Levelling the Playing Field: Exploring Methods of Reducing Weed Competition to Improve the Establishment of Native Ground Cover on Former Agricultural Land

Thesis submitted in fulfilment of the requirements for the Doctor of Philosophy (Faculty of Science)

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DECLARATION

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

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ABSTRACT

In order to restore endangered native ecosystems such as Grey Box grassy woodlands, research is required on the establishment of native groundcover to ensure functioning ecosystems with greater structural complexity are created. Invasive, exotic plant species (weeds) are a major threat to restoration efforts. This study explores a variety of potential methods and strategies to control weeds in an effort to determine which ones may be effective in promoting the reestablishment of native understorey on previously farmed land.

The three main strategies tested were; carbon addition, topsoil removal and tree canopy planting. Carbon addition reduces the available soil nitrate and can inhibit nitrophilic weeds, topsoil removal depletes the weedy soil seed bank as well as soil nutrients, and trees are believed to outcompete weeds in some situations and facilitate native understorey establishment.

A glasshouse study was carried out to test the effects of these methods directly on common invasive annual grasses. Topsoil removal, tested by planting the grasses in subsoil collected from the field, reduced biomass of three out of four invasive annual grass species when compared with plants grown in topsoil. All four species had biomasses over 60% lower than the control when carbon was added, whereas shade had no effect.

This was followed by a field experiment at Glenthorne Farm, O'Halloran Hill, South Australia. Plots were set up with five treatments; carbon addition, topsoil removal, artificial shade, natural shade under tree canopies and a control. Plots were cleared initially and the reestablishment of weeds monitored for 18 months. Native seeds were also sown and the survival of any germinants monitored. Carbon addition plots had decreased weed cover but no increase in native species establishment compared to the controls. Topsoil removal plots had the lowest percentage weed cover and also the
highest number and cover of native plants. Shade and tree canopy treatments did not reduce the cover of weeds and native species establishment was very low.

Topsoil removal was the most effective method in the above experiment, and a second trial was set up at Glenthorne Farm to test this method further. Larger tracts of land were scalped and some of these were also sprayed with herbicide three weeks after topsoil removal to further reduce the number of weeds subsequently germinating from the soil seed bank. Total weed cover was not significantly reduced on topsoil removal plots compared to controls, but native species establishment was higher.

After analysing these results, some of my recommendations for improving the revegetation of native groundcover from seed on former agricultural land include:

- Ensuring topsoil removal is to a relevant depth and following up with herbicide applications or other weed removal methods to ensure the soil seed bank is sufficiently depleted before planting;
- Where native species already grow, carbon addition can be used to inhibit some weeds, such as nitrophilic annual grasses, although it may not be appropriate to use while native species are germinating;
- Groundcover species should be planted at the same time or before tree species as the shade and increased fertility under tree canopies can promote the growth of some weed species.
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Chapter 1: Introduction

HABITAT RESTORATION

Australia has suffered an extensive loss of native vegetation due to land clearance for agriculture, mining and urbanisation in the last 200 years. Some areas now have less than 5% of their original native land cover (Yates and Hobbs 1997) and this problem is exacerbated by the fragmented and degraded state of these remnants (Prober and Smith 2009). This loss of habitat has also led to extinctions and range restrictions for a significant proportion of the native fauna (Bennett and Ford 1997; Brown et al. 2008) and it is important that these remnants are protected if Australia’s biodiversity is to be maintained. However, simply protecting what is left is unlikely to be sufficient to stop regional extinctions of more native species and losing important ecosystem functions (Vesk and Mac Nally 2006). In order to safeguard our natural environment for the future, there needs to be attempts to restore degraded land to more natural, functioning ecosystems. This may involve restoring existing remnants that have been disturbed, re-establishing vegetation around remnants as buffer zones or corridors to link fragments, or recreating native ecosystems on land that has been cleared for farms and mines. The amount of input required to achieve these outcomes will depend on the extent to which the site has been disturbed (Yates and Hobbs 1997).

Yates and Hobbs (1997) describe three approaches to ecological restoration, each requiring different levels of resource input from managers. Approach 1 involves the removal of disturbance agents such as grazing livestock or invasive weeds. Approach 2 includes the removal of disturbance agents but also involves the reintroduction of native plant species that are no longer able to regenerate or recolonise naturally. This is usually done via direct seeding or planting seedlings. Approach 3 also involves the removal of disturbing agents and plant reintroductions, but combines these with some form of site amelioration that aims to restore a damaged ecosystem function. This is usually required where a site has become so degraded that it has reached an abiotic threshold that prevents it from recovering (Hobbs and Cramer 2008) such as changes in soil pH,
available nutrients, compaction or hydrology. When considering the conversion of cleared land that has been used for agriculture back to a native, functioning ecosystem, Approach 3 is generally required due to the severity of the impacts of cropping systems and intensive livestock grazing (Cramer et al. 2008). This approach also requires a high level of initial resource input and ongoing management.

Revegetation

Revegetation with native plant species has been carried out throughout Australia for a variety of different purposes including the mitigation of salinity (Kavanagh et al. 2005) and soil erosion (Smith 2008), mine site restoration (Loney 1990; Odermatt 1990), and timber production (Kavanagh et al. 2005; Munro et al. 2009). For these purposes, high diversity plantings are not necessarily required, so most of these revegetation projects involve plantings of a small number of woody species that are easily grown (Munro et al. 2009; Wilkins et al. 2003; Yates and Hobbs 1997). However if the aim is ecological restoration, where the goal is to recreate the vegetation community as it was before land clearance (Munro et al. 2007) or at least to provide habitat and restore ecosystem functions, then much more complex and diverse plantings are necessary.

A definite trend in revegetation in Australia from simple woodlot plantings towards more diverse ecological restoration can be seen in the literature. The proceedings from a direct seeding conference held by Greening Australia in 1990 suggested that the majority of revegetation works undertaken in Australia in the 1980’s involved the planting of native trees and shrubs (particularly eucalypts and acacias) for mine site rehabilitation (Loney 1990; Odermatt 1990) and the planting of chenopods and pasture grasses for livestock fodder and erosion control (Tatnell 1990; Walker 1990). However, one paper did discuss the use of native kangaroo grass to replace weeds in the understorey of woodlands to help restore ecosystem functions (Stafford 1990). Revegetation in the 1980’s and 1990’s often involved a single species or single structural layer of species (e.g. just trees) that were not always native to the local area (Bennett et al. 2000; Prober and Smith 2009). In a review of revegetation in the central wheatbelt of Western Australia, Smith (2008) noted that although salinity mitigation was the main
driver behind revegetation projects previously, the last decade had seen a shift in attitude with nature conservation becoming the most common motive. However, the author also noted that there is a limit to how much land farmers were willing or able to revegetate due to the subsequent loss of income, so there was also a trend towards planting species that provided a product or income.

Even when only considering plantings that were motivated by nature conservation, many projects have concentrated on woody trees and shrubs and have not included any ground cover species (Munro et al. 2009; Wilkins et al. 2003). This may have been because it was believed that these smaller life forms would be capable of recolonising on their own once the overstorey was established (Munro et al. 2009). This theory may be applicable to less disturbed sites that were adjacent to remnant native ecosystems which provide viable seed for colonisation. However, in Australia, revegetation is often carried out on highly disturbed sites where there is little or no seed available for recruitment and colonisation. Many of these ground cover species do not produce long term seed banks in the soil (Lunt 1995; Morgan 1998a) and there may not be any other populations left within seed dispersal range due to widespread land clearing, grazing and urbanisation. Even where there is seed available, the conditions are often no longer suitable for germination due to changes in soil pH, soil compaction, water availability, nutrients, shading by competitors, herbivory or altered fire regimes (Standish et al. 2007). Studies have shown that the native species richness of plantings often does not increase over time indicating that there is no successful colonisation occurring (Munro et al. 2009; Wilkins et al. 2003). Thus it is important that a wide range of species are included in revegetation efforts where the goal is to create diverse vegetation communities capable of providing habitat for a wide range of native fauna.

**Ground Cover**

Ground cover plants are a diverse group including grasses, rushes, sedges, forbs and small woody shrubs. They often contribute a large proportion of the overall diversity in a community and provide important ecosystem functions and resources for fauna (Prober et al. 2005). Ground cover plants help protect the soil from erosion, especially grasses as they have fibrous root systems which bind the soil.
Due to the extensive use of land for livestock grazing in Australia and the cultivation of a number of exotic pasture and crop species, often seemingly intact native woodlands have a highly altered ground cover layer. Grazing by livestock leads to changes in the plant community due to selective herbivory, where palatable species are replaced by non-palatable species (Tremont and McIntyre 1994). The trampling and soil compaction caused by the hooves of livestock can also lead to an increase in those plants that are adapted to disturbance whereas other species may be adversely affected and decline (Yates and Hobbs 1997). Unfortunately, often the species that are best adapted to disturbance are exotic (Tremont and McIntyre 1994), and they will often heavily infect disturbed areas, outcompeting the local native plants.

Until recently, ground cover species have been poorly represented in scientific papers discussing revegetation in Australia. The methods and benefits of revegetating agricultural land with woody perennials such as eucalypts, acacias and chenopods are well documented as they tend to provide other benefits to farmers such as lowering of the water table to reduce dryland salinisation, reducing soil erosion, acting as wind breaks and shelter for livestock (Smith 2009). Some native species also provide a direct economic incentive as products such as timber, oils, grazing forage or bush tucker that can be harvested and sold. Some native ground cover species (generally grasses) have also been well researched due to their forage value for grazing livestock (Garden et al. 1996; Reed et al. 2008; Waters et al. 2005) or ability to reduce the toxicity of mine rehabilitation sites (Grant et al. 2002; Lottermoser et al. 2009). However, where habitat restoration and conservation have been the main aims of a revegetation project, ground cover species are often overlooked.

