest level of P addition.

L. albus species responded to increasing P by increasing individual leaf size, while *P. macrocephalus and P. polystachyus* species responded by increasing leaf number. This study clearly showed that the growth response of *L. albus* species to high P levels is greater than the response of *P. macrocephalus and P. polystachyus* species (Figure 5.5; Table 5.1). This may be due to the shape and form of the leaves' size in *P. macrocephalus and P. polystachyus*, which is smaller than in *L. albus*.

There was sufficient response by the species to increased soil P levels, and shoots and root P uptake of all three species were all subsequently effected. The highest shoot P concentration was found at *P. macrocephalus* at high soil P levels, which was significantly higher than *P. polystachyus* and *L. albus* ($p<0.05$; Figure 5.7; Table 5.3). In the medium P level soil, the highest shoot P concentration was in *P. macrocephalus* but this was not significantly different from *P. polystachyus*. This trend declined at lower P levels in the soil where there was no significant differences between all three species in regard to shoot P uptake.

There was little difference in root P concentration among species at low levels of soil P application, while root P concentration increased at the medium and high soil P in all three species. It reached very high values at the highest soil P level. Many Australian native plants have evolved in severely P-impoverished environments (Handreck, 1997) and efficient P acquisition mechanisms appear to be poorly regulated at high P availability in some of these species (Lambers *et al.,* 2008; Ryan *et al.,* 2009; Shane *et al.,* 2004). The ability to accumulate high concentrations of P in roots and shoots might be related to a low capacity to down-regulate P uptake as found in *Hakea prostrata* (Shane *et al.,* 2004; Pang *et al.,* 2010a).

In regards to soil P concentration, two main hypotheses were posited and tested. The first (null) hypothesis is that the addition of Phoslock® to soils high in P will not appreciably lower the available P, thus facilitating native plant growth. This null hypothesis was accepted. The results obtained showed that all Phoslock treatments (Phoslock 100,

Phoslock 500 and Phoslock 1500) had no significant effects on the soil P reduction at low and medium soil P levels. Similarly, there was no effect of Phoslock 100 and Phoslock 500 treatments on P reduction in high P level soils (Figure 5.9). However, Geurts *et al.* (2011) showed that the addition of Phoslock® to flooded soils could be expected to be effective due to it forming an active layer on top of the soil and thus reducing phosphate mobilization into the mobile water. But in this current study, the addition of Phoslock® to the soil was not effective in low and medium soil P levels. Haghseresht (2005) showed that the Phoslock® binds various forms of phosphorus and precipitates as stable mineral rhabdophane which is characterized by a very low solubility product.

In contrast, the effect of Phoslock 1500 treatment was about 50% more than Phoslock 100 and Phoslock 500 treatments at high P level soil (Figure 5.9). It is thus clear that the Phoslock® treatments only reduced the P level in high P level soil when the treatment concentration is at the highest level (1500 kg/m^2). The reduction of soil P by this method may not be economically satisfactory because the cost effect of this treatment is prohibitive, and thus it does not provide an ideal management method for soil P reduction in the terrestrial ecosystem.

The second hypothesis was that seeding and harvesting areas high in P with P-absorbing plants will not appreciably lower the available P content in the soil. This hypothesis was rejected in the case of the two native species *P. macrocephalus* and *P. polystachyus*. This was due to the demonstrated effective phosphorus absorption from the three levels of soil P, and consequently there was reduction of the phosphorus in the soil at all P levels.

The response of *L. albus* and *P. polystachyus* species to high phosphorus soil levels was significant, with the highest shoot dry weight, plant height and shoot: root ratio being recorded on *P. polystachyus* species at medium and high P level soils. The highest root dry weight and leaf area were recorded on *L. albus* species at high and low P level soils. Several studies have shown the positive relationship between plant growth and P uptake; for instance Brennan *et al.* (2000) showed significant results with *P. exaltatus* species

growth by increasing the phosphorus level in the soil. Similar results were showed by Ryan *et al*. (2009) for *P. polystachyus*, where there was an increasing shoot dry weight and root dry weight at low and high phosphorus level soils. Lelei and Onwonga (2014b) showed an increase of growth response as well as phosphorus uptake of *L. albus* species at high phosphorus level soils. However, in this current study, this relationship was not positive in regards to *L. albus* species, since mean shoot and root P uptake was very low at all three levels of soil P (Figures 5.7 and 5.8). It could be argued that whilst *L. albus* species had access to the P at all P level soils, it did not take it up but rather maintained a constant homeostasis and P concentration in the tissues (Watt & Evans, 2003).

In contrast, the growth measurement of *P. macrocephalus* (shoot dry weight, root dry weight, leaf area, plant height and shoot: root ratio) was lower than *L. albus* and *P. polystachyus*. The phosphorus shoot and root uptake was higher than *P. polystachyus* and *L. albus* respectively (Figures 5.7 and 5.8). The lack of response of *P. macrocephalus* to the P levels can be explained in terms of the inherent genetic differences in the development, and the root construction of the species.

In this study, it is implied that the use of low and medium concentrations of Phoslock® does not affect the reduction of available soil P at different soil P levels. However, the high concentration of Phoslock® (1500 kg/m^2) could reduce the available phosphorus in the soil by binding it without removing it from the soil. It is also implied by these results that *L. albus* is not an effective management tool for phosphorus reduction for ex-arable cropping land in the VVP grassland even though *L. albus* has long been predicted as a species with strong, specialized P-responsive physiological adaptations such as producing cluster roots that release large quantities of citrate and malate (Gardner *et al., 1983*; Gerke*,* 1994; Sas *et al.,* 2001). Finally, it is implied that the *P. macrocephalus* and *P. polystachyus* species have the capability to accumulate high concentrations of P in the shoot and root tissues whilst maintaining biomass production, which makes it eminently suitable for such a phytoremediation role (Ryan *et al.,* 2009). It is thus an eminently suitable native species

for restoration management of ex-arable cropping area within the VVP grasslands. This is especially so when abundant P in soil raises an environmental threat to native species and needs to be removed from the system (Novak & Chan, 2002; Tibbett & Diaz, 2005; Sharma *et al.,* 2007).

5.6 Conclusion

This chapter has covered an investigation of the effectiveness of reducing available soil phosphorus by either binding it within the soil as an insoluble matrix, or by biologically extracting the phosphorus using suitable native plants. These two methods have been assessed during a glasshouse trial which was designed to investigate two main (null) hypotheses.

The first hypothesis is that the addition of Phoslock® to soils high in P will not appreciably lower the available P, thus being unable to facilitate native plant growth. The second hypothesis is that seeding and harvesting areas high in P with P-absorbing species will not appreciably lower the available P content in the soil.

The results indicated that whilst the use of Phoslock® treatments in high concentration could reduce the available P level at very high soil P levels, it is not practically useful in low and medium concentrations. However, both *Ptilotus* species are useful management tools for phosphorus reduction in the soil due to their ability to absorb high amounts of soil P during growth period at different soil P levels (low, medium and high).

Chapter 6. Synthesis of key findings, management implications, and future research directions

6.1 Introduction

This thesis has been carried out in the domain of grassland research, understanding that grasslands occupy and constitute approximately 70% of the world's agricultural areas (Suttie *et al.*, 2005). As a consequence, grasslands are an integral and central part of the global ecosphere, and thus it is essential that any remaining native grasslands need to be protected, and further, degraded grasslands should be, wherever possible, restored to be fully functioning ecosystems.

It was noted earlier that in Victoria, native grasslands are one of the most endangered ecosystems in the country. Approximately 99% of these grasslands have been disturbed in the last 150 years (Scarlett *et al.,* 1992), and, as a result, the VVP grasslands, which are an integral ecological element in this region, are one of the most endangered vegetation formations in Australia (Muir, 1994). A detailed review of the national and international literature found that several studies have examined the effectiveness of grassland restoration (Lunt & Morgan, 2002; Prober *et al.,* 2009; Cole *et al.,* 2016; Kendal *et al.,* 2017; Van Daele *et al.,* 2017) in Australia and other parts of the world. However, very few studies have investigated the approaches to overcome biotic and abiotic barriers in ex-cropland environments. Understanding that there are two broad classes of barriers which prevent the recovery of degraded grassland—biotic and abiotic issues—this thesis has addressed a significant knowledge gap by addressing biotic and abiotic barriers individually, and hence observing the relative importance of each barrier to grassland restoration. In doing so, I investigated the efficacy of various techniques for overcoming these observed barriers.

This final chapter synthesises the key findings, summarises and explores the implications of the research findings presented in this thesis and also provides some future research directions in this critical area of research. Its objectives are to: (i) summarise the main

findings of the research; (ii) highlight the major theoretical advances of the work; (iii) summarise the main implications and management recommendations, and (iii) highlight and discuss future research implications.

6.2 Synthesis of key findings

In the following section all the key findings as reported in Chapter 2 to 5 of this thesis are presented. The main research question of this study was: *What are the relative influences of nitrogen, phosphorus and exotic weeds as barriers to grassland restoration from former cropland?* In order to address this question, which by its nature is multifaceted, a series of inter-related experiments were conducted to answer the overarching research question. It was anticipated that successfully addressing this question will advance our theoretical understandings of the barriers to grassland restoration, which will prove crucial to the development of new and viable landscape scale restoration approaches. In designing this work, it was understood that grasslands are a complex system, and they have been historically disturbed in such a way that they are far from their natural equilibrium state. As a consequence, the restoration work carried out in this thesis has been done in a series of steps in an attempt to move the system toward the equilibrium state thorough abiotic and biotic barriers.

Establishing a baseline condition

In Chapter 2, I measured various environmental parameters to evaluate the current state of the ex-cropland areas to provide a clear baseline for the study. First, the difference in vegetation cover between the effected land and a nearby undisturbed site was carried out, and this showed that (i) there were few native species present in the experimental site, but there were many aggressive exotic species, and (ii) whilst there were more native species in the reference site, there was evidence of invasion of exotic species, possibly by wind-borne seeds or through animal or human intervention.

Next, I examined the current soil seed bank species composition in the reference and experimental sites. This study shows that the soil stored seed bank in the experimental site is dominated by exotic species, and this again supported the observation that few, if any, native species remained in the seedbank. This demonstrated that any restoration attempts will need to introduce new propagules of native species, either by seed or tube stock, particularly given natural dispersal of native species to isolated grassland patches is expected to be very low.