GREY BOX GRASSY WOODLANDS

The Grey Box (Eucalyptus microcarpa (Maiden) Maiden) grassy woodlands and derived native grasslands of south-eastern Australia have recently been listed as endangered under the Australian Commonwealth’s EPBC Act 1999 due to a substantial decline in distribution and degradation of the community (Threatened Species Scientific
Committee 2010a). As with a number of other grassland and woodland ecosystems, Grey Box grassy woodlands have been extensively cleared for agriculture and are now highly fragmented (Threatened Species Scientific Committee 2010b). Even where an overstorey of native trees remain, the ground cover layer has often been largely replaced by exotic crop, pasture or weed species (Threatened Species Scientific Committee 2010b). These Grey Box communities are also under threat from degrading processes such as grazing, fertiliser application and inappropriate herbicide use. A number of threatened species occur in Grey Box grassy woodlands with 17 plant and 13 animal species listed as vulnerable or endangered under the EPBC Act (Threatened Species Scientific Committee 2010b). Grey Box grassy woodlands are associated with dry temperate climates and occur in a relatively contiguous belt from central New South Wales through Victoria and into the south-east of South Australia. There are also more isolated patches in South Australia in the Mount Lofty and Flinders Ranges. Grey Box grassy woodlands often occur on good agricultural land and have been extensively cleared and used for livestock pasture. The Commonwealth government has now listed a number of priority actions to support the recovery of Grey Box grassy woodlands including active revegetation with native species undertaken in an appropriate manner at sites with limited natural regeneration (Threatened Species Scientific Committee 2010a).

Grey Box grassy woodlands have been described as:

“A tree canopy dominated by *Eucalyptus microcarpa* (Grey Box) is typically present. A range of other associated tree species may be present but do not dominate the ecological community. The understorey comprises a sparse shrub layer and a species-rich ground layer of grasses and herbs.” (Threatened Species Scientific Committee 2010a)

Therefore restoring the community on cleared land involves revegetation of *E. microcarpa* and other trees, as well as some shrubs and a diverse range of grasses and herbs. Successful methods for planting native trees and shrubs are now widely known and documented, but how do we return the native ground layer? The groundcover of eucalypt woodlands and grasslands is known to be highly diverse (Lunt 1990; Yates and Hobbs 1997) with a great number of plants per square metre (Caetano de Abreu 2009), making this vegetation layer particularly hard to recreate. There are also a number of
factors that can restrict the establishment of ground cover species that need to be overcome.

**Limitations to Ground Cover Establishment**

*Seed Availability and Germination*

One of the main limitations to native ground cover establishment and the reason active revegetation is often required is the lack of native seed and propagules. Most Australian native herbaceous species do not create persistent soil seed banks (Clarke *et al.* 2000; Lunt 1995; Morgan 1998a), so in areas that have been cleared, cropped or grazed for decades, there is unlikely to be a sufficient soil seed bank for natural regeneration. This problem is exacerbated where the land is fragmented or isolated from remnant areas of native vegetation, so external seed input (seed rain) is also limited. Standish *et al.* (2007) found that a lack of seed dispersal limited native recolonisation of old-fields in Western Australia. There was reduced seed rain and a reduced native soil seed bank only 50 m from remnant vegetation, 45 years after abandonment.

Germination is also an issue with some native species having complex dormancy mechanisms that require specific moisture, temperature or light conditions in order to germinate (Morgan 1998a). This makes the propagation of these species quite difficult and a lot of seed can be wasted when these cues are not understood. Even though many native herbaceous species have less specific germination cues and can germinate readily in a range of conditions (Morgan 1998a), the timing of germination can still be an issue where non-native species are also present. Non-native seed often germinates more rapidly than native seed providing a strong competitive advantage as the non-natives are able to pre-emptively acquire resources and space (Fisher *et al.* 2009; Standish *et al.* 2007), reducing the germination success of native species.

*Weed Competition*

One of the major threats to Australian native ecosystems has been the introduction of exotic plant species which successfully compete with native species for resources and displace them (Yates and Hobbs 1997). Plants compete for space, water, light and
nutrients and are most vulnerable during the germination and establishment phases (Smallbone et al. 2007). The addition of a limiting resource to a system, such as heavy rainfall (water) or a canopy opening due to a disturbance (light), will often cause a germination event. Many seeds may germinate at once, but those individuals that are more efficient at using the available resources will become established at the cost of the less efficient individuals. Unfortunately, in Australian landscapes today, these successful individuals are often exotic invasive species (Smallbone et al. 2007; Tremont and McIntyre 1994), especially annual grasses (Lenz et al. 2003). Fisher et al. (2009) showed that even in near pristine Banksia woodland, the soil seed bank held large numbers of seed from non-native species that were able to germinate much quicker than native species and therefore were likely to dominate after a disturbance. Invasive species usually produce large amounts of seed and germinate quickly, allowing them to take over a site in a short amount of time, particularly after a disturbance (Blumenthal et al. 2003).

On agricultural land, weed “invasion” is usually due to an active removal of native species and sowing of exotic crop and pasture species by humans. Whether deliberate or accidental, once these species have invaded, the ability of local native species to re-establish is often limited. Cramer et al. (2008) suggested that systems that have been dominated by an invasive species for decades or more have often reached an alternative stable state that is self-maintained and highly resistant to change. This is supported by evidence that recolonisation by native species on abandoned farmland in the Western Australian wheatbelt was slow to non-existent, even when directly adjacent to remnant vegetation (Standish et al. 2006; Standish et al. 2007; Yates and Hobbs 1997). Therefore, the removal of these exotic species is generally essential to allow native species the chance to germinate and establish. This weed removal may involve herbicide application, mechanical or manual pulling, slashing or fire. Restoration sites are likely to be reinvaded by weed propagules from surrounding populations or weeds may re-establish from reserves of seeds in the soil seed bank. So beyond the initial weed removal, other tactics will also be necessary to allow a native plant community to re-establish, remain competitive and able to resist reinvasion.
**Increased Nutrients**

Weed removal is essential for revegetation to be successful, but ecological invasions are often symptoms of underlying changes to ecosystem functions or abiotic conditions. If a restored community is to remain resistant to future invasions (which are highly likely due to the proliferation of seed by many introduced plants and their pervasiveness in the Australian landscape), these underlying changes also need to be addressed (Perry et al. 2010; Prober et al. 2005). One of the main abiotic changes that has been detected in land used for agriculture is the increase in available nutrients due to the application of fertilisers (Cramer et al. 2008; McLauchlan 2006; Prober and Smith 2009; Prober et al. 2002b; Prober et al. 2005; Standish et al. 2008). High levels of nutrients such as nitrogen (N) can promote the establishment and spread of fast growing weedy species that are adapted to high resource conditions (Paschke et al. 2000; Perry et al. 2010). In a lot of cases, the ecosystems that need to be recreated are at a late successional stage with mainly perennial plant species, often adapted to low nutrient environments (Perry et al. 2010; Prober et al. 2005; Smallbone et al. 2007). When a disturbance or input of resources occurs, these systems become susceptible to invasion (Morghan and Seastedt 1999) by early successional species that can grow quickly and use up the available resources (Perry et al. 2010; Redente et al. 1992). If the high resource conditions continue, the invasive species may remain dominant indefinitely (Cramer et al. 2008) as the native species that are better adapted to low resource levels are suppressed (Perry et al. 2010).

Prober et al. (2002b) describe a positive feedback loop between understorey composition and nitrogen cycling that allows annual exotics to remain dominant by maintaining seasonal nitrate peaks during their dormant season that improves their competitive establishment. However, native perennial grasses also exhibit positive feedback mechanisms by reabsorbing nitrogen from senescing tissues to create low N litter that maintains low soil nitrate levels which may provide resistance to invasion by annuals (Prober et al. 2002b; Prober et al. 2005). Thus at sites where annual exotic plants have become dominant, intervention may be required to break this positive feedback loop by reducing soil nitrate to allow native perennials to establish and be more competitive. Once established, low N perennials may be able to keep nitrate levels
low enough to inhibit reinvansion by invasive exotic species. A number of studies have shown that by reducing the level of available nitrogen in the soil, invasive species (in particular annuals) may show reduced growth rates (Prober et al. 2005; Redente et al. 1992), reduced cover (Rowe et al. 2009), reduced biomass (Blumenthal et al. 2003) or a reduced competitive effect (Smallbone et al. 2007), so providing any low-nutrient adapted species a competitive advantage.

Tree Canopies

As well as exotic weeds, trees can also be highly competitive with ground cover plants growing nearby due to their extensive root systems and canopies which are effective at capturing light, water and nutrients. Historically, trees have been assumed to reduce pasture productivity leading to widespread clearing of trees on pastoral land in Australia (Le Brocque et al. 2009; Walker et al. 1972) and there is evidence that herbaceous yield is decreased under tree canopies, particularly in Queensland (Scanlan and Burrows 1990). However, in subtropical savannas in Kenya, tree canopies were also found to increase productivity by facilitation (Belsky 1994; Belsky et al. 1993). As well as competition, trees can also facilitate the growth of herbaceous plants due to increased nutrient and litter accumulation under their canopies, improved soil structure and improved water relations (Simmons et al. 2008). Gea-Izquierdo et al. (2009) suggested that whether facilitation or competition occurs, depends upon whether the limiting factor of an ecosystem was light, soil moisture or soil nutrients. In their study, trees had a positive effect on pasture production when nutrients were limiting due to the increased fertility under tree canopies. In dry years, when water was the limiting factor, the canopy effect became neutral as the increased nutrients could not be used and shade became beneficial for water relations. In the case of revegetation, it is useful to understand the effect of any established trees on native plantings and to consider whether native trees should be planted before herbaceous species if they provide a facilitative effect, or if the herbaceous species should be established first so they have a better chance of competing.
Grazing

Usually one of the first actions taken when restoring degraded habitat is the reduction of large herbivore grazing. Fencing of areas to be restored to restrict access by grazers such as livestock, kangaroos and rabbits is an important step in allowing native species to germinate and establish. In some cases where native propagules are in adequate supply, this may be all that is required to begin to see improvement in native ground cover (Briggs et al. 2008). However it is likely that only a small number of species will be able to recover/recolonise and this will be dependent on the availability of seed (Lunt et al. 2007; Yates and Hobbs 1997; Yates et al. 2000). Revegetation is still likely to be required, but the restriction of herbivores will greatly increase the successful establishment of native ground cover species (Clarke and Davison 2004).