Examination of the current level of soil nutrients and soil bulk density variables in the reference and ex-cropland site grasslands also showed considerable differences. In particular, it was found that the ex-cropland site was very high in nutrient content.

Overall, this initial work showed that there was a high number of exotic weeds, a lack of native species, very high nutrient levels (especially phosphorus and nitrogen), and an absence of organic carbon, all due to conversion of grassland into cultivated land. Each of these conditions have been shown by previous studies to mitigate against the introduction and sustainability of native species.

Evaluation of grassland restoration techniques

In Chapter 3, the investigations moved to the design and comparative evaluation of four methods of overcoming biotic and abiotic barriers to begin the restoration initiatives. These experiments were: (i) the use of urban green waste (oversize) as a cheap and readily available source of compost, which was applied to heat up and physically destroy the resident exotic seed bank; (ii) the use of carbon (in the form of cold green waste and sugar) to stimulate essential microbial activity in the soil and to temporarily reduce the soil nitrogen levels; (3) the application of Phoslock® to the topsoil in an attempt to reduce the levels of available phosphorus by chemical complexing; and (4) scalping the area of affected topsoil (top 10 cm).

It was found that the use of hot green waste alone, a combination of hot green waste plus mulch), and a mixture of hot green waste plus sugar, all led to a re-establishment of native species (*Austrostipa semibarbata, Bothriochloa macra, Rytidosperma* spp. and *Themeda triandra*) when the topsoil was deliberately seeded. In addition, the hot green waste + sugar treatment was seen to be more effective in reducing the exotic species cover directly after its application, and observations indicated significant establishment of native species at the end of the three year study period. This treatment also has a significant effect on the topsoil by increasing the organic matter level, which had a positive effect on the desirable microbial activity. However, the treatment had a negative effect on soil nitrogen, phosphorus and potassium levels which were seen to increase dramatically. As such, one of the main barriers to restoration of Australian grasslands (i.e. high nutrient status) is exacerbated by this treatment, despite the other positive effects of the treatment. This may, in time, facilitate the re-invasion of exotic plant species. However, there were no negative effects of this treatment on soil pH levels and soil bulk density during sampling period.

In regards to the use of other added carbon resources, it was found that cold green waste and sugar treatments have reduced the vegetation cover of exotic weeds and have facilitated the recruitment of broadcasted native species. There was also a significant increase in the level of organic matter in the soil, and this was especially so for the cold green waste treatment which was dramatically increased in the second assessment year (2016). This approach has had a positive effect on the microbial activity within the soil, and has also reduced the amount of ammonium nitrogen in the first assessment year (2015). This was especially so for the sugar addition treatment, which led to the lowest ammonium nitrogen level recorded (5.2 ppm). However, it was noted that the amount of ammonium nitrogen increased in the second year (2016), but nevertheless was still smaller than levels achieved by the use of hot green waste alone.

In contrast, the amount of nitrate nitrogen was shown to be highest for the cold green waste treatments, while the lowest nitrate nitrogen levels were recorded for the sugar treatment after the first assessment year (2015). Both phosphorus and potassium levels were increased dramatically by the cold green waste treatments, but again there was no effect of these treatments on soil pH or soil bulk density levels.

In regard to the use of Phoslock® addition to the topsoil in an effort to reduce available phosphorus by binding the free phosphorus in a bentonite clay matrix to prevent the uptake of phosphorus by plants, it was found that this treatment did not have an effective influence on the exotic species cover or recruitment of seeded native mix. There was little effect on reduction of the amount of phosphorus amount in the soil, but it did appear to reduce ammonium nitrogen levels during the second assessment year (2016). The lowest ammonium nitrogen amount (4.7 ppm) was recorded in this treatment. There were no observable effects of this treatment on nitrate nitrogen, organic matter, potassium amount and pH level in the soil, nor was there an effect on soil bulk density level and soil microbial activity.

In regards to the scalping treatment, it was found that the exotic species cover in scalped plots declined substantially during spring 2014 and autumn 2015, but it was seen that this rate increased during spring 2015, with 31% regrowth of exotic species in the plots. It is thought that this unexpected increase is largely due to wind-blown seeds from outside the study area. Clearly, scalping has a positive effect in removing all the exotic weeds in the soil seed bank, thus preventing their re-emergence, however, whilst this treatment is an effective tool to reduce or remove the unwanted high levels of nitrogen and phosphorus levels required for native species re-establishment in the soil, it also dramatically decreases the organic matter level and removes all microsites for microbial activity. As a consequence, it reduces the microbial activity as well as increasing the soil pH level. It has also a negative effect on soil bulk density, since the remaining subsoil had a much higher density than the removed material that likely due to the heavy clay soil.

Breaking seed dormancy in two *Ptilotus* species

In Chapter 4, pre-treatments were conducted in order to examine a series of methods and techniques to break the seed dormancy, and to find the optimum germination condition of selected species (*Ptilotus macrocephalus* and *Ptilotus polystachyus*), which were required in the next investigation of phosphorous mitigation. This practical problem of dealing with seed dormancy needed to be addressed before using these species for the later glasshouse experiments, and also to prepare seeds in restoration trials to make reseeding more financially viable. The investigation involved a determination of these seeds' response to a number of seed pre-treatments, which were exposing those target species seed to (i) smoke water, (ii) heating shock, (iii) cold stratification and (iv) gibberellic acid solution. Results were compared with RO water (which represents a control with no pre-treatment). It was found that the use of untreated seed of both *P. machrocephalus* and *P. polystachyus* to reseed cleared land would not be an economical possibility because the germination percentages are low. The use of smoke water and heat shock ($60^{O}C$) somewhat increased the germination rate of *P. machrocephalus* species, while the germination rate of *P. polystachyus* seeds remained at zero. The cold stratification without bracts increased the germination rate of *P. polystachyus* species significantly, but the germination rate of *P. machrocephalus* was very low. Finally, the use of gibberellic acid with a concentration of 500ppm was sufficient to increase the germination rate of both species significantly. It is found that the highest germination values were observed when the seeds of *P. macrocephalus* (62.37 ± 1.16) and *P. polystachyus* (38.11 ± 0.67) were pre-treated with GA500 and exposed to a temperature range of ($20/18^{O}C$) and a 12h dark/12h light regime, so this combination, with the gibberellic acid, was used in the following glasshouse study.

Methods for reducing soil phosphorus

Chapter 5 describes the glasshouse study, which was designed as an investigation of the effectiveness of reducing available soil phosphorus by either (i) binding it within the soil

as an insoluble matrix with Phoslock®, or (ii) by biologically extracting the phosphorus using suitable *Lupin albus, Ptilotus macrocephalus* and *Ptilotus polystachyus*. It was found that whereas the use of Phoslock® treatments in high concentration could reduce the available P level at very high soil P concentrations, it is not practically useful in low and medium concentrations in terms of phosphorous reduction or economic viability. However, it is found that both *Ptilotus* species (*P. macrocephalus* and *P. polystachyus*) are useful management tools for phosphorus reduction in the soil, due to their ability to absorb high amounts of soil P during growth period at different soil P levels (low, medium and high), and thus several years of seeding and harvesting of these plants is anticipated to provide a useful phosphorous reduction mechanism.

6.3 The theoretical contribution of this work

6.3.1 A summary of contributions

The main theoretical contribution of this thesis was in the identification of possible treatments to deal with the biotic and abiotic barriers which currently prevent the spontaneous restoration of ex-cropland native grassland.

First, the initial observation of the differences in vegetation cover, soil seed bank composition, current soil nutrient condition and soil bulk density between the ex-cropland and native grassland showed conclusively that the likelihood of grassland revering to its natural state without intervention is very low.

Second, the comparison between four methods of overcoming biotic and abiotic barriers to restoration initiatives in order to allow the re-establishment of native grassland species, has indicated that a careful and sequential application of treatments is required to develop the appropriate levels of mineral and nutrients in damaged soil. One application of any particular treatment, whilst contributing to part of the restoration process, is not able to achieve the overcoming of both biotic and abiotic barriers.

Third, the examination of methods and techniques to break the seed dormancy to find the optimum germination condition of *P. macrocephalus* and *P. polystachyus* species have

provided an important guide to reseeding technology. The biology of native seeds indicates that they are resistant to mass germination, and pre-treatment is essential to support economically feasible restoration attempts.

Finally, the investigation of the effectiveness of reducing available soil phosphorus by either binding it within the soil as an insoluble matrix with Phoslock®, or by biologically extracting the phosphorus using suitable native plants, has indicated that chemical chelation is less than useful in phosphorous reduction and is expensive, but cropping the effected areas with *P. macrocephalus* and *P. polystachyus,* seems to be a very practical, and possibly profitable approach to this problem.

6.3.2 Some reflective comments

The analysis of vegetation cover, soil seed bank, soil nutrients and soil bulk density knowledge described in Chapter 2, were useful contributions to the main study area. Other authors (Tisdall & Oades, 1982; Tisdall, 1994; Six *et al.,* 2000; McLauchlan, 2006; Coleman *et al.,* 2017; Nunes *et al.,* 2018), based on their investigations, have emphasized that conventional cultivation has been shown to harm soil horizons, increase soil erosion, change nutrient accessibility and mineral compositions, compact soil, and decrease the percentage and formation of soil macro aggregates. The current study has supported these authors' findings, highlighting (i) the main differences between the native grassland and ex-cropland in regards to vegetation cover, soil seed bank composition, soil bulk density and the negative relationship between introduced soil nutrients and native species, mainly with respect to phosphorous and nitrogen, with the result that exotic species tend to dominate altered grasslands that carry an excess of these minerals (Morgan, 1998; Dorrough *et al.,* 2004; Prober *et al.,* 2005; McIntyre & Lavorel, 2007), and (ii) there is a surprisingly low correspondence between the composition of the above-ground vegetation and the seed bank (Williams, 1984; Wisheu & Keddy, 1991; Lunt, 1997; Pardo *et al.,* 2018).

The correspondence of this current project and published knowledge of aspects of ex-agricultural land contributed by the study of other authors was addressed in Chapter 3. Here, this current work based treatment techniques on previous studies that investigated (i) the effect of soil nutrients on species-richness (Janssens *et al.,* 1998), (ii) methods of reducing the nutrient status of soils to improve success in natural grassland establishment (McCrea *et al*., 2001) and (iii) multisite experiments comparing soil pre-treatments, natural regeneration, nurse crops, and seed mixes (Pywell *et al.,* 2002). Whilst due recognition is given to these authors, the restoration management techniques used in this current work have added to our knowledge regarding the use of a novel method for restoration of ex-cropland in VVP grassland, which involved application of hot green waste, which reduced the exotic weed cover and the related soil seed bank.

Previous studies conducted in grassland restoration have shown that using carbon resources to reduce the exotic weed infestation in above ground and below ground cover has been demonstrated (Dalenberg & Jager, 1981; Morghan, & Seastedt, 1999; Magill & Abel, 2000; Blumenthal *et al.,* 2003; Prober *et al.,* 2005; Eschen *et al.,* 2006; Prober *et al.,* 2009; Faithfull *et al.,* 2010), where they indicted that addition of biologically available carbon as sugar or sawdust can increase microbial activity which helps to draw down the nitrogen level. It has been shown that addition of carbon can also reduce inorganic nitrogen and exotic plant biomass, whilst other studies have shown that addition of sugar has a significant impact on reducing the germination of exotic weed seeds within the soil seed bank. It has also been shown that the particular source of carbon added to the soil is important as its influence on soil condition and plant growth is governed by the rate at which the carbon becomes available to microorganisms (Eschen *et al.,* 2006). For instance, a readily available carbon source, such as sugar, may stimulate microbial activity within hours (Dalenberg & Jager, 1981), however other sources, such as more complex molecules, have structures that need more time to decay (Magill & Abel, 2000). Eschen *et al*. (2007) showed that carbon addition during grassland restoration is a useful

management technique to reduce nitrogen availability on ex-cropping land. The results of this current investigation have concurred with these comments, and indicate that addition of carbon resource to ex-arable cropland to redress its condition, is an essential consideration.

Using Phoslock® treatments as a useful management tool to reduce the available soil phosphorus for terrestrial purposes, was demonstrated by the previous studies of Geurts *et al.* (2011) which implied that the addition of Phoslock® is expected to be especially effective in flooded soils, because it can form an active layer on top of the soil that reduces phosphate mobilization to the water layer. However, the findings of the current investigation are that the use of Phoslock® as a phosphorous removal agent have not been encouraging. Both the small reductions in phosphorous level and the cost of the material are factors which might suggest that its use in restoration management is marginal.

Finally, in this respect, I concur with the claim that stringent exotic weed control is an important factor in the successful restoration of highly degraded sites to grassland which requires native species seeding (Cole & Lunt, 2005), and that adding a mixture of native species seeds, post ground treatment, increased species richness and abundance after the exotic species cover has been substantially reduced (Morris & Gibson-Roy, 2018). It has been previously reported that the scalping treatment is an effective tool for the restoration of degraded grassland on the VVP (Gibson-Roy, 2010a; Morris & Gibson-Roy, 2018), where their results clearly showed that the physical removal of topsoil for 10 cm is sufficient to remove all unwanted exotic seeds and excessive inorganic nutrients. However, I have commented that this path introduces its own problems, with the expense of removal of the soil, the location of a suitable collection site, and the interference to scalping by rocks and other matter. This suggests that other treatments investigated by this work may be more likely to provide practical management options, both in terms of expense and efficiency of work.

Breaking the seed dormancy (Chapter 4) has been the subject of many other studies, and a range of techniques have been used. These include (i) low temperature (cold) and hormonal treatments (gibberellic acid) (Taylor *et al.,* 1993; Schneider & Gifford, 1994; Nicolas *et al.,* 1996; Clarke *et al.,* 2000; Walck *et al.*, 2000; Kaye & Kuykendall, 2001; Merritt *et al.,* 2007); (ii) high temperatures up to 80^{O}C, (Mott, 1974b; Hagon, 1976; Tieu *et al.,* 2001); and (iii) smoke water, which is widely agreed to be a key agent for the release of seed dormancy in a range of native Australian species (Dixon *et al.,* 1995; Roche *et al.,* 1997a, b; Smith *et al.,* 1999). This current study has supported the findings of the studies by identifying the best methods and techniques to break seed dormancy and finding the optimum germination condition of *P. macrocephalus* and *P. polystachyus*. Other authors have demonstrated that temperature and light both play a part in the control of dormancy and germination rate (Benech-Arnold *et al.,* 2000; Batlla *et al.,* 2004). Williams *et al.* (1989) studied methods to overcome the seed dormancy of *P. exaltatus*, and asserted that scarification of the seed husk and application of gibberellic acid will stimulate effective germination. In the current investigation, I am confident that the recommended approach using gibberellic acid is the most effective treatment to ensure maximum germination rates.

The two approaches used (Chapter 5) to reduce the individual effect of phosphorus, using either Phoslock® or using native species to reduce the available soil phosphorus, have been drawn from the work of many authors (Trinick, 1977; Bates, 1990; Saito & Kato, 1994; Islam *et al*., 1999; Brennan *et al.,* 2000; Veneklaas *et al.,* 2003; Watt & Evans, 2003; Le Bayon *et al.,* 2006; Gilbert *et al*,. 2009; Ryan *et al*., 2009; Geurts *et al.,* 2011; Lelei & Onwonga, 2014a). The contribution of the current study was to demonstrate that the use of native species such as *P. machrocephalus* and *P. polystachyus* were useful management tools for phosphorus reduction in the soil. This finding was in agreement with the general findings of authors (Islam *et al*., 1999; Ryan *et al*., 2009), whilst the idea of using *L. albus* as a tool for reducing available soil phosphorus (Gardner *et al.,*1983;

Veneklaas *et al.,* 2003; Lelei & Onwonga, 2014a, b) was rejected because there was not significant differences of phosphorus level in soil planted with this species. The use of Phoslock® in terrestrial ecosystem (Haghseresht, 2005; Geurts *et al.,* 2011) also was not supported due to the Phoslock® treatments poor ability to reduce the phosphorous level, even in high P level soils. As a consequence, this approach to reduction of soil P is not economically useful, because the cost effect of this treatments is negative, and thus it does not provide an ideal management method for soil P reduction in terrestrial ecosystems.

6.4 Implications and management recommendations

The main implications and management recommendations of this thesis for restoration of grasslands are as follows:

(i) Clearly, restoration needs to initially address abiotic barriers, followed by biotic barriers, and then be managed to allow the area to come to a natural equilibrium;

(ii) Scalping is costly to be a useful management tool for large areas;

(iii) Windborne and animal carried exotic seeds will always be a problem, and must be addressed, possibly by decreasing these exotic species entering the management area through controlling the source of weed seeds within a reasonable distance around the management area;

(iv) Ingress of fauna will need to be factored into the restoration of area, being restricted until native plants are robust enough to survive grazing by the installation of fencing;

(v) Interim cropping with those two species (*P. machrocephalus* and *P. polystachyus*) and sale as fodder of the interim phosphorous-absorbing plants could be used to offset restoration costs as well as removing phosphorous from the area; and

(vi) In the longer-term, added native plants will have to be seeded, particularly with more early and fast growing native species. It is also recommended to use an increased rate of seeding with a larger number of native species in the mix to catalyse the seeding process

and to establish a more healthy and representative native cover community. This seeding addition could be repeated 3-4 times in order to cover a wider area with successfully grown native species.

Regarding the breaking of the seed dormancy and germination of both *Ptilotus* species (*P. machrocephalus* and *P. polystachyus*), it is recommended that the best pre-treatment, optimum temperature and light period for both species is GA 500 (20/18^{O}C) and 12h dark/ 12h light regimes.

Finally, regarding the management implications of phosphorus reduction in the soil, It is implied that the both *Ptilotus* species (*P. machrocephalus* and *P. polystachyus*) are useful management tools for phosphorus reduction due to absorbing high amount of soil P at growth period in different soil P levels (low, medium and high) either by harvesting the plants after growing or by grazing of cattle.

6.5 Future research implications

I. Restoration cannot be achieved all at once; thus a systematic long-term strategy needs to be applied;
II. There may be several years needed to bring the land back to an abiotic equilibrium;
III. Finer attention needs to be given to the mix of native plants for seeding;
IV. Care must be taken with the gradual introduction of native animals;
V. Exotic seed ingress will always be a problem, which demands that action will be needed in the future to control local outbreaks.

6.6 Conclusion

The findings of this thesis have significantly advanced our current knowledge of the restoration of ex-cropland. It has determined the impacts of biotic and abiotic barriers to the Victorian Volcanic Plains grassland, and has demonstrated some practical approaches to begin the restoration process. I suggest that many of the methods and

techniques used in this study could be useful techniques across broad areas of grassland within Australia as well as in similar situations in temperate climate conditions around the world.

References

Adair, R. J., & Groves, R. H. (1997). Impact of environmental weeds on biodiversity: a review and development of a methodology. Biodiversity Group, Environment Australia, Canberra.

Afsar, A., & Groves, S. (2009). *Toxicity Assessment of Phoslock® and Lanthanum to Human Health*. Phoslock Water Solutions Limited: *Report Number:* TR 023/09. Australia.

Aguiar, M. R. (2005). Biodiversity in grasslands. Current changes and future scenarios. *Grasslands: Developments, Opportunities, Perspectives*, 261-280.

Allison, M., & Ausden, M. (2004). Successful use of topsoil removal and soil amelioration to create heathland vegetation. *Biological Conservation*, *120*(2), 221-228.

Allison, V. J., Miller, R. M., Jastrow, J. D., Matamala, R., & Zak, D. R. (2005). Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil *Science Society of America Journal, 69(5),* 1412-1421

Alpert, P., & Maron, J. L. (2000). Carbon addition as a countermeasure against biological invasion by plants. *Biological Invasions, 2*(1), 33-40.

Bakker, J. P., & Berendse, F. (1999). Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in ecology and evolution, 14*(2), 63-68.

Barabesi, L., & Fattorini, L. (1998). The use of replicated plot, line and point sampling for estimating species abundance and ecological diversity. *Environmental and Ecological Statistics, 5*(4)*,* 353-370.

Barberis, E., Marsan, F. A., Scalenghe, R., Lammers, A., Schwertmann, U., Edwards, A. C. & Torrent, J. (1995). European soils over fertilized with phosphorus: Part 1. Basic properties. *Fertilizer Research, 45*(3)*,* 199-207.