GLENTHORNE FARM

Glenthorne Farm is an example of land that was historically a Grey Box grassy woodland but has been cleared and farmed since the 1840’s (Caetano de Abreu 2009). The 208 ha farm is located in a peri-urban area 17 km south of the Adelaide CBD in South Australia. The topography is gently undulating with mainly loam over clay on rock soils (Caetano de Abreu 2009). Glenthorne Farm is situated in the southern Mount Lofty Ranges in the temperate climate zone of South Australia with warm, dry summers and cold, wet winters and an average annual rainfall of 635 mm (Bureau of Meteorology 2014a). It was settled in 1838 and has mainly been used for grazing sheep, cattle and horses, cropping and for agricultural research. The property is now owned by the University of Adelaide and provides an excellent opportunity for broad scale restoration of a Grey Box grassy woodland. However, the land has been drastically changed by 170 years of farming, so restoring the groundcover layer in particular will not be easy.

There is very little native vegetation remaining on Glenthorne Farm and limited opportunity for seed dispersal from nearby remnant Grey Box grassy woodlands. There are remnant woodlands surrounding the nearby Happy Valley Reservoir, but there is a multi-lane highway and residential areas separating them from Glenthorne Farm making
it unlikely that sufficient seed dispersal could occur for natural recolonisation. To restore Glenthorne Farm to a Grey Box grassy woodland, it is therefore necessary to introduce native species as seedlings or by direct seeding. Tree species including *Eucalyptus microcarpa* have already begun to be introduced into a 5 ha fenced site at Glenthorne Farm as seedlings and are growing well. For the groundcover species, the numbers required make it difficult financially and logistically to plant them as seedlings, although seedlings may provide the best success rates (Morgan 1999) and may be the most efficient use of seed if availability is limited. For large scale plantings, direct seeding may be the most appropriate choice for ground cover revegetation, but optimising germination and establishment success will be imperative to avoid seed wastage.

Glenthorne Farm now has only 10 remnant *Eucalyptus microcarpa* trees with no evidence of new recruitment. The vegetation is predominantly introduced annual grasses plus a range of broadleaf herbaceous weeds. Common weeds include *Avena barbata* Pott ex Link (bearded oat), *Hordeum leporinum* Link (barley-grass) and *Echium plantagineum* L. (salvation Jane, Paterson’s curse). The dominance of weeds on the site is likely to be the main factor limiting restoration success and a lot of effort will be required to control them.

Although Grey Box grassy woodlands are known to grow on fertile soils (Threatened Species Scientific Committee 2010b), it is likely that the soil nutrient levels at Glenthorne Farm have been elevated through agricultural processes such as fertiliser application and livestock grazing. The continued dominance of annual weeds can also maintain high soil nitrate levels as their litter is high in nitrogen (Prober et al. 2002b). Leguminous weeds such as clovers (*Trifolium* spp.) and annual medics (*Medicago* spp.) are also common at Glenthorne Farm and can increase soil nitrogen by rhizobium nitrogen fixation (Bowman et al. 2004; Dear et al. 1999; Temprano et al. 2008). Any increased nutrient levels, in particular nitrogen, may need to be lowered in order to reduce the dominance of fast growing annual weeds that are capable of rapid uptake of soil nutrients (Prober et al. 2002b).
In order to optimise establishment, potential risk factors need to be acknowledged and methods of mitigating them adopted or researched. The risk factors identified for the revegetation of ground cover at Glenthorne Farm are:

1) Increased soil nutrients, in particular nitrogen, which could favour the growth of weed species over native species;
2) Competition by introduced exotic weed species;
3) Low germination or high mortality rates of native seedlings;
4) Shading and other impacts of planted overstorey trees; and
5) Grazing by large herbivores such as sheep, rabbits and kangaroos.

The grazing risk has already been reduced by fencing the revegetation site to exclude large herbivores. Overcoming the other four risks will not be as simple, with a number of theories and methods being researched, but no conclusive solutions found yet. After considering the literature, the following four areas were chosen for testing at Glenthorne Farm to determine whether they could improve the establishment of native groundcover vegetation and increase our understanding of the limitations that need to be overcome for revegetation to be successful:

1) Carbon addition, to reduce soil nutrients;
2) Topsoil removal, to reduce nutrients and weed seed banks;
3) Density of plantings; and
4) Tree canopy effects.

Methods and Strategies for Improving Ground Cover Establishment

Carbon Addition

There are a number of methods that have been used to reduce the levels of available nitrogen in the soil in areas that have been nutrient enriched, for the purposes of ecological restoration. Perry et al. (2010) reviews the most common strategies of immobilising nitrogen to control plant invasions including carbon (C) addition, topsoil removal, burning, grazing and the establishment of species adapted to low nitrogen conditions. They found that C addition, usually in the form of sucrose or sawdust, was one of the most reliable methods for lowering N availability and it generally favoured desired species over invasive species. Carbon addition works by increasing the
abundance of heterotrophic soil microbes, which in turn increases the amount of nitrogen taken up by the microbes. This leads to a decrease in the amount of inorganic nitrogen available for plant uptake, although this nitrogen will eventually be released into the soil again through microbial respiration and decomposition (Perry et al. 2010). This decrease in available nitrogen may lead to a decrease in the growth or abundance of nitrophilic invasive species and therefore a reduction in the competitive stresses on native species.

Carbon addition has been studied quite extensively, particularly on the grasslands of North America (Blumenthal 2009; Blumenthal et al. 2003; Corbin and D'Antonio 2004; Morghan and Seastedt 1999; Paschke et al. 2000; Redente et al. 1992; Rowe et al. 2009; Vinton and Goergen 2006) and Europe (Eschen et al. 2007; Kardol et al. 2008) but with varied results. Most of the studies showed that carbon addition decreased the levels of available nitrogen in the soil (Blumenthal 2009; Blumenthal et al. 2003; Eschen et al. 2007; Morghan and Seastedt 1999; Paschke et al. 2000; Prober et al. 2005) and had a negative effect on the biomass (Blumenthal 2009; Blumenthal et al. 2003; Paschke et al. 2000), cover (Kardol et al. 2008; Rowe et al. 2009; Smallbone et al. 2007) or abundance (Prober et al. 2005) of invasive species. There is also substantial evidence that in some ecosystems this negative effect on invasive species will lead to a positive effect on the growth and establishment of native species (Blumenthal et al. 2003; Paschke et al. 2000; Prober et al. 2005; Rowe et al. 2009; Smallbone et al. 2007). However, there are some cases where carbon addition produced no significant effects and Blumenthal (2003) suggests that for carbon addition to aid or accelerate the restoration of native species, three conditions need to be met: 1) without carbon addition, native species need to be suppressed by weeds, 2) weeds must be more nitrophilic than native species, and 3) there needs to be a decrease in soil available nitrogen that is of a duration and magnitude such that the competitive balance between weeds and native species is altered. These conditions are supported by those studies that did not show a positive effect on native species through soil carbon supplements, as one or more of the conditions was usually not met. For example, not enough carbon was added to significantly increase nitrogen immobilisation (Corbin and D'Antonio 2004; Morghan and Seastedt 1999; Vinton and Goergen 2006) or there was no significant competitive
suppression by weeds in the control treatments (Kardol et al. 2008). Carbon addition also seems to work best when the invasive species are annuals and the desired species are perennial (which is the case in most instances) as annuals tend to require and to be more competitive under high nitrogen conditions whereas slower growing perennials are usually better adapted to cope with low nitrogen levels (Paschke et al. 2000; Perry et al. 2010).

Despite these promising findings, carbon addition may have limited applications for broad scale restoration efforts as it can be expensive and difficult to apply and only provides short term benefits (Perry et al. 2010). A number of products with high C:N ratios can be used to increase nitrogen immobilisation, with the most commonly used and most effective being sucrose, sawdust or a combination of the two. Depending on the soil conditions, quite large quantities of carbon (>1000 g C/m²) are often required to tip the balance between invasive and native species (Blumenthal et al. 2003), and the effects may only last a few months (Perry et al. 2010). However, if repeatedly applying carbon for a year or two improves the establishment and growth of native plants and allows them to become dominant over invasive species, the effort may be worthwhile in the long term as less money and effort is required to continually control invasive species. This strategy will be more successful if low nitrogen adapted species are established that are capable of maintaining plant-soil feedbacks to keep soil nitrogen levels low (Perry et al. 2010; Prober et al. 2005). Despite the number of studies and success of carbon addition overseas, it has only had limited consideration in Australia, but with promising results (Prober et al. 2005; Smallbone et al. 2007). As Australia has a large number of nutrient enriched old fields that originally supported plants adapted to quite low nutrients (e.g. <1mg kg⁻¹ nitrate, Prober et al. 2002a; Prober et al. 2002b) and invasive annual plants are a major threat to restoration efforts (Yates and Hobbs 1997), carbon addition is a strategy that deserves further attention.

Topsoil Removal

When restoring a site that has been severely degraded by human use over decades or centuries, sometimes it may be necessary to wipe the slate clean and start over.
Removal of topsoil (scalping) is an attempt at removing decades of human inputs from the soil and leaving a relatively ‘blank canvas’ for native reintroductions. Although not a particularly complicated method, it is quite destructive and may only be suitable for certain sites, such as areas where little native or desirable vegetation remains. However there is evidence that it can significantly promote restoration efforts.