Baskin, C. C., & Baskin, J. M. (1998). *Seeds: ecology, biogeography, and, evolution of dormancy and germination*. New York, NY, USA: Academic Press.

Baskin, C. C., & Baskin, J. M. (2014). *Seeds ecology, biogeography, and evolution of dormancy and germination* (2nd ed.). San Diego, CA: Academic Press.

Baskin, J. M., & Baskin, C. C. (2004). A classification system for seed dormancy. *Seed Science Research, 14*(1), 1-16.

Bates, T. E. (1990). Prediction of phosphorus availability from 88 Ontario soils using five phosphorus soil tests. *Communications in Soil Science and Plant Analysis, 21*(13-16), 1009-1023.

Batlla, D., Kruk, B. C., & Benech-Arnold, R. L. (2004). Modelling changes in dormancy in weed soil seed banks: implications for the prediction of weed emergence. *Handbook of seed physiology. Applications to agriculture. New York: Haworth Press Inc,* 245-264.

Beadle, N. C. W. (1954). Soil phosphate and the delimitation of plant communities in eastern Australia. *Ecology*, *35*(3), 370-375.

Bellairs, S. M., & Bell2, D. T. (1993). Seed stores for restoration of species-rich shrubland vegetation following mining in Western Australia. *Restoration Ecology*, *1*(4), 231-240.

Benech-Arnold, R. L., Sánchez, R. A., Forcella, F., Kruk, B. C., & Ghersa, C. M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Research, 67*(2), 105-122.

Benl, G. (1971). A key for the determination of the genus *Ptilotus* R. Br. (Amaranthaceae). *Mitteilungen der Botanischen Staatssammlung Munchen*, *9*, 135-176.

Bennett, J. R. (2014). Comparison of native and exotic distribution and richness models across scales reveals essential conservation lessons. *Ecography, 37*(2), 120-129.

Bewley, J. D., Black, M., & Halmer, P. (Eds.). (2006). *The Encyclopedia of Seeds: Science, Technology and Uses*. CAB International, Cambridge, USA.

Bing-Ru, L. I. U., Guo-Mei, J. I. A., Jian, C. H. E. N., & Gang, W. A. N. G. (2006). A Review of Methods for Studying Microbial Diversity in Soils1. *Pedosphere*, *16*(1), 18-24.

Blumenthal, D. M., Jordan, N. R., & Russelle, M. P. (2003). Soil carbon addition controls weeds and facilitates prairie restoration. *Ecological Applications*, *13*(3), 605-615.

Brown, S. L., Reid, N., Reid, J., Smith, R., Whalley, R. W., & Carr, D. (2017). Topsoil removal and carbon addition for weed control and native grass recruitment in a temperate-derived grassland in northern New South Wales. *The Rangeland Journal*, *39*(4), 355-361.

Brennan, R. F., Webb, M. G., & Crowhurst, A. M. (2000). Yield responses of mulla mulla (*Ptilotus exaltatus Nees*.) seedlings to additions of nitrogen, potassium and phosphorus fertiliser. *Australian Journal of Experimental Agriculture*, *40*(6), 867-871.

Brereton, R. & Backhouse, G. (2003). Southern Lined Earless Dragon Tympanocryptis lineata. Flora and Fauna Guarantee Action Statement No. 63, Department of Sustainability and Environment, East Melbourne, 6 pp. Australia. Retrieved from https://www.environment.vic.gov.au/_data/assets/pdf_file/0022/32395/Grassland_Earless_Dragon_Tympanocryptis_lineata-lineata.pdf.

Burke, M. J., & Grime, J. P. (1996). An experimental study of plant community invisibility. *Ecology, 77*(3)*,* 776-790.

Carr, G. W., Yugovic, J. V., & Robinson, K. E. (1992). *Environmental weed invasions in Victoria: conservation and management implications*. Department of Conservation and Environment and Ecological Horticulture Pty Limited.

Carta, A., Probert, R., Moretti, M., Peruzzi, L., & Bedini, G. (2014). Seed dormancy and germination in three Crocus ser. *Verni* species (Iridaceae): implications for evolution of dormancy within the genus. *Plant Biology, 16*(6), 1065-1074.

Cavigelli, M. A., & Robertson, G. P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology, 81*(5), 1402-1414.

Chalker-Scott, L. (2007). Impact of mulches on landscape plants and the environment—a review. *Journal of Environmental Horticulture*, *25*(4), 239-249.

Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. (2008). Agronomic values of green waste biochar as a soil amendment. *Soil Research, 45*(8), 629-634.

Chaudhari, P. R., Ahire, D. V., Ahire, V. D., Chkravarty, M., & Maity, S. (2013). Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, *3*(2), 1-8.

Clarke, P. J., Davison, E. A., & Fulloon, L. (2000). Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold. *Australian Journal of Botany, 48*(6), 687-699.

Cleveland, C. C., & Liptzin, D. (2007). C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry*, *85*(3), 235-252.

Cofie, P., Koolen, A. J., & Perdok, U. D. (2000). Measurement of stress–strain relationship of beech roots and calculation of the reinforcement effect of tree roots in soil–wheel systems. *Soil and Tillage Research*, *57*(1-2), 1-12.

Cole, B. I., & Lunt, I. D. (2005). Restoring Kangaroo Grass (*Themeda triandra*) to grassland and woodland understoreys: a review of establishment requirements and restoration exercises in south-east Australia. *Ecological Management and Restoration*, *6*(1), 28-33.

Cole, I. A., Prober, S., Lunt, I., & Koen, T. B. (2016). Nutrient versus seed bank depletion approaches to controlling exotic annuals in threatened Box Gum woodlands. *Austral Ecology*, *41*(1), 40-52.

Cole, I., Lunt, I. D., & Koen, T. (2004). Effects of soil disturbance, weed control and mulch treatments on establishment of *Themeda triandra* (Poaceae) in a degraded white box (*Eucalyptus albens*) woodland in central western New South Wales. *Australian Journal of Botany*, *52*(5), 629-637.

Coleman, D. C., Callaham, M. A., & Crossley Jr, D. A. (2017). *Fundamentals of Soil Ecology*. Academic press.

Coleman, M. J., Sindel, B. M., Kristiansen, P., & Henderson, C. W. (2015). Survey of weed impact, management, and research priorities in Australian cucurbit production. *Plant Protection Quarterly*, *30*(1), 12

Colwell, J. D. (1963). The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. *Australian Journal of Experimental Agriculture*, *3*(10), 190-197.

Commander, L. E., Merritt, D. J., Rokich, D. P., & Dixon, K. W. (2009). Seed biology of Australian arid zone species: germination of 18 species used for rehabilitation. *Journal of Arid Environments*, *73*(6-7), 617-625.

Coor, K. (2003). *Revegetation Techniques: A Guide for Establishing Native Vegetation in Victoria.* Greening Australia Victoria, Melbourne.

Copeland, L. O., & McDonald, M. F. (2012). *Principles of seed science and technology*. Springer Science & Business Media.

Copley, J. (2000). Ecology goes underground. *Nature, 406*(6795)*,* 452-454.

Correll, R. L., Harch, B. D., Kirkby, C. A., O'Brien, K., & Pankhurst, C. E. (1997). Statistical analysis of reduction in tensile strength of cotton strips as a measure of soil microbial activity. *Journal of Microbiological Methods, 31(1-2)*, 9-17.

Cronk, Q. C., & Fuller, J. L. (2001). *Plant Invaders: The Threat to Natural Ecosystems*. London: Routledge. https://www.taylorfrancis.com/books/9781134203581.

Curtis, R. O., & Post, B. W. (1964). Estimating Bulk Density from Organic-Matter Content in Some Vermont Forest Soils 1. *Soil Science Society of America Journal*, *28*(2), 285-286.

Dalenberg, J. W., & Jager, G. (1981). Priming effect of small glucose additions to 14 C-labelled soil. *Soil Biology and Biochemistry, 13*(3), 219-223.

Day, N. J., & Buckley, H. L. (2013). Twenty-five years of plant community dynamics and invasion in New Zealand tussock grasslands. *Austral Ecology, 38*(6), 688-699.

DELWP. (2008). NV1750_EVCBCS https://www.data.vic.gov.au/data /dataset/native-vegetation-modelled-1750-ecological -vegetation-classes.

Díaz-Villa, M. D., Marañón, T., Arroyo, J., & Garrido, B. (2003). Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. *Journal of Vegetation Science*, *14*(5), 701-709.

Dinkelaker, B., Römheld, V., & Marschner, H. (1989). Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant, Cell and Environment*, *12*(3), 285-292.

Dixon, K. W., Roche, S., & Pate, J. S. (1995). The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia, 101*(2), 185-192.

Dobson, A. (2005). Monitoring global rates of biodiversity change: challenges that arise in meeting the Convention on Biological Diversity (CBD) 2010 goals. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *360*(1454), 229-241.

Donath, T. W., Bissels, S., Hölzel, N., & Otte, A. (2007). Large scale application of diaspore transfer with plant material in restoration practice–Impact of seed and microsite limitation. *Biological Conservation*, *138*(1-2), 224-234.

Duong, T. T., Verma, S. L., Penfold, C., & Marschner, P. (2013). Nutrient release from composts into the surrounding soil. *Geoderma, 195*, 42-47.

Dorrough, J., & Scroggie, M. P. (2008). Plant responses to agricultural intensification. *Journal of Applied Ecology, 45*(4)*,* 1274-1283.

Dorrough, J., Ash, J., & McIntyre, S. (2004). Plant responses to livestock grazing frequency in an Australian temperate grassland. *Ecography, 27*(6)*,* 798-810*.*

Dorrough, J., McIntyre, S., & Scroggie, M. P. (2011). Individual plant species responses to phosphorus and livestock grazing. *Australian Journal of Botany*, *59*(7), 670-681.

Dorrough, J., Moxham, C., Turner, V., & Sutter, G. (2006). Soil phosphorus and tree cover modify the effects of livestock grazing on plant species richness in Australian grassy woodland. *Biological Conservation*, *130*(3), 394-405.

Douglas, G. B. (2002). *U.S. Patent No. 6,350,383*. Washington, DC: U.S. Patent and Trademark Office.

Douglas, G. B., Adeney, J. A., & Robb, M. S. (1999). A novel technique for reducing bioavailable phosphorus in water and sediments. In *International Association Water Quality Conference on Diffuse Pollution,* (pp. 517-523).

Douglas, G. B., Dodd, M. B., & Power, I. L. (2007). Potential of direct seeding for establishing native plants into pastoral land in New Zealand. *New Zealand Journal of Ecology*, *31*(2)*,* 143-153.

Douglas, G. D., Adeney, J. A., & Zappia, L. R. (2000). *Sediment remediation project: 1998/9 laboratory trial report CSIRO Land* and *Water*. Technical report no. 6/00. CSIRO, Clayton South, Australia.

Ehrenfeld, J. G. (2003). Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems, 6*(6)*,* 503-523.

Eira, M. T., & Caldas, L. S. (2000). Seed dormancy and germination as concurrent processes. *Revista Brasileira de Fisiologia Vegetal*, *12*(Edicao Especial), 85-104.

Ellis, E. C., Klein Goldewijk, K., Siebert, S., Lightman, D., & Ramankutty, N. (2010). Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, *19*(5), 589-606.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H.& Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135-1142.

Erickson, T., Barrett, R., Merritt, D., & Dixon, K. (Eds.). (2016). Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO PUBLISHING.

Eschen, R., Mortimer, S. R., Lawson, C. S., Edwards, A. R., Brook, A. J., Igual, J. M., Schaffner, U. (2007). Carbon addition alters vegetation composition on ex-arable fields. *Journal of Applied Ecology, 44*(1), 95-104.

Eschen, R., Muller-Schärer, H., & Schaffner, U. (2006). Soil carbon addition affects plant growth in a species-specific way. *Journal of Applied Ecology, 43*(1), 35-42.

Faithfull, I. G., Hocking, C., McLaren, D. A., & Zydenbos, S. M. (2010, September). Chilean needle grass (*Nassella neesiana*) in the native grasslands of south-eastern Australia: biodiversity effects, invasion drivers and impact mechanisms. In *Papers and Proceedings 17th Australasian Weeds Conference*, (pp.431-434).

Ferrari, F. N., & Parera, C. A. (2015). Germination of six native perennial grasses that can be used as potential soil cover crops in drip-irrigated vineyards in semiarid environs of Argentina. *Journal of Arid Environment*s. 113, 1-5.

Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist, 171*(3), 501-523.

Flory, S. L., & Clay, K. (2010). Non-native grass invasion alters native plant composition in experimental communities. *Biological Invasions, 12*(5)*,* 1285-1294.

Gardner, C.A. (1934). Mulla Mulla (*Trichinium alopecuroideum,* Lindl.): a native forage plant. *Journal of the Department of Agriculture of Western Australia, 11*: 530-531.

Gardner, W. K., & Boundy, K. A. (1983). The acquisition of phosphorus by *Lupinus albus* L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. *Plant and Soil,* 391-402.

Geneve, R. L. (2005). Some common misconceptions about seed dormancy. In *combined proceedings- International Plant Propagators Society* (Vol. 55, p. 327). IPPS; 1998.

Gerke, J. (1994). Kinetics of soil phosphate desorption as effected by citric acid. *Zeitschrift für Pflanzenernährung und Bodenkunde*, *157*(1), 17-22.

Geurts, J. J., Van de Wouw, P. A., Smolders, A. J., Roelofs, J. G., & Lamers, L. P. (2011). Ecological restoration on former agricultural soils: feasibility of in situ phosphate fixation as an alternative to topsoil removal. *Ecological Engineering, 37*(11), 1620-1629.

Gibson-Roy, P. (2000). *An investigation of the germination and establishment potential of selected species from the basalt plains grassland community as indigenous seed mixtures* (Honours Thesis). The University of Melbourne, Melbourne.

Gibson-Roy, P., Moore, G., & Delpratt, J. (2010a). Testing methods for reducing weed loads in preparation for reconstructing species-rich native grassland by direct seeding. *Ecological Management and Restoration, 11*(2)*,* 135-139.

Gibson-Roy, P., Moore, G., Delpratt, J., & Gardner, J. (2010b). Expanding horizons for herbaceous ecosystem restoration: The grassy groundcover restoration project. *Ecological Management and Restoration, 11*(3), 176-186.

Gilbert, J., Gowing, D., & Wallace, H. (2009). Available soil phosphorus in semi-natural grasslands: assessment methods and community tolerances. *Biological Conservation, 142*(5), 1074-1083.

Grice, A. C. (2004). Weeds and the monitoring of biodiversity in Australian rangelands. *Austral Ecology*, *29*(1), 51-58.

Grime, J. P. (1974). Vegetation classification by reference to strategies. *Nature*, *250*(5461), 26-31.

Groves, R. H., Austin, M. P., & Kaye, P. E. (2003). Competition between Australian native and introduced grasses along a nutrient gradient. *Austral Ecology*, *28*(5), 491-498.

Gu, B., Lv, Z. W., Fang, H. L., Li, Q., & Hao, G. J. (2009). Effects of Green Plant Waste Compost on Soil Remediation in Urban Greenbelts [J]. *Soils*, *41*(6), 940-946.

Güsewell, S. (2004). N: P ratios in terrestrial plants: Variation and functional significance. *New Phytologist, 164*(2)*,* 243-266.

Haddad, N. M., Haarstad, J., & Tilman, D. (2000). The effects of long-term nitrogen loading on grassland insect communities. *Oecologia, 124*(1), 73-84.

Haghseresht, F. (2005). A Revolution in Phosphorous Removal. *Phoslock Water Solutions Ltd*. *In*, 21. http://www.phoslock.com.au.

Hagon, M. W. (1976). Germination and dormancy of *Themeda australis*, *Danthonia spp*., *Stipa bigeniculata* and *Bothriochloa macra*. *Australian Journal of Botany*, *24*(3), 319-327.

Handreck, K. A. (1997). Phosphorus requirements of Australian native plants. *Soil Research*, *35*(2), 241-290.

Harris, R. B. (2010). Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. *Journal of Arid Environments, 74*(1), 1-12.

Hitchmough, J. (1994). The reconstruction of semi-natural vegetation. *Urban Landscape Management*, pp. 425-430, Inkata, Melbourne.

Hobbs, R. J., & Harris, J. A. (2001). Restoration ecology: repairing the earth's ecosystems in the new millennium. *Restoration Ecology*, *9*(2), 239-246.

Horn, R., & Rostek, J. (2000). Subsoil compaction processes-state of knowledge. *Advances in GeoEcology,* (32), 44-54.

Huenneke, L. F., Hamburg, S. P., Koide, R., Mooney, H. A., & Vitousek, P. M. (1990). Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology*, *71*(2), 478-491.

Hurtt, G. C., & Pacala, S. W. (1995). The consequences of recruitment limitation: reconciling chance, history and competitive differences between plants. *Journal of Theoretical Biology*, *176*(1), 1-12.

Hutchinson, C. M., & McGiffen, M. E. (2000). Cowpea cover crop mulch for weed control in desert pepper production. *HortScience*, *35*(2), 196-198.

Islam, M., Turner, D., & Adams, M. (1999). Phosphorus availability and the growth, mineral composition and nutritive value of ephemeral forbs and associated perennials from the Pilbara, Western Australia. *Australian Journal of Experimental Agriculture, 39*(2), 149-159.

Janssens, F., Peeters, A., Tallowin, J. R. B., Bakker, J. P., Bekker, R. M., Fillat, F., & Oomes, M. J. M. (1998). Relationship between soil chemical factors and grassland diversity. *Plant and Soil*, *202*(1), 69-78.

Jastrow, J. D., Miller, R. M., & Lussenhop, J. (1998). Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. *Soil Biology and Biochemistry*, *30*(7), 905-916.

Jaunatre, R., Buisson, E., & Dutoit, T. (2014). Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, southeast France). *Applied Vegetation Science*, *17*(2), 236-245.

Johnston, S., & Ryan, M. (2000). Occurrence of arbuscular mycorrhizal fungi across a range of alpine humus soil conditions in Kosciuszko National Park, Australia. *Arctic, Antarctic, and Alpine Research*, *32*(3), 255-261.

Jonasson, S., Michelsen, A., Schmidt, I. K., Nielsen, E. V., & Callaghan, T. V. (1996). Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. *Oecologia*, *106*(4), 507-515.

Jorajuria, D., Draghi, L., & Aragon, A. (1997). The effect of vehicle weight on the distribution of compaction with depth and the yield of *Lolium/Trifolium* grassland. *Soil and Tillage Research*, *41*(1-2), 1-12.

Kambizi, L., Adebola, P. O., & Afolayan, A. J. (2006). Effects of temperature, pre-chilling and light on seed germination of *Withania somnifera*; a high value medicinal plant. *South African Journal of Botany*, *72*(1), 11-14.

Kaye, T. N., & Kuykendall, K. (2001). Effects of scarification and cold stratification on seed germination of *Lupinus sulphureus* ssp. kincaidi. *Seed Science and Technology*, *29*(3), 663-668.

Keeling, A. A., McCallum, K. R., & Beckwith, C. P. (2003). Mature green waste compost enhances growth and nitrogen uptake in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) through the action of water-extractable factors. *Bioresource Technology, 90*(2), 127-132.

Kendal, D., Zeeman, B. J., Ikin, K., Lunt, I. D., McDonnell, M. J., Farrar, A. & Morgan, J. W. (2017). The importance of small urban reserves for plant conservation. *Biological Conservation*, *213*, 146-153.

Khan, I., Khan, M. A., Khan, M. I., Saeed, M., Hanif, Z., Khan, S. M., & Ali, M. (2016). Weed management in maize (*Zea mays* L.) through different control strategies. *Pakistan Journal of Weed Science Research*, *22*(1).

Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L., & Hölzel, N. (2010). Species introduction in restoration projects–Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology*, *11*(4), 285-299.

Kirkpatrick, J. B., Gilfedder, L., Bridle, K., & Zacharek, A. (2005). The positive and negative conservation impacts of sheep grazing and other disturbances on the vascular plant species and vegetation of lowland subhumid Tasmania. *Ecological Management and Restoration, 6*(1), 51-60.

Kirmizi, S., Güleryüz, G., Arslan, H., & Sakar, F. S. (2010). Effects of moist chilling, gibberellic acid, and scarification on seed dormancy in the rare endemic *Pedicularis olympica* (Scrophulariaceae). *Turkish Journal of Botany*, *34*(3), 225-232.