In the review by Perry et al. (2010), topsoil removal was found to be the fastest and most effective method for reducing available nitrogen. A number of studies have shown that topsoil removal can reduce soil nutrients and change other soil conditions such as pH so that these soils resemble natural reference sites more closely (Allison and Ausden 2004; Kiehl and Pfadenhauer 2007; Rasran et al. 2007; Tallowin and Smith 2001). Although the specific conditions that were limiting local native vegetation from establishing varied between the studies, the target vegetation generally preferred low nutrient soils and were inhibited by the eutrophication that had occurred. A reduction in soil fertility often inhibits invasive non-target species and can allow bare patches to persist for a few years for further colonisation by native species (Allison and Ausden 2004; Rasran et al. 2007). Topsoil removal was not as successful on sandy soils where the mineral substrate was too infertile and inhibited plant establishment (Choi and Pavlovic 1998; Kardol et al. 2008). Most of these studies removed all of the topsoil (from 15-40cm) so this problem may be overcome if only part of the topsoil is removed.

Another important benefit of topsoil removal is that most of the soil seed bank is also removed (Rasran et al. 2007). On sites where the vegetation has been dominated by invasive species, the removal of their propagules, as well as the plants themselves is a major advantage when trying to establish native, usually less competitive species. Of course any native seeds in the seed bank will also be removed so restoration will rely on inputs of seeds or seedlings by managers. But this is usually the case anyway as native seeds are often lacking from weed invaded sites or are unable to germinate successfully with more competitive seeds in the soil (Fisher et al. 2009; Rasran et al. 2007; Standish et al. 2007). Removing the soil seed bank before revegetation greatly decreases the amount of ongoing weed management required by decreasing the abundance of
undesired species (Allison and Ausden 2004; Choi and Pavlovic 1998; Kiehl and Pfadenhauer 2007; Pywell et al. 2007; Rasran et al. 2007).

A number of studies have shown that topsoil removal (either by lowering nutrients, removing the seed bank or a combination of these and other mechanisms) is able to increase native plant diversity (Allison and Ausden 2004; Pywell et al. 2007), establishment (Choi and Pavlovic 1998; Cole et al. 2005; Tallowin and Smith 2001), persistence (Kiehl and Pfadenhauer 2007) or all of the above (Rasran et al. 2007). Most of these studies were carried out on grasslands or heathlands in Europe, but there is sufficient evidence to suggest that similar methods may be useful for the restoration of Australian low nutrient woodlands and grasslands that have been used for farmland and highly degraded. Gibson-Roy et al. (2007) were able to establish 43 grassland species on subsoil in the Victorian Western (Basalt) Plains which is very encouraging. Of course such a destructive method does have some potentially negative impacts associated with it. The main issue is the loss of important soil biota that reside in the organic topsoil. Topsoil removal significantly reduces the abundance of soil fauna (Frouz et al. 2009) and arbuscular mycorrhizae (Bellgard 1993; Vergeer et al. 2006) which can affect ecosystem functioning as well as plant establishment. This may also be overcome by only removing part of the topsoil (not scraping as deeply), or alternatively by leaving sections of topsoil intact for recolonisation by soil biota (which may also reduce the cost), or by soil inoculation.

**Planting Density**

Competition between neighbouring plants is an important driver of community composition and can influence the diversity, abundance and spatial arrangement of different species (Pyke and Archer 1991). When plants grow closely together, they are more likely to experience competition (Yurkonis et al. 2010) as their pool of available resources overlaps that of their neighbours. Thus planting at a low density should, in theory, promote a high growth rate and low mortality of seedlings. However, in an ecological setting, there are a number of other factors to consider. In a low density planting, there will be relatively large gaps between individuals which can be colonised
by invasive species (Pyke and Archer 1991). For ecological restorations where exotic weeds are a problem, this bare ground needs to be minimised to avoid reinvasion. High density plantings are more likely to resist invasions by limiting the space and resources available for colonisers (Bakker and Wilson 2004). Therefore, when considering planting densities for ecological restoration, there needs to be a balance between promoting the competitive exclusion of undesirable species and reducing the competition between planted individuals that may lead to poor growth or survivorship.

Although the amount of seed or seedlings used for revegetation will often be limited by cost or availability, the planting densities can be adjusted to improve establishment. For example, Morgan and Scacco (2006) found that high density populations of Button Wrinklewort (*Rutidosis leptorrhynchoidea*) had a greater percentage seed set than low density populations of the same size, which was probably due to an improvement in the quantity and/or quality of pollination events. They suggested that where planting size is limited, establishing small dense populations is likely to be more successful in the long term than sparse populations. A study of grassland restoration plantings in the USA found that high density plantings of dominant grasses inhibited the cover and biomass of planted forbs (Dickson and Busby 2009). The forbs in this system grew better when there were no or low density grass seeds planted with them. Therefore the species diversity of some plantings may be increased if dominant species are planted in separate patches or at lower densities than less competitive species. The resulting patchiness may also more closely resemble the heterogeneity of natural communities (Dickson and Busby 2009).

**Tree Planting**

The presence of established or planted trees can also greatly impact the revegetation of ground cover species. The belief that trees are strong competitors that will decrease the productivity of nearby herbaceous plants has been carried over from pastoralists to revegetation managers. It has been predicted in the past that establishing trees will starve exotic herbaceous plants of water, nutrients and light and facilitate their replacement by natives (Davies and Christie 2001; Nichols et al. 2010). Whilst there is
some evidence of this occurring (e.g. in NSW riparian communities, Harris et al. 2011),
there are more cases where there was no improvement in the native understorey
following tree planting (Florentine et al. 2013; Munro et al. 2009; Nichols 2005; Nichols
et al. 2010; Wilkins et al. 2003). None of these studies involved the revegetation of
ground cover species and the lack of recruitment was likely to be due to the absence of
available propagules, rather than the presence of trees. There are no published studies
that compare the establishment of Australian native ground cover species planted with
and without trees. Studies comparing the plants growing under tree canopies with those
in open spaces in remnant native Australian woodland highlight the variability in species
responses. There is often a different suite of species growing under tree canopies as
some species may be facilitated by trees whilst others are inhibited. A common finding
in these studies is that summer active (C4) grasses such as Aristida ramosa and Themeda
triandra dominate in open areas, whilst shade tolerant C3 grasses such as Microlaena
stipoides and Poa sieberiana are dominant under mature tree canopies (Gibbs et al.
1999; Prober et al. 2002a; Scanlan and Burrows 1990). Further generalisations such as
these are difficult to make as the results vary, depending on the species, the climate and
the soil of the area. It is also difficult to predict the effects of newly planted trees
compared with mature established trees on understorey establishment.

SUMMARY

Extensive clearing of temperate woodlands in Australia has led to a number of
ecosystems such as Grey Box grassy woodlands being listed as endangered under the
EPBC Act 1999. Work has already begun to try to restore the overstorey of these
woodlands but effective methods for re-establishing native understorey are not widely
understood. Trying to return native species to cleared, highly altered agricultural land is
particularly challenging. There are a number of factors that can limit the success of
ground cover revegetation efforts, these include grazing, the presence of exotic invasive
weeds, changes to the soil including increased nutrient loads due to fertiliser
application, the presence of mature or planted trees and low germination and survival
rates of native species.
This thesis explores some of these limiting factors and tests methods that may help to overcome them. Chapter 2 details a glasshouse experiment to test the effects of carbon addition, topsoil removal and shading on the growth of common annual grass weeds. These treatments, as well as the effects of mature tree canopies, were then tested in the field at Glenthorne Farm to determine their impact on weed control and the establishment of native groundcover species from seed (Chapter 3). Topsoil removal is then explored in more detail with another planting experiment at Glenthorne Farm where it is combined with herbicide application (Chapter 4). The findings from these experiments are then used to generate a list of recommendations for establishing ground cover at Glenthorne Farm and restoring grassy woodlands more generally (Chapter 5).

INTRODUCTION

Invasive exotic plants pose a serious threat to the reestablishment of native vegetation, affecting both natural processes and human assisted revegetation efforts. Australia has over 2000 species of exotic plants that have naturalised, and around half of these are considered environmental weeds (Csurhes and Edwards 1998). Problem weeds generally have traits such as fast growth, high reproductive output and an increased ability to exploit available resources (Vourlitis and Kroon 2013). These traits allow them to colonise new areas, aggressively compete with native species and reduce the successful establishment of new individuals (Holzner 1982).

Exotic annual grasses commonly become problem weeds as they are actively transported to new areas by humans, either accidentally or for cropping or pasture improvement, and they can alter plant communities and affect ecosystem processes (D’Antonio and Vitousek 1992). They are generally fast growing, quick to germinate, create large numbers of seed and are able to outcompete natives due to their large combined biomass (Davies 2000). They have been shown to reduce the establishment of native trees and understorey species (Clary 2012; Lenz and Facelli 2005; Standish et al. 2008; Windsor 2000) and will often remain dominant following invasion, particularly in native perennial grasslands (Clary 2012; D’Antonio and Vitousek 1992; Lenz and Facelli 2006; Seabloom et al. 2003) and abandoned farmlands (Cramer et al. 2008; Standish et al. 2008; Standish et al. 2007). Long term persistence of invasive annual grasses usually occurs when there is ongoing disturbance or an abiotic threshold is reached which prevents the system from recovering (Cramer et al. 2008; Hobbs and Cramer 2008). This often relates to increases in soil nutrients, particularly nitrogen (N), due to fertiliser addition, livestock grazing or atmospheric N-fixation (Perry et al. 2010).
Annual grasses are generally adapted to high resource availability (Lowe et al. 2003; Mazzola et al. 2011; Paschke et al. 2000) and are more likely to invade and become dominant in areas where resources such as light, nutrients or water are plentiful (Lenz and Facelli 2006; Mazzola et al. 2011). This means that they are better suited to take advantage of increased nutrient availability than native species which are often slower growing, perennial species adapted to low resource availability (Lowe et al. 2003; Mangla et al. 2011; Prober et al. 2005). Annual grasses also tend to create positive plant-soil feedbacks that maintain high levels of N due to the production of high N litter and enables them to stay dominant (Lenz et al. 2003; Perry et al. 2010; Prober et al. 2002a). Therefore, to control annual grass weeds, underlying changes to resource availability need to be reversed to break the cycle of dominance.