Kisetu, E., & Teveli, C. N. M. (2013). Response of green gram (*Vigna radiata* L.) to an application of Minjingu Mazao fertilizer grown on Olasiti soils from Minjingu Manyara, Tanzania. *Pakistan Journal of Biological Science*, *16*(22), 1601-1604.

Kiss, R., Deák, B., Török, P., Tóthmérész, B., & Valkó, O. (2018). Grassland seed bank and community resilience in a changing climate. *Restoration Ecology*. *26*(S2), 141-150.

Krebstein, K., Von Janowsky, K., Reintam, E., Horn, R., Leeduks, J., & Kuht, J. (2013). Soil compaction in a Cambisol under grassland in Estonia. *Zemdirbyste-Agriculture*, *100*(1), 33-38.

Kucera, B., Cohn, M. A., & Leubner-Metzger, G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research, 15*(4), 281-307.

Lambers, H., Raven, J. A., Shaver, G. R., & Smith, S. E. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution*, *23*(2), 95-103.

Larney, F. J., & Blackshaw, R. E. (2003). Weed seed viability in composted beef cattle feedlot manure. *Journal of Environmental Quality*, *32*(3), 1105-1113.

Lategan, M. J., Korbel, K., & Hose, G. C. (2010). Is cotton-strip tensile strength a surrogate for microbial activity in groundwater? *Marine and Freshwater Research*, *61*(3), 351-356.

Le Bayon, R., Weisskopf, L., Martinoia, E., Jansa, J., Frossard, E., Keller, F., Gobat, J. (2006). Soil phosphorus uptake by continuously cropped *Lupinus albus*: A new microcosm design. *Plant and Soil, 283*(1-2), 309–321.

Lee, K. K., Harrison, D. K., Johnston, M. E., & Williams, R. R. (2007). Molecular taxonomic clarification of *Ptilotus exaltatus* and *Ptilotus nobilis* (Amaranthaceae). *Australian Systematic Botany*, *20*(1), 72-81.

Leeper, G. (1970). The Australian environment (4th ed.). Commonwealth Scientific and Industrial Research Organization, Australia, in association with Melbourne University Press. Melbourne.

Lelei, J. J., & Onwonga, R. N. (2014a). White lupin (*Lupinus albus* L. cv. Amiga) increases solubility of Minjingu phosphate rock, phosphorus balances and maize yields in Njoro Kenya. *Sustainable Agriculture Research, 3*(3), 37.

Lelei, J. J., & Onwonga, R. N. (2014b). Response of microbial populations, soil available, P and yield of Lupin (*Lupinus albus* L cv. Amiga) to application of Minjingu phosphate rock-A green house study. *International Journal of Current Microbiology and Applied Sciences*, *3*(4), 671-684.

Levine, J. M., Vila, M., Antonio, C. M., Dukes, J. S., Grigulis, K., & Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*(1517), 775-781.

Lipiec, J., & Hatano, R. (2003). Quantification of compaction effects on soil physical properties and crop growth. *Geoderma*, *116*(1), 107-136.

Logsdon, S. D., & Karlen, D. L. (2004). Bulk density as a soil quality indicator during conversion to no-tillage. *Soil and Tillage Research*, *78*(2), 143-149.

Lunt, I. D. (1990). The soil seed bank of a long-grazed *Themeda triandra* grassland in Victoria. *Proceedings of the Royal Society of Victoria*, *102*(1), 53-57.

Lunt, I. D. (1997). Germinable soil seed banks of anthropogenic native grasslands and grassy forest remnants in temperate south-eastern Australia. *Plant Ecology*, *130*(1), 21-34.

Lunt, I. D., & Morgan, J. W. (2002). Grasslands of south-eastern Australia. *Flammable Australia: The fire regimes and biodiversity of a continent*, Cambridge University Press, Cambridge, pp. 177-196.

MacDougall, A. S., & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, *86*(1), 42-55.

MacDougall, A. S., & Turkington, R. (2007). Does the Type of Disturbance Matter When Restoring Disturbance-Dependent Grasslands? *Restoration Ecology*, *15*(2), 263-272.

Mack, R. N., & Lonsdale, W. M. (2001). Humans as Global Plant Dispersers: Getting More Than We Bargained For: Current introductions of species for aesthetic purposes present the largest single challenge for predicting which plant immigrants will become future pests. *American Institute of Biological Sciences Bulletin*, *51*(2), 95-102.

Madsen, M. D., Davies, K. W., Boyd, C. S., Kerby, J. D., & Svejcar, T. J. (2016). Emerging seed enhancement technologies for overcoming barriers to restoration. *Restoration Ecology*, 24 (S2), 77-84.

Magill, A. H., & Aber, J. D. (2000). Variation in soil net mineralization rates with dissolved organic carbon additions. *Soil Biology and Biochemistry*, *32*(5), 597-601.

Major, J., & Pyott, W. T. (1966). Buried, viable seeds in two California bunchgrass sites and their bearing on the definition of a flora. *Plant Ecology*, *13*(5), 253-282.

Mann, L. K. (1986). Changes in soil carbon storage after cultivation. *Soil Science*, *142*(5), 279-288.

Marrs, R. H. (1985). Techniques for reducing soil fertility for nature conservation purposes: A review in relation to research at roper's heath, Suffolk, *England. Biological Conservation, 34*(4)*,* 307-332*.*

Martinkova, Z., Honek, A., & Lukas, J. (2006). Seed age and storage conditions influence germination of barnyard grass (*Echinochloa crus*-galli). *Weed science, 54*(2), 298-304.

Matamala, R., Jastrow, J. D., Miller, R. M., & Garten, C. (2008). Temporal changes in C and N stocks of restored prairie: Implications for C sequestration strategies. *Ecological Applications, 18*(6), 1470-1488.

Mathur, S. P. (1998). Composting processes. In: Martin A.M. (eds). In *Bioconversion of Waste Materials to Industrial Products*. Springer, Boston, MA, pp. 154-193.

McCrea, A. R., Trueman, I. C., & Fullen, M. A. (2001). A comparison of the effects of four arable crops on the fertility depletion of a sandy silt loam destined for grassland habitat creation. *Biological Conservation, 97*(2), 181-187.

McDonald, J. H. (2009). *Handbook of biological statistics* (Vol. 2), Baltimore, MD: Sparky House Publishing.

McDougall, K. L., & Morgan, J. W. (2005). Establishment of native grassland vegetation at organ pipes national park near Melbourne, Victoria: Vegetation changes from 1989 to 2003. *Ecological Management and Restoration, 6*(1), 34-42.

McIntyre, S., & Hobbs, R. (1999). A framework for conceptualizing human effects on landscapes and its relevance to management and research models. *Conservation Biology, 13*(6), 1282-1292.

McIntyre, S., & Lavorel, S. (1994). How environmental and disturbance factors influence species composition in temperate Australian grasslands. *Journal of Vegetation Science, 5*(3), 373-384.

McIntyre, S., & Lavorel, S. (2007). A conceptual model of land use effects on the structure and function of herbaceous vegetation. *Agriculture, Ecosystems and Environment, 119*(1)*,* 11-21.

McKinley, V., Peacock, A., & White, D. (2005). Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biology and Biochemistry, 37*(10), 1946-1958.

McLauchlan, K. K. (2006). Effects of soil texture on soil carbon and nitrogen dynamics after cessation of agriculture. *Geoderma*, *136*(1-2), 289-299.

McLendon, T., & Redente, E. F. (1992). Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. *Oecologia*, *91*(3), 312-317.

Merritt, D. J., Turner, S. R., Clarke, S., & Dixon, K. W. (2007). Seed dormancy and germination stimulation syndromes for Australian temperate species. *Australian Journal of Botany*, *55*(3), 336-344.

Miller, E. M., & Seastedt, T. R. (2009). Impacts of woodchip amendments and soil nutrient availability on understory vegetation establishment following thinning of a ponderosa pine forest. *Forest Ecology and Management*, *258*(3), 263-272.

Minitab, I. (2014). MINITAB release 17: statistical software for windows. *Minitab Inc, USA*.

Mitchell, M. (2003). Native grasses: an example of using on-farm biodiversity. *Kangaroo*, *10*(27), 55-80.

Moral, R., Paredes, C., Bustamante, M. A., Marhuenda-Egea, F., & Bernal, M. P. (2009). Utilisation of manure composts by high-value crops: Safety and environmental challenges. *Bioresource Technology*, *100*(22), 5454-5460.

Morgan, J. W. (1998). Composition and seasonal flux of the soil seed bank of species-rich *Themeda triandra* grasslands in relation to burning history. *Journal of Vegetation Science, 9*(2)*,* 145-156.

Morgan, J. W. (1995). Ecological studies of the endangered *Rutidosis leptorhynchoides*. II. Patterns of seedling emergence and survival in a native grassland. *Australian Journal of Botany, 43*(1)*,* 13-24.

Morgan, J. W. (1999). Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. *Conservation Biology*, *13*(2), 266-273.

Morgan, J.W. (1996). Secondary juvenile period and community recovery following late-spring burning of a kangaroo grass *Themeda triandra* grassland. *Victorian Naturalist, 113*(2), 47-57.

Morghan, K. J., & Seastedt, T. R. (1999). Effects of soil nitrogen reduction on non-native plants in restored grasslands. *Restoration Ecology*, *7*(1), 51-55.

Morris, E. C. (2000). Germination response of seven east Australian *Grevillea* species (Proteaceae) to smoke, heat exposure and scarification. *Australian Journal of Botany, 48*(2), 179-189.

Morris, E. C., & De Barse, M. (2013). Carbon, fire and seed addition favour native over exotic species in a grassy woodland. *Austral Ecology*, *38*(4), 413-426.

Morris, E. C., & Gibson-Roy, P. (2018). Comparison of biomass removal, nutrient manipulation and native seed addition to restore the ground layer of a degraded grassy woodland. *Australian Journal of Botany*, *66*(1), 1-12.

Morris, E. C., Barse, M., & Sanders, J. (2016). Effects of burning and rainfall on former agricultural land with remnant grassy woodland flora. *Austral Ecology*, *41*(1), 74-86.

Mott, J. J. (1974a). Factors affecting seed germination in three annual species from an arid region of Western Australia. *The Journal of Ecology*, 699-709.

Mott, J. J. (1974b). Mechanisms controlling dormancy in the arid zone grass *Aristida contorta.* I. Physiology and mechanisms of dormancy. *Australian Journal of Botany, 22*(4), 635-645.

Muir, A. (1994). *Western (Basalt) Plains Grassland Community*. Flora and Fauna Guarantee Action Statement No. 53. Department of Sustainability and Environment, Melbourne.

Munzbergova, Z., Kr, M., Bucharová, A., & Juklíc, V. (2005). Ramet performance in two tussock plants- do the tussock-level parameters matter? *Flora-Morphology, Distribution, Functional Ecology of Plants*, *200*(3), 275-284.

Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeler, C., Römheld, V., & Martinoia, E. (2000). Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). *Annals of Botany*, *85*(6), 909-919.

Nicolas, C., Nicolas, G., & Rodríguez, D. (1996). Antagonistic effects of abscisic acid and gibberellic acid on the breaking of dormancy of Fagus sylvatica seeds. *Physiologia Plantarum*, *96*(2), 244-250

Nikolaeva, M. G. (1977). Factors controlling the seed dormancy pattern. In: Khan, A.A. (ed.) *The Physiology and Biochemistry of Seed dormancy and Germination*, North Holland Publishing, Amsterdam, pp. 51-74.

Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W., & Tanner, E. V. (2009). Soil priming by sugar and leaf-litter substrates: a link to microbial groups. *Applied Soil Ecology*, *42*(3), 183-190.

Novak, J. M., & Chan, A. S. K. (2002). Development of P-hyperaccumulator plant strategies to remediate soils with excess P concentrations. *Critical Reviews in Plant Sciences*, *21*(5), 493-509.

Nunes, M. R., Van Es, H. M., Schindelbeck, R., Ristow, A. J., & Ryan, M. (2018). No-till and cropping system diversification improve soil health and crop yield. *Geoderma, 328*, 30-43.

Offord, C. A., & Meagher, P. F. (Eds.). (2009). *Plant germplasm conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections*. Canberra, Australian Capital Territory, Australia: Australian Network for Plant Conservation.

Olsen, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. U.S. Department of Agriculture, Washington.

Orians, G. H., & Milewski, A. V. (2007). Ecology of Australia: the effects of nutrient-poor soils and intense fires. *Biological Reviews*, *82*(3), 393-423.

Palmer, S. M., Driscoll, C. T., & Johnson, C. E. (2004). Long-term trends in soil solution and stream water chemistry at the Hubbard Brook Experimental Forest: relationship with landscape position. *Biogeochemistry*, *68*(1), 51-70.

Pang, J., Tibbett, M., Denton, M. D., Lambers, H., Siddique, K. H., Bolland, M. D. & Ryan, M. H. (2010a). Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. *Plant and Soil*, *328*(1-2), 133-143.

Pang, J., Ryan, M. H., Tibbett, M., Cawthray, G. R., Siddique, K. H., Bolland, M. D. & Lambers, H. (2010b). Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant and Soil*, *331*(1-2), 241-255.

Pardo, J., Mondaca, P., Celis-Diez, J. L., Ginocchio, R., Navarro-Villarroel, C., & Neaman, A. (2018). Assessment of revegetation of an acidic metal (loid)-polluted soils six years after the incorporation of lime with and without compost. *Geoderma*, *331*, 81-86.

Paschke, M. W., McLendon, T., & Redente, E. F. (2000). Nitrogen availability and old-field succession in a shortgrass steppe. *Ecosystems*, *3*(2), 144-158.

Payne, R.W. ed. (2013). The Guide to the GenStat ® Command Language (Release 16). Part 2: Statistics. VSN International, Hemel Hempstead, UK.

Perry, L. G., Blumenthal, D. M., Monaco, T. A., Paschke, M. W., & Redente, E. F. (2010). Immobilizing nitrogen to control plant invasion. *Oecologia*, *163*(1), 13-24.

Peters, J. (2000). Tetrazolium testing handbook: contribution no. 29 to the handbook on seed testing. *Lincoln, NE: Association of Official Seed Analysts*, *32*.

Plummer, J. A., Crawford, A. D., & Taylor, S. K. (1995). Germination of *Lomandra sonderi* (Dasypogonaceae) Promoted by Pericarp Removal and Chemical-Stimulation of the Embryo. *Australian Journal of Botany*, *43*(2), 223-230.

Pons, T. L. (2000). Seed responses to light. *Seeds: the ecology of regeneration in plant communities*, Wallingford, UK: CAB International, pp. 237–260.

Portlock, C. C., Shea, S. R., Majer, J. D., & Bell, D. T. (1990). Stimulation of germination of *Acacia pulchella:* laboratory basis for forest management options. *Journal of Applied Ecology*, 319-324.

Prober, S. M., Lunt, I. D., & Morgan, J. W. (2009). Rapid internal plant-soil feedbacks lead to alternative stable states in temperate Australian grassy woodlands. *New Models for Ecosystem Dynamics and Restoration*, Island Press, Washington, USA, pp. 156-168.

Prober, S. M., Thiele, K. R., Lunt, I. D., & Koen, T. B. (2005). Restoring ecological function in temperate grassy woodlands: manipulating soil nutrients, exotic annuals and native perennial grasses through carbon supplements and spring burns. *Journal of Applied Ecology*, *42*(6), 1073-1085.

Pywell, R. F., Bullock, J. M., Hopkins, A., Walker, K. J., Sparks, T. H., Burke, M. J., & Peel, S. (2002). Restoration of species-rich grassland on arable land: assessing the

limiting processes using a multi-site experiment. *Journal of Applied Ecology*, *39*(2), 294-309.

Pywell, R. F., Bullock, J. M., Tallowin, J. B., Walker, K. J., Warman, E. A., & Masters, G. (2007). Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology*, *44*(1), 81-94.

Radford, J., Williams, J., & Park, G. (2007). Effective landscape restoration for native biodiversity in northern Victoria. *North Central Catchment Management Authority, Victoria*.

Read, T. R., Bellairs, S. M., Mulligan, D. R., & Lamb, D. (2000). Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology*, *25*(1), 48-57.

Reitzel, K., Lotter, S., Dubke, M., Egemose, S., Jensen, H. S., & Andersen, F. (2013). Effects of Phoslock® treatment and chironomids on the exchange of nutrients between sediment and water. *Hydrobiologia, 703*(1), 189-202.

Robb, M., Greenop, B., Goss, Z., Douglas, G., & Adeney, J. (2003). Application of Phoslock, an innovative phosphorus binding clay, to two Western Australian waterways: preliminary findings. In: Kronvang B. (eds). *The Interactions between Sediments and Water.* Developments in Hydrobiology, vol 169, Springer, Dordrecht, pp. 237-243.

Roche, S., Dixon, K. W., & Pate, J. S. (1997a). Seed Ageing and Smoke: Partner Cuesin the Amelioration of Seed Dormancy in Selected Australian Native Species. *Australian Journal of Botany, 45*(5), 783-815.

Roche, S., Koch, J. M., & Dixon, K. W. (1997b). Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restoration Ecology, 5*(3), 191-203.

Ross, G., Haghseresht, F., & Cloete, T. E. (2008). The effect of pH and anoxia on the performance of Phoslock®, a phosphorus binding clay. *Harmful Algae*, *7*(4), 545-550.

Rossiter, N., Setterfield, S., Douglas, M., Hutley, L., & Cook, G. (2006). The impact of exotic grass invasions on nitrogen cycling: a mini-review. In *Managing Weeds in a Changing Climate', Proceedings of the 15th Australian Weeds Conference, Adelaide, SA* (Vol. 815).

Rotundo, J. L., & Aguiar, M. R. (2005). Litter effects on plant regeneration in arid lands: a complex balance between seed retention, seed longevity and soil–seed contact. *Journal of Ecology, 93*(4), 829-838.

Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., & Munch, J. (2006). Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. *Soil Biology and Biochemistry, 38*(2), 263-274.

Ryan, M. H., Ehrenberg, S., Bennett, R. G., & Tibbett, M. (2009). Putting the P in *Ptilotus*: A phosphorus-accumulating herb native to Australia. *Annals of Botany, 103*(6), 901-911.

Ryan, M. H., McCully, M. E., & Huang, C. X. (2003). Location and quantification of phosphorus and other elements in fully hydrated, soil-grown arbuscular mycorrhizas: a cryo-analytical scanning electron microscopy study. *New Phytologist, 160(*2), 429-441.

Saatkamp, A., Affre, L., Dutoit, T., & Poschlod, P. (2011). Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. *Annals of Botany, 107*(3), 415-426.

Saito, M., & Kato, T. (1994). Relationships between dry matter production, nitrogen and phosphorus accumulation in soybean grown under cool weather conditions. S*oil Science and Plant Nutrition, 40*(1), 73-81.

Sanchez, R. A., & Mella, R. A. (2004). The exit from dormancy and the induction of germination: physiological and molecular aspects. *Handbook of seed physiology. Applications to agriculture. New York: Food Product Press and the Haworth Press Inc*, pp. 221-243.

Scarlett, N. H., Wallbrink, S. J., & McDougall, K. (Eds.). (1992). *Field Guide to Victoria's Native Grasslands: Native Plants of Victorian Lowland Plants*. Victoria Press.

Schimel, J., Balser, T. C., & Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology, 88(6),* 1386-1394.

Schneider, W. L., & Gifford, D. J. (1994). Loblolly pine seed dormancy. I. The relationship between protein synthesis and the loss of dormancy. *Physiologia Plantarum, 90*(2), 246-252.

Scott, K. (2006). Effect of heat on the dormancy and viability of *Parkinsonia* seeds: Implications for management. *Ecological Management and Restoration, 7*(2), 153-163.

Scotti, R., Pane, C., Spaccini, R., Palese, A. M., Piccolo, A., Celano, G., & Zaccardelli, M. (2016). On-farm compost: a useful tool to improve soil quality under intensive farming systems. *Applied Soil Ecology*, *107*, 13-23.

Seabloom, E.W., Harpole, W.S., Reichman, O.J. and Tilman, D. (2003). Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences (USA) 100*(23), 13384-13389.

Shane, M. W., De Vos, M., De Roock, S., & Lambers, H. (2003). Shoot P status regulates cluster-root growth and citrate exudation in *Lupinus albus* grown with a divided root system. *Plant, Cell and Environment*, *26*(2), 265-273.