Reducing the available N in the soil has been widely suggested as a method for controlling annual weeds in order to improve the restoration of native species (James et al. 2011; Paschke et al. 2000; Prober et al. 2005; Vinton and Goergen 2006). This can be done in a number of ways, including burning, grazing, biomass removal, carbon addition and topsoil removal. These techniques are reviewed by Perry et al. (2010) and each have their own advantages and disadvantages and will be suitable for different situations. For example, topsoil removal (scalping) may not be appropriate where some native species are already present and need to be retained. Whereas carbon addition can be used on areas of disturbed native vegetation to reduce weed biomass, with ideally minimal negative effects on perennial natives that are better adapted to low N environments.

Carbon addition can reduce N availability by increasing the abundance and activity of heterotrophic microbes, increasing their uptake of soil N and thus reducing N availability for plants (Perry et al. 2010). It usually involves the spreading of high carbon, low N material such as sugar or sawdust on the soil. The effect is not permanent and the carbon source needs to be re-applied every 3-6 months to maintain low N levels in the soil (Perry et al. 2010). Topsoil removal is more destructive but is very effective at reducing available nitrogen as it involves the removal of standing vegetation as well as all the nutrients stored in the top layers of the soil.
In theory, the reduction of light availability should also suppress weed growth by limiting their ability to photosynthesise. However the relationship between light and plant growth can be a lot more complex. In warm, arid environments, shade can also be beneficial as it reduces air and soil temperatures and evapotranspiration (Belsky et al. 1993; Lin 2010; Royer et al. 2012). The effects of shade are further confounded by the trees that provide it. Trees have been shown to increase soil fertility under their canopy (Belsky et al. 1993; Jackson and Ash 2001; Prober et al. 2002a), alter water availability (Gea-Izquierdo et al. 2009; Scholes and Archer 1997) and also compete strongly for underground resources (Belsky 1994; Monk and Gabrielson 1985; Simmons et al. 2008). Whether these effects add up to be advantageous or unfavourable to herbaceous plants will depend upon the species and the environment in question (Belsky 1994; Scholes and Archer 1997). For this study, I wanted to quantify the direct effects of shade on invasive annual grass growth, without the confounding canopy effects of nutrient and water availability and competition; so that the results could be used by land managers more widely, taking into account the conditions of their system. For revegetation purposes, if invasive annual grasses are inhibited by shade, it may be worthwhile planting canopy species first to reduce their competitiveness and make it easier to establish ground cover species.

To determine the direct effects of some of these weed suppression tactics on invasive annual grasses, a simple glasshouse experiment was undertaken. Two methods of nutrient limitation, carbon addition and topsoil removal, were tested as well as a shade treatment to test light limitation. Four species of exotic annual grasses (Avena barbata Pott ex Link, Hordeum leporinum Link, Lolium rigidum Gaudin and Vulpia bromoides (L.) Gray) were grown from seed in pots containing soil from Glenthorne Farm, an agricultural field site at O’Halloran Hill, South Australia. I hypothesised that the biomass of the grasses would be reduced in all three treatments when compared to a control due to a reduction in available resources. My aims were to 1) compare the effects of two different methods for limiting nutrients on the biomass of invasive annual grasses in the absence of competition and 2) to measure the effects of light limitation (shade) on the growth of invasive annual grasses.
METHODS

A glasshouse experiment was set up in autumn 2011 to measure the effects of different resource limiting treatments on the growth of exotic invasive grasses. Four different treatments were tested using four different exotic annual grasses that are common in Australia, including the University of Adelaide field site, Glenthorne Farm. Soil was collected from across the study site at Glenthorne Farm, O’Halloran Hill, South Australia in April 2011 and separated by depth into topsoil (0–5 cm) and subsoil (5-10 cm). All the samples from each depth were pooled together and well mixed. This soil was then used to fill seedling containers (50 x 50 x 120 mm) as per the following treatments:

1) Topsoil (Control)
2) Subsoil (Topsoil removal)
3) Carbon Addition – Topsoil plus ¼ teaspoon of sugar (roughly equivalent to 400gm$^2$ or 168g C m$^2$)
4) Shade – Topsoil surrounded by 70% shade cloth

For the Carbon Addition treatment, white table sugar (sucrose) was added to the soil surface in the seedling pots, watered in, lightly tilled and left for a day or two before germinating seeds were added. The shade cloth for the Shade treatment was erected around trays of 50 seedling containers so that the top and sides were covered but there was room for seedlings to grow.

Originally, the experiment was planned to test the treatment effects on 2 native (Rytidosperma geniculatum (J.M.Black) Connor & Edgar and Microlaena stipoides (Labill.) R.Br.) and 2 invasive grass species (Avena barbata and Hordeum leporinum, but due to very low germination of the native species (despite high germination rates in a pilot study), these were not able to be included in this experiment. The remaining pots were planted with another 2 exotic species (Vulpia bromoides and Lolium rigidum) instead, although with less replicates than A. barbata and H. leporinum (Table 2.1). Grasses were germinated in trays from seed collected from the Waite Conservation Reserve or germinated from soil collected from Glenthorne Farm. Seeds were transferred to the treatment pots (one seed per pot) once the cotyledon had emerged and all seeds were
transferred within 7 days of each other. Seedlings were watered regularly using an automated sprinkler system and repositioned within the glasshouse each week to minimise positional effects. Any other seedlings germinating from the soil in the pots were removed immediately so that each pot only contained one seedling. There were a few early mortalities of seedlings but these were not believed to be due to treatment effects.

After 11 weeks, the seedlings were harvested so their shoot and root dry biomass could be measured. The roots of each plant were removed and washed and shoots and roots were dried in an oven at 70°C for 72 hours and then weighed. The height (length from base of plant to tip of longest leaf) and number of tillers of each plant was also measured during harvesting.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>Topsoil</td>
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<tr>
<td></td>
<td>Subsoil</td>
</tr>
<tr>
<td></td>
<td>Carbon</td>
</tr>
<tr>
<td></td>
<td>Addition</td>
</tr>
<tr>
<td></td>
<td>Shade</td>
</tr>
<tr>
<td>Avena barbata</td>
<td>15</td>
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<td></td>
<td>15</td>
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<tr>
<td></td>
<td>15</td>
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<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Hordeum leporinum</td>
<td>15</td>
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<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>15</td>
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<td></td>
<td>14</td>
</tr>
<tr>
<td>Vulpia bromoides</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>10</td>
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<tr>
<td></td>
<td>9</td>
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<td></td>
<td>9</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>9</td>
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<td></td>
<td>8</td>
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<td></td>
<td>8</td>
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<td>8</td>
</tr>
</tbody>
</table>

**Table 2.1:** The numbers of replicates of each plant species in each treatment that were successfully planted and harvested after 11 weeks growth in the glasshouse.

**Analysis**

For each species, one-way ANOVA was carried out using SPSS (IBM Corp. 2010) to compare the means of the plant growth variables between treatments. Where significant differences were found ($P < 0.05$), Tukey’s HSD tests were used to identify which treatments were dissimilar. Some samples required square root or log transformations to meet the assumptions of normality and homoscedascity.
RESULTS

For all four grass species, the means of total, shoot and root biomass were significantly different between treatments ($P < 0.001$; Table 2.2). Analysis revealed significant differences for the root:shoot ratios of three of the species, but only for *V. bromoides* were any of the treatment means different to the control mean. Height was also significantly different between treatments for all species ($P < 0.001$) but the mean number of tillers per plant was only affected by treatment for *H. leporinum* and *V. bromoides*.

**Carbon Addition**

The carbon addition treatment had the largest overall effect on annual grass biomass in this experiment. The means of total, shoot and root dry biomass for all four species were significantly lower for plants grown in soil with sugar added, than in the topsoil control (Fig. 2.1). *Vulpia bromoides* showed the strongest response with an 82.4% decrease in total dry biomass (Carbon, 0.091 ± 0.062 g; Topsoil, 0.515 ± 0.239 g). The other three species showed reductions in total biomass of 63-66% of the control means. Both the shoot masses and root masses were affected to similar extents by carbon addition, except for *V. bromoides* whose roots were affected more strongly, resulting in a significantly smaller root:shoot ratio than the plants grown in untreated topsoil (Carbon Addition 0.86 ± 0.09, Topsoil 1.837 ± 0.18, $P < 0.001$). *Vulpia bromoides* also had less tillers in carbon addition treatments than in topsoil treatments (Carbon Addition 4.56 ± 3.12, Topsoil 10.67 ± 4.42) as well as *H. leporinum* (Carbon Addition 2.40 ± 0.99, Topsoil 3.87 ± 1.06).

**Subsoil**

Growing plants in subsoil (>5 cm depth) compared with topsoil also had a significant effect on the biomass of all four species, although the effect was slightly smaller and less consistent across species than the carbon addition treatment. Mean total and shoot biomasses were lower in the subsoil treatment than the topsoil treatment for all four species (Fig. 2.1). *Hordeum leporinum* showed the greatest reduction in total biomass with the subsoil mean 64.6% lower than the topsoil mean. The other species had 55.5%
(L. rigidum), 45.9% (A. barbata) and 37.0% (V. bromoides) lower means. Root biomasses were also lower for all species but the difference was not significant for V. bromoides (P = 0.067, Tukey HSD on square root transformed data). There was a significant difference in the mean number of tillers for both V. bromoides (Subsoil 5.50 ± 1.58, Topsoil 10.67 ± 4.42) and H. leporinum (Subsoil 1.73 ± 0.7, Topsoil 3.87 ± 1.06) and also a reduction in the mean height of H. leporinum (Subsoil 138.6 ± 32.7mm, Topsoil 220.5 ± 54.9 mm).