Shane, M. W., Dixon, K. W., & Lambers, H. (2005). The occurrence of dauciform roots amongst Western Australian reeds, rushes and sedges, and the impact of

phosphorus supply on dauciform-root development in *Schoenus unispiculatus* (Cyperaceae). *New Phytologist*, *165*(3), 887-898.

Shane, M. W., McCully, M. E., & Lambers, H. (2004). Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *Journal of Experimental Botany*, *55*(399), 1033-1044.

Sharma, N. C., Starnes, D. L., & Sahi, S. V. (2007). Phytoextraction of excess soil phosphorus. *Environmental Pollution*, *146*(1), 120-127.

Sharp, S. (1997). *Diversity, patterns and processes of vegetation and invertebrate orders in natural temperate grassland in the Australian Capital Territory* (Doctoral dissertation, University of Canberra).

Sinden, J., Jones, R., Hester, S., Odom, D., Kalisch, C., James, R. & Griffith, G. (2004). The economic impact of weeds in Australia. *Technical Series*, *8*, Glen Osmond, SA, Australian Weed Management.

Six, J., Paustian, K., Elliott, E. T., & Combrink, C. (2000). Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society of America Journal*, *64*(2), 681-689.

Smith, J. L., Collins, H. P., & Bailey, V. L. (2010). The effect of young biochar on soil respiration. *Soil Biology and Biochemistry, 42*(12), 2345-2347.

Smith, M. A., Bell, D. T., & Loneragan, W. A. (1999). Comparative seed germination ecology of *Austrostipa compressa* and *Ehrharta calycina* (Poaceae) in a Western Australian Banksia woodland. *Austral Ecology*, *24*(1), 35-42.

Smolders, A. J., Lucassen, E. C., Van Der Aalst, M., Lamers, L. P., & Roelofs, J. G. (2008). Decreasing the abundance of juncus effusus on former agricultural lands with noncalcareous sandy soils: Possible effects of liming and soil removal. *Restoration Ecology, 16*(2), 240-248.

Soane, B. D., & Van Ouwerkerk, C. (1994). Soil compaction problems in world agriculture. In *Developments in agricultural engineering*. *Elsevier*, *11*, 1-21.

Steenwerth, K. L., Jackson, L. E., Calderón, F. J., Stromberg, M. R., & Scow, K. M. (2002). Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry*, *34*(11), 1599-1611.

Steinbeiss, S., Gleixner, G., & Antonietti, M. (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry, 41*(6), 1301-1310.

Suding, K. N., & Hobbs, R. J. (2009). Threshold models in restoration and conservation: a developing framework. *Trends in Ecology and Evolution*, *24*(5), 271-279.

Suttie, J. M., Reynolds, S. G., & Batello, C. (2005). Grasslands of the world (Plant Production and Protection Series No. 34). *Food and Agriculture Organization of the United Nations (FAO), Rome*.

Sweedman, L., & Merritt, D. (Eds.). (2006). Australian seeds: a guide to their collection, identification and biology. CSIRO PUBLISHING.

Taylor, M. A., Davies, H. V., Smith, S. B., Abruzzese, A., & Gosling, P. G. (1993). Cold-induced changes in gene expression during dormancy breakage in seeds of Douglas fir (*Pseudotsuga menziesii*). *Journal of Plant Physiology*, *142*(1), 120-123.

Teasdale, J. R., & Mohler, C. L. (2000). The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Science*, *48*(3), 385-392.

Tibbett, M., & Diaz, A. (2005). Are sulfurous soil amendments (S^0, $Fe^{(II)}SO_4$, $Fe^{(III)}SO_4$) an effective tool in the restoration of heathland and acidic grassland after four decades of rock phosphate fertilization? *Restoration Ecology*, *13*(1), 83-91.

Tiegs, S. D., Clapcott, J. E., Griffiths, N. A., & Boulton, A. J. (2013). A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators*, *32*, 131-139.

Tieu, A., Dixon, K. W., Meney, K. A., & Sivasithamparam, K. (2001). The interaction of heat and smoke in the release of seed dormancy in seven species from southwestern Western Australia. *Annals of Botany*, *88*(2), 259-265.

Tisdall, J. M. (1994). Possible role of soil microorganisms in aggregation in soils. *Plant and Soil*, *159*(1), 115-121.

Tisdall, J. M., & Oades, J. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, *33*(2), 141-163.

Török, P., Miglécz, T., Valkó, O., Kelemen, A., Deák, B., Lengyel, S., & Tóthmérész, B. (2012). Recovery of native grass biodiversity by sowing on former croplands: is weed suppression a feasible goal for grassland restoration? *Journal for Nature Conservation*, *20*(1), 41-48.

Török, P., Vida, E., Deák, B., Lengyel, S., & Tóthmérész, B. (2011). Grassland restoration on former croplands in Europe: an assessment of applicability of techniques and costs. *Biodiversity and Conservation*, *20*(11), 2311-2332.

Traill, B., & Porter, C. (2001). *Nature conservation review Victoria 2001*. Melbourne: Victorian National Parks Association.

Trinick, M. (1977). Vesicular-Arbuscular Infection and Soil Phosphorus Utilization in *Lupinus spp*. *New Phytologist, 78*(2), 297-304.

TSSC (Threatened Species Scientific Committee) (2008) Advice to the Minister for the Environment, Heritage and the Arts from the Threatened Species Scientific Committee (the Committee) on Amendment to the list of Threatened Ecological Communities under the Environment Protection and Biodiversity Conservation Act

1999 (EPBC Act): Natural Temperate Grassland of the Victorian Volcanic Plains. Canberra, ACT.

Tsuyuzaki, S., & Miyoshi, C. (2009). Effects of smoke, heat, darkness and cold stratification on seed germination of 40 species in a cool temperate zone in northern Japan. *Plant Biology*, *11*(3), 369-378.

Van Daele, F., Wasof, S., Demey, A., Schelfhout, S., De Schrijver, A., Baeten, L., & Verheyen, K. (2017). Quantifying establishment limitations during the ecological restoration of species-rich Nardus grassland. *Applied Vegetation Science*, *20*(4), 594-607.

Van Waes, J. M., & Debergh, P. C. (1986). Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some Western European orchids. *Physiologia Plantarum*, *66*(3), 435-442.

Veneklaas, E. J., Stevens, J., Cawthray, G. R., Turner, S., Grigg, A. M., & Lambers, H. (2003). Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. *Plant and Soil*, *248*(1-2), 187-197.

Vidler, S. J. (2004). Using your cute and furries: the role of threatened species in weed awareness. In *Weed management: balancing people, planet, profit. 14th Australian Weeds Conference, Wagga Wagga, New South Wales, Australia, 6-9 September 2004: papers and proceedings* (pp. 652-658). Weed Society of New South Wales.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*(5325), 494-499.

Walck, J. L., Baskin, C. C., & Baskin, J. M. (2000). Seeds of *Thalictrum mirabile* (Ranunculaceae) require cold stratification for loss of non-deep simple morpho-physiological dormancy. *Canadian Journal of Botany*, *77*(12), 1769-1776.

Walker, K. J., Stevens, P. A., Stevens, D. P., Mountford, J. O., Manchester, S. J., & Pywell, R. F. (2004). The restoration and re-creation of species-rich lowland grassland on

land formerly managed for intensive agriculture in the UK. *Biological Conservation*, *119*(1),1-18.

Walsh, N. G., & Entwisle, T. J. (1996). Flora of Victoria Volume 3: Dicotoledons; Winteraceae to Mytaceae.

Watt, M., & Evans, J. R. (2003). Phosphorus acquisition from soil by white lupin (*Lupinus albus L.*) and soybean (*Glycine max* L.), species with contrasting root development. *Plant and Soil, 248*(1-2), 271-283.

Wedin, D. A., & Tilman, D. (1996). Influence of nitrogen loading and species composition on the carbon balance of grasslands. *Science*, *274*(5293), 1720-1723.

Whisenant, S. (1999). *Repairing damaged wildlands: a process-orientated, landscape-scale approach* (Vol. 1). Cambridge University Press.

Wijesuriya, S., & Hocking, C. (1999). Why do weeds grow when you dig up native grasslands? The effects of physical disturbance on available nutrients, mineralisation and weed invasion in grassland soils. In *Down to Grass Roots. Proceedings of Conference on the Management of Grassy Ecosystems'.* (*Eds V. Craigie and C. Hocking*), pp. 31-37.

Wikum, D. A., & Shanholtzer, G. F. (1978). Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. *Environmental Management*, *2*(4), 323-329.

Willems, J. H., Peet, R. K., & Bik, L. (1993). Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. *Journal of Vegetation Science*, *4*(2), 203-212.

Williams, E. D. (1984). Changes during 3 years in the size and composition of the seed bank beneath a long-term pasture as influenced by defoliation and fertilizer regime. *Journal of Applied Ecology*, 603-615.

Williams, R. R., Holliday, K. C., & Bennell, M. R. (1989). Cultivation of the pink mulla mulla, *Ptilotus exaltatus Nees*. 1. Seed germination and dormancy. *Scientia Horticulturae*, *40*(3), 267-274.

Williamson, W. M., Wardle, D. A., & Yeates, G. W. (2005). Changes in soil microbial and nematode communities during ecosystem decline across a long-term chronosequence. *Soil Biology and Biochemistry, 37(7),* 1289-1301

Wilson, S. D., & Gerry, A. K. (1995). Strategies for mixed-grass prairie restoration: herbicide, tilling, and nitrogen manipulation. *Restoration Ecology*, *3*(4), 290-298.

Wisheu, I. C., & Keddy, P. A. (1991). Seed banks of a rare wetland plant community: distribution patterns and effects of human-induced disturbance. *Journal of Vegetation Science*, *2*(2), 181-188.

Wong, M. R., Morgan, J. W., Wong, N. K., & Cavagnaro, T. R. (2015). The incorporation of fungal to bacterial ratios and plant ecosystem effect traits into a state-and-transition model of land-use change in semi-arid grasslands. *Agriculture, Ecosystems and Environment*, *201*, 11-19.

Yates, C. J., & Hobbs, R. J. (2000). Temperate eucalypt woodlands in Australia-an overview. *Temperate eucalypt woodlands in Australia: biology, conservation, management and restoration*, 1-5.