Table 2.2: One-Way ANOVA of treatment effects on different growth parameters for four invasive annual grass species grown in a glasshouse for 11 weeks. Treatments were topsoil (control), carbon addition, shade and topsoil removal (subsoil).

<table>
<thead>
<tr>
<th>Species</th>
<th>F-value</th>
<th>d.f.</th>
<th>p-value</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total dry biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata</td>
<td>16.408</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Hordeum leporinumA</td>
<td>25.985</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Vulpia bromoidesA</td>
<td>25.039</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>12.915</td>
<td>3,29</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td><strong>Shoot dry biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata A</td>
<td>15.881</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Hordeum leporinumB</td>
<td>20.045</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Vulpia bromoides</td>
<td>13.694</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>12.34</td>
<td>3,29</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td><strong>Root dry biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata</td>
<td>16.452</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Hordeum leporinumA</td>
<td>30.198</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Vulpia bromoidesA</td>
<td>25.437</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>10.758</td>
<td>3,29</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td><strong>Root:Shoot ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata</td>
<td>4.784</td>
<td>3,55</td>
<td>0.005</td>
<td>**</td>
</tr>
<tr>
<td>Hordeum leporinum</td>
<td>0.535</td>
<td>3,55</td>
<td>0.660</td>
<td>n.s.</td>
</tr>
<tr>
<td>Vulpia bromoides</td>
<td>16.955</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>2.966</td>
<td>3,29</td>
<td>0.048</td>
<td>*</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata A</td>
<td>18.293</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Hordeum leporinum</td>
<td>48.114</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Vulpia bromoides</td>
<td>9.925</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidumA</td>
<td>11.385</td>
<td>3,29</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td><strong>Tillers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avena barbata</td>
<td>1.299</td>
<td>3,55</td>
<td>0.284</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hordeum leporinum</td>
<td>15.247</td>
<td>3,55</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Vulpia bromoidesA</td>
<td>8.99</td>
<td>3,33</td>
<td>&lt;0.001</td>
<td>***</td>
</tr>
<tr>
<td>Lolium rigidum</td>
<td>2.849</td>
<td>3,29</td>
<td>0.055</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001, n.s. not significant

A Samples were square root transformed for analysis.
B Samples were log transformed for analysis.
Figure 2.1: Effects of four different treatments on the biomass and growth of four invasive annual grass species. Germinating seeds were planted in plots individually with either topsoil (Topsoil), topsoil plus ¼ teaspoon of sucrose (Carbon), topsoil under shade cloth (Shade) or subsoil >5 cm depth (Subsoil). Soil was collected from Glenthorne Farm, O’Halloran Hill, South Australia and plants were grown in a greenhouse and watered regularly for 11 weeks before harvesting and drying at 70°C for 72 hrs. Figures compare means of (a) shoot biomass, (b) root biomass, (c) total plant biomass, (d) root to shoot ratio, e) height and f) number of tillers. Error bars are ± s.e.. Sample sizes range from 8 to 15 (Table 2.1). Different letters above bars for each species show significant differences at α = 0.05. ^ Samples were square root transformed for analysis. ^^ Samples were log transformed for analysis.
Shade

The mean plant heights for all four species were significantly higher in the shade treatment than in the other treatments. When compared with the control means, A. barbata, H. leporinum and L. rigidum plants were over 50% taller and V. bromoides plants were 37% taller when grown under 70% shade cloth. However, there was no associated increase in shoot biomass for any of the species. Mean shoot and total dry biomasses in the shade treatment were lower than the control means, but the differences were not significant. The root masses of A. barbata and L. rigidum were significantly lower (31.1% and 33.7% respectively), but only V. bromoides had a significant difference in root:shoot ratio (Shade 1.15 ± 0.24 , Topsoil 1.84 ± 0.55, P = 0.001).

Comparison Between Treatments

In most of the analyses for A. barbata, H. leporinum and L. rigidum, the means for Carbon Addition and Subsoil were not significantly different from each other, suggesting the plants were affected to a similar extent in the two treatments. However, V. bromoides showed significant differences in growth between the two treatments (Fig. 2.1). Root, shoot and total dry biomass, as well as root:shoot ratio were much lower for V. bromoides plants grown with carbon addition than plants grown in subsoil.

DISCUSSION

Carbon Addition

The results of this experiment suggest that there is the potential for carbon addition and topsoil removal to be useful methods for controlling invasive annual grasses. All four species showed significantly reduced biomasses after 11 weeks when grown in field soil that had carbon added. Although nitrogen levels were not measured in this study, it is most likely that these effects were due to a reduced availability of nitrogen as no other factors differed from the control treatment and sugar addition has been shown to reduce nitrogen availability in many other studies (Blumenthal 2009; Blumenthal et al. 2003; Eschen et al. 2007; Kirkpatrick and Lubetkin 2011; Mazzola et al. 2011; Morghan...
Vulpia bromoides plants had a mean total biomass 82.4% lower than the control mean when grown in soil where carbon was added. The other three species were over 60% smaller. This effect may well be increased when competition between plants is involved. However, in this experiment the plants were growing in a small closed system which may also have exaggerated the effects of carbon addition.

Although carbon addition was not tested on native grasses in this study due to poor germination, it may be expected that nitrogen limitation would also have a negative effect on their growth. However, the effects are likely to be less severe as most Australian native grasses are perennial, slower growing species that are often better adapted to or more competitive in lower nutrient soils than invasive annual grasses (Prober et al. 2005). Further tests that quantify the direct effects of carbon addition on native species in comparison with undesirable weedy species would help to predict whether it could be a viable weed control method. Other studies have shown that carbon addition in the field can have a positive (Blumenthal et al. 2003; Paschke et al. 2000; Prober et al. 2005; Smallbone et al. 2007) or neutral (Kirkpatrick and Lubetkin 2011) effect on Australian and North American native, perennial grassland species. Their results suggest that any possible negative effects from carbon addition are often outweighed by the benefits gained from a reduction in weed growth.

Carbon addition is not expected to kill invasive weeds, however it can significantly decrease the biomass of nitrophilic plants (this study, Paschke et al. 2000). This gives natives more access to space and light and may provide them with the opportunity to mature and set seed. Carbon addition could help “level the playing field” for native species by slowing down the growth of normally fast growing, competitive annual grasses. If nutrients are depleted enough, it may also reduce the reproductive capacity of exotic annual species (Harper and Ogden 1970; Tungate et al. 2006; Tungate et al. 2002) thus reducing the number of successive germinants over time. Although this experiment has its limitations, there is evidence of carbon addition having an inhibitive effect on a number of common invasive annual grasses and it would be worthwhile testing carbon addition in the field. It will not be suitable for all situations but is worth
considering, especially where nitrogen enrichment has occurred (e.g. old farms) and where native species are already present making more invasive or destructive weed control methods unsuitable.

**Topsoil Removal**

Three species of exotic annual grasses (*A. barbata*, *H. leporinum*, *L. rigidum*) grown in subsoil from Glenthorne Farm showed significantly reduced total, shoot and root biomass when compared to plants grown in topsoil. There was no competition for resources for these individually grown plants, and water and light were not limiting, so it is likely that nutrient availability was limiting the growth of these species. Topsoil removal has been shown to lower the nutrient levels in European grasslands and heathlands (Allison and Ausden 2004; Frouz *et al.* 2009; Kiehl and Pfadenhauer 2007; Rasran *et al.* 2007; Tallowin and Smith 2001) although there is very little published data from other ecosystems. A review by Perry *et al.* (2010, p. 19) found that: “Topsoil removal is the fastest and most effective method for removing N, because typically most of the total N pool is held in the uppermost layers of soil”. This is supported by the results of soil analysis from Glenthorne Farm which suggest that the subsoil generally had lower levels of nutrients such as nitrogen, phosphorous, potassium and sulphur as well as organic carbon than the topsoil, although these results were not statistically significant (Chapter 3).

The soil at the study site within Glenthorne Farm is a red duplex soil (Atlas of South Australia 2013) of thick sand over clay (Caetano de Abreu 2009). Once the thin topsoil is removed, the subsoil is a cracking clay which is very hard when dry and may inhibit the growth of roots (MacEwan *et al.* 2010). Cole *et al.* (2005) found at a study site in New South Wales, Australia, that although topsoil removal successfully increased establishment of a native perennial grass (*Themeda triandra*), it also exposed the poorly structured subsoil which became compacted and hard and the plants were more heavily grazed by a range of herbivores. Similar issues may be encountered in the field at sites such as Glenthorne Farm. However in this glasshouse experiment, none of the species
showed a disproportionately greater loss of root biomass compared with shoot biomass in the subsoil treatment.

Topsoil removal could have quite varied results in the field depending on the chemical and physical properties of the subsoil, but there is an important benefit to this method of weed management that was not tested in this glasshouse experiment. That is the removal of the soil weed seed bank. Weedy species generally produce large amounts of seed which is mainly stored in the litter and topsoil (Fisher et al. 2009). Topsoil removal has the potential to severely deplete the soil seed bank (Rasran et al. 2007), and thus reduce the potential for weed reinvasions following treatment. Only a few studies involving topsoil removal from Australia could be found in the literature, however they show that it can be used to help establish native grasses (Cole et al. 2004; Cole et al. 2005), forbs (Gibson-Roy et al. 2010b) and trees (Bai et al. 2012) and reduce weed regrowth (Gibson-Roy et al. 2010a). There are three ways in which topsoil removal can impact weed cover; removal of standing vegetation, depletion of the soil seed bank and reduction of soil nutrients, making it a powerful revegetation tool that reduces the requirements for ongoing weed management. Although such a destructive method will not be suitable in some situations, there is strong cause for further research and field trials.

**Shade**

In this study, annual grasses were significantly taller when grown under 70% shade cloth in a glasshouse, compared with unshaded plants. However, there was no significant increase or decrease in total or shoot dry biomass after 11 weeks compared to plants in the control treatment. These results suggest that the grasses are growing taller trying to find more light, but are not inhibited by a 70% reduction in light availability. A review of the literature shows that most plants that exhibited symptoms of shade inhibition were subjected to light levels of less than or equal to 5% solar radiation (Cole and Weltzin 2005; Funk and McDaniel 2010; Siemann and Rogers 2003), so annual grasses may only be inhibited by extreme shading. In Australian temperate woodlands and grassy woodlands, the canopy species are usually *Eucalyptus* or *Acacia* which do not often
intercept such high levels of radiation and therefore are unlikely to inhibit weed growth unless weeds are highly shade intolerant. Other studies of tropical pastoral grasses grown under moderate shade showed a significant reduction in tiller number and root:shoot ratio (Jackson and Ash 2001; Wong et al. 1985) which corresponds to the results of this study, although the differences were not always statistically significant.

In the past, revegetating with dominant trees has been believed to facilitate the restoration of native ground cover, due to the inhibition of weed species (Nichols et al. 2010). This is rarely the case, with the evidence showing little or no improvement in native species richness or reduced weed abundance following tree planting (Munro et al. 2009; Nichols et al. 2010; Wilkins et al. 2003). Rather than being inhibited, the increased height of exotic annual grasses is more likely to shade and spatially restrict other germinating plants and seedlings and their negative effect on native seedling establishment may be amplified. The results of this experiment suggest that establishing native ground cover species may be more successful if carried out before tree and shrub planting. However the effects of shade on native plants and the other effects of tree canopies such as litter accumulation, hydrology and nutrient availability have not been explored.

CONCLUSION

Invasive annual grasses cause serious problems for restoration ecologists and land managers around the world due to their rapid spread and their potential for remaining dominant following invasion. Their ability to produce large numbers of seed and to alter the environment to suit them, makes them very difficult to successfully eradicate and makes revegetation and restoration efforts expensive and challenging. Often more than one method is required and having a range of options to suit different situations is valuable. Limiting the availability of soil nutrients can inhibit invasive annual grasses. Topsoil removal may be an effective method to use before revegetation efforts to clear the land of weeds, weed seed and excess nutrients. Carbon addition can be used where native species still exist or have already been planted to help them establish
successfully, especially where soil nitrogen levels have been elevated due to human activities. Shading does not inhibit invasive annual grasses, so planting canopy species to help control annual grasses for groundcover revegetation is not recommended. However further trials of these methods in the field and their effects on native species as well as broadleaf and perennial weeds will provide more insight into their value for groundcover revegetation (Chapter 3).
Chapter 3: Testing methods of weed inhibition in the field to improve groundcover revegetation success.

INTRODUCTION

Native ground cover species are often overlooked in revegetation projects, but as the aims of revegetation move away from creating native wood lots and towards restoring native habitats and functional, complex ecosystems, more information is required to determine how to re-establish this important component of vegetation. Whilst the revegetation of native trees in Australia is now common and often successful (Smith 2008), the gaps between trees are often left as bare ground for exotic species to reinvade (Munro et al. 2009; Wilkins et al. 2003). In the past, researchers have believed that native ground cover species would re-establish on their own once the trees were planted, but this is rarely the case (Florentine et al. 2013; Munro et al. 2009; Nichols 2005; Nichols et al. 2010). Native grasses and forbs seldom form persistent soil seed banks (Lunt 1995; Morgan 1998a; Scott and Morgan 2012) and where the land has been used for agriculture, seed bearing plants may have been absent for many decades. Thus deliberate reintroductions of native ground cover species via seed or seedlings are required. Due to the large species diversity and density of herbaceous plants in the understorey, particularly in grassy woodlands and grasslands (Caetano de Abreu 2009; Tremont and McIntyre 1994), using tubestock seedlings may not be practical or economical. Direct seeding may be the preferred option, but more research is needed to find effective methods for broad scale establishment (Tonkinson et al. 1999).

The presence of exotic invasive plant species (weeds) is often the main hurdle that needs to be overcome for native revegetation to be successful. Revegetation sites are usually covered in a range of weeds including pastoral and cropping species, as well as agricultural and environmental weeds (otherwise revegetation probably would not be necessary). Some of these species can be extremely difficult to get rid of as they are fast growing, disturbance tolerant and have high reproductive rates, either via abundant seed or extensive vegetative reproduction. These traits make them strong competitors that are able to dominate and exclude most native species, which are generally slower
growing (Seabloom et al. 2003; Standish et al. 2008). In agricultural areas, the soil conditions have often been changed to favour these invasive weeds through actions such as fertiliser application, livestock grazing, tilling, and irrigation (Cramer et al. 2008). Therefore, in order to re-establish native ground cover in areas cleared or altered by agriculture, not only do the species need to be reintroduced and the weeds eradicated, but the soil conditions may also need to be ameliorated (Yates and Hobbs 1997). Where increased soil nitrogen levels have encouraged weed dominance, methods such as carbon addition and scalping may help to control weeds by reducing nitrogen availability (Perry et al. 2010).

Carbon addition is a method of weed control that is currently being trialled around the world with varying levels of success (Perry et al. 2010). Carbon sources such as sucrose or sawdust are applied to the soil which causes an increase in the abundance of heterotrophic soil organisms (Baer et al. 2003). These organisms also take up inorganic nitrogen (N) from the soil, making it less available to plants (Baer et al. 2003). This reduction in available N can reduce the growth of nitrophilic weeds, thus potentially benefitting native plant species that are better adapted to low N availability (Blumenthal et al. 2003). However this will depend on the species present (Eschen et al. 2006) and the soil conditions (Blumenthal 2009) as well as the duration and volume of carbon added (Blumenthal et al. 2003). The aim is to find a balance where the benefits of reducing weed competition outweigh the negative effects that reducing N availability might normally have on native species (Eschen et al. 2006; Smallbone et al. 2007). Carbon addition has been shown to reduce the cover, abundance or biomass of weeds in Australian grassy woodlands (Morris and de Barse 2013; Prober et al. 2005) and American prairies (Averett et al. 2004; Blumenthal 2009; Burke et al. 2013; Kirkpatrick and Lubetkin 2011; Paschke et al. 2000; Rowe et al. 2009) with a subsequent increase in native (usually perennial) species (Blumenthal et al. 2003; Burke et al. 2013; Paschke et al. 2000; Prober et al. 2005). Results from Europe were less convincing (Eschen et al. 2007; Kardol et al. 2008), but this may be due to differences in limiting factors or native species being more nitrophilic. However, all of these studies have been on restoration sites where some natives were still present and were not usually reintroduced as part of the study. The potential for carbon addition to be used for revegetation projects,
particularly where native ground cover species are introduced by direct seeding, has only received limited attention. Blumenthal et al. (2003) measured a 700% increase in prairie biomass when native prairie species and weeds were grown together in bare plots treated with carbon, and Smallbone et al. (2007) found an increase in initial germination and survival of Australian native forbs when sown in degraded native grasslands where sugar had been applied. Carbon addition has only a short term effect on soil N availability and needs to be regularly applied (Perry et al. 2010), but if used during the crucial germination and establishment phase when plants are most vulnerable (Smallbone et al. 2007), it could create an extended period of low competition from weeds following seeding, increasing native revegetation success.

Scalping is a method of weed control that whilst generally unsuitable for restoration purposes, could be very successful for revegetation. Scalping involves the removal of part or all of the topsoil, usually using a grader blade, excavator or other machinery. Scalping can be particularly effective as it not only removes any extant vegetation, but also greatly reduces the soil seed bank (Rasran et al. 2007) and can reduce soil nutrients (Allison and Ausden 2004; Bai et al. 2012; Kiehl et al. 2010; Tallowin and Smith 2001). Although not extensively trialled yet, scalping has been shown to reduce the cover or abundance of undesirable species in North America (Choi and Pavlovic 1998), Europe (Kiehl and Pfadenhauer 2007) and Australia (Gibson-Roy et al. 2010a), as well as increase grassland diversity (Pywell et al. 2007) and improve native species establishment (Choi and Pavlovic 1998; Cole et al. 2005; Gibson-Roy et al. 2010b). Whilst unsuitable for the restoration of remnant vegetation for obvious reasons, it has great potential as a broad scale weed control method for the revegetation of cleared agricultural land.

Revegetation in the past has often involved a small number of species, usually trees and shrubs, evenly spaced and planted simultaneously (Munro et al. 2009; Wilkins et al. 2003; Yates and Hobbs 1997). As our ideas of revegetation move towards recreating complex habitats from scratch, there is a requirement for more knowledge on how different species or functional groups of plants will interact when all planted together on bare substrate. It is difficult to predict how late successional species (which are usually
the plants that ultimately need to be re-established) will interact in a primary succession environment, especially if early colonising invasive species are also present as weeds.

One of the questions that should be answered before planting takes place is: will the increasing shade caused by planted trees have a negative, positive or neutral effect on the establishment and growth of native ground cover species? As weed competition also plays a major role in ground cover establishment, the answer will require a greater understanding of the effects of tree canopy on weed species as well. Shading is usually considered inhibitive to understorey plants by increasing competition for light, but it can also have a positive effect in hot, dry environments by lowering soil temperatures and reducing evapotranspiration (Belsky et al. 1993). Of course shading is not the only impact trees can have on ground cover species, they are also highly competitive for resources and can affect soil moisture, nutrient cycling and litter cover (Belsky et al. 1993; Scholes and Archer 1997; Simmons et al. 2008).

The effect of trees on understorey productivity has been researched in a range of different ecosystems, although not usually in the context of revegetation. In environments with extreme shading (>95%, e.g. closed forests) there is strong evidence that understorey growth is inhibited (Cole and Weltzin 2005; Monk and Gabrielson 1985; Siemann and Rogers 2003), but where tree shading is less severe, such as grassy woodlands, savannahs and pastures, the patterns are less clear. Historically, trees have been cleared from grazing systems as their competitive effect was believed to reduce the productivity of grasses (Le Brocque et al. 2009) and there is evidence of this in a number of systems (Scanlan and Burrows 1990; Wahlen and Gisiger 1937; Walker et al. 1972). But the effects can often vary depending on climatic conditions such as annual rainfall and temperatures (Belsky 1994; Belsky et al. 1993; Gea-Izquierdo et al. 2009) as well as soil fertility (Jackson and Ash 2001). Whether trees are facilitative or competitive with herbaceous understorey will also change with the type and species of herbs considered. For example, grasses may be less inhibited than forbs (Gea-Izquierdo et al. 2009), or invasive species more inhibited than natives (Harris et al. 2011). These differences will often result in dissimilar species assemblages under tree canopies compared with nearby open areas (Harris et al. 2011; Prober et al. 2002a; Treydte et al.)
These effects may also change as the trees mature, i.e. the effects of tree seedlings are likely to be different to larger trees with higher litter deposition (Florentine et al. 2013) and more extensive root systems (Gibbs et al. 1999). There are no clear patterns in the literature for determining the likely response of herbaceous vegetation to tree canopies in different environments. There are a number of complex interactions going on, but for revegetation purposes, we are interested in whether the overall effect of canopy species is likely to be positive (facilitative) or negative (competitive) for the understorey community. This knowledge will help revegetation managers to decide whether overstorey species should be planted at the same time as understorey species, or if there is some benefit in planting them earlier or later.

The aims of this study were to improve our understanding of native groundcover establishment by investigating a number of methods of weed inhibition. Weed control is often the most important factor influencing the success of revegetation efforts (Corr 2003) and finding methods that are effective after planting without damaging natives can be difficult. Carbon addition, scalping and shading from tree canopies can potentially continue to inhibit weeds following planting to improve the competitiveness of native species. These methods were tested in the field on cleared agricultural land that was previously a temperate eucalypt grassy woodland. The effects of these treatments on both herbaceous weeds and native groundcover species sown from seed were investigated to determine which methods would improve groundcover revegetation success.

METHODS

Site Description

The study was carried out on Glenthorne Farm at O’Halloran Hill, South Australia (S35°03’37 E138°33’11’), 17km south of the city centre of Adelaide. Glenthorne Farm is 208 ha and has been used for various farming practices since the 1830’s including cattle, sheep and horse grazing, cropping and research (Caetano de Abreu 2009). It is now owned by the University of Adelaide and is predominantly used to graze sheep. It is
situated in the southern Mount Lofty Ranges, 200 m above sea level and has an undulating, hilly topography. It is in the temperate zone of southern Australia and experiences hot, dry summers and cool, wet winters with an average annual rainfall of 635mm (Bureau of Meteorology 2014a). The vegetation was originally Grey Box grassy woodlands (Kraehenbuehl 1996), but other than some eucalypt trees, relatively little native vegetation remains and the property is now covered in exotic pasture grasses and weeds. The soil is generally a thick sand or loam over clay (Caetano de Abreu 2009). The experimental treatments were carried out within a 5 ha section of Glenthorne Farm that had been fenced to exclude livestock and rabbits as part of a Grey Box eucalypt revegetation project.

Experimental Design

A field experiment to test different methods of herbaceous weed control and the effects on native grassland revegetation by seed was conducted at Glenthorne Farm from May 2011 until October 2012. The treatments tested were: 1) Control, 2) Carbon Addition, 3) Scalping (topsoil removal), 4) Shade and 5) Canopy (remnant trees). For treatments 1 - 4, 40 blocks (3 m x 4.2 m) were marked out across the 5 ha Glenthorne Farm site. These were allocated a treatment sequentially, with 10 blocks per treatment. Prior to the experiment, the Glenthorne Farm site was covered in herbaceous weeds, these were killed with herbicide and removed from each block prior to beginning the experiment. The blocks for each treatment were prepared in the following ways prior to seeding:

1) Control - sprayed with a glyphosate herbicide (Weedmaster Duo™ at 1L/100L water) and subsequent dead plant matter removed from the surface of the soil using mattocks and spades.

2) Carbon Addition (+Carbon) – as per Control, then 500g/m² of white sugar (sucrose) and 500g/m² of sawdust was added to the surface of the soil. The addition of sugar and sawdust was repeated 3, 6, 12 and 15 months after seeding. Sugar only was added after 9 months due to the accumulation of sawdust during summer.
3) Shade – as per Control. A shade structure was then built over the block using 1.65m fence droppers and 70% green shade cloth. The shade cloth was attached to the tops of the droppers so that it lay horizontally, approximately 1.2m off the ground and was 3.6m x 4.8m (slightly larger than the blocks). Blocks were not completely shaded for the whole day, but this was meant to replicate the shading expected from an open eucalypt woodland canopy.

4) Scalping - the top 5 cm of soil and all plant matter was removed using a ‘Dingo’ mini loader with a backfill attachment.

The Canopy treatments were set up the same as the Control treatments but were situated under two small patches of extant mature eucalypts on opposite sides of the site. Due to limited space under the canopies, only 8 blocks were used (4 in the northern end, 4 in the southern end) and they were only 3 m x 3 m each. Due to the sparse and irregular nature of the canopies and the movement of the sun during the day, plots were under shade for variable proportions of each day and these changed with the seasons. However each plot was under shade for a large part of each day, and the conditions were similar to those experienced under grassy eucalypt woodland canopies.

Glyphosate spraying and scalping was carried out in April 2011. The blocks were then left for a few weeks to allow the herbicide to work before the standing vegetation was removed using mattocks and spades. The sugar and sawdust was applied to the Carbon Addition blocks a few days prior to planting and the shade structures were erected over the Shade blocks two weeks after planting. Seed application occurred in May 2011, four weeks after spraying.

Within each block, six 0.75 x 0.75 m plots were marked out for seed application and monitoring (Fig. 3.1), except for Canopy blocks which only contained four plots each due to limited space. There was a 0.5 m buffer between each of the plots and between the plots and the edge of the treatment block. Seeds were planted in the plots at three different densities; High - 240 seeds (equivalent to 428 seeds/m²), Low - 120 seeds (equivalent to 214 seeds/m²) and No Seed, with two replicates of each density within each block. Canopy blocks only had High density and No Seed treatments due to limited
space. Plots were randomly allocated a seed density. Seeds of 12 native species commonly found in local Grey Box grassy woodlands were purchased from a local collector/supplier (Blackwood Seeds). The species names, growth form and number of seeds per plot are shown in Table 3.1. Variations in seed numbers are due to the availability of seed, but the high and low density plots retained the same species ratios, with twice as many seeds of each species in the High density plots. Grass seeds generally retained their awns (some fell off during counting but none were deliberately removed). *Kennedia prostrata* seeds were scarified by soaking in hot water overnight prior to planting.

![Diagram showing plot positioning](image)

**Figure 3.1:** Positioning of six plots sown with different seed densities within one treatment block.

Before planting, the soil in each plot was loosened using a mattock and any large stones were removed. The top centimetre or two of soil was then raked to the side and the seeds were scattered across the surface by hand, being careful to try to avoid seeds being blown away by the wind. The soil was then carefully raked back across the plot to cover the seeds and pressed down firmly using a compression board to minimise seed loss.
Table 3.1: A list of the native Australian grassland species used for this study and the number of seeds planted per 0.75 m x 0.75 m plot in low density and high density seed treatments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
<th># Seeds Low Density</th>
<th># Seeds - High Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Themeda triandra</td>
<td>C4 Grass</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Microlaena stipoides</td>
<td>C3 Grass</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Rytidosperma geniculatum</td>
<td>C3 Grass</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Rytidosperma setaceum</td>
<td>C3 Grass</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Rytidosperma pilosum</td>
<td>C3 Grass</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Austrostipa mollis</td>
<td>C3 Grass</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Lomandra multiflora ssp. dura</td>
<td>Rush</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Dianella revoluta</td>
<td>Lily</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Arthropodium strictum</td>
<td>Lily</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Burchardia umbellata</td>
<td>Lily</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Vittadinia gracilis</td>
<td>Daisy</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Kennedia prostrata</td>
<td>Legume</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>120</strong></td>
<td><strong>240</strong></td>
</tr>
</tbody>
</table>

The vegetation cover of each plot was recorded every two months from August 2011 to October 2012. This included estimating the percentage cover of living non-native plants (weeds), seeded native plants, bare ground and dead plant material (litter) using a 5x5 grid of 15 cm x 15 cm squares. Each species present (and alive) in each plot was identified.

The Glenthorne Farm site was slashed in July and November 2011 for fire management purposes, but not within any of the treatment blocks. The area received slightly above average rainfall during the study, with total annual rainfall of 701 mm and 698 mm in 2011 and 2012 respectively (Bureau of Meteorology 2014a).

**Soil Testing**

Soil samples were collected from eight blocks each from four of the treatments (Control, +Carbon, Scalping, Canopy) for analysis in October 2011. Soil was collected from the corridors between seed application plots within the treatment blocks. Samples were
tested for nitrate N, ammonium N, phosphorous, potassium, sulphur, organic carbon, conductivity and pH levels by CSBP Laboratories.

Analysis

None of the data met the assumptions of normality and homoscedacity required for parametric tests, even after transformation, so non-parametric Kruskal-Wallis tests were used to determine differences amongst treatments. Multiple pairwise comparisons with Bonferroni adjustments were used to show which treatments were different. All analyses were carried out in SPSS (IBM Corp. 2010). One of the Scalped blocks was flooded during most of the study, so was not included in the analyses. For clarity and concision, I have concentrated on the results for four of the count dates, each four months apart; October 2011 (spring), February 2012 (summer), June 2012 (winter) and October 2012 (spring).

RESULTS

Soil

Soils from under mature tree canopies had the highest levels of all nutrients tested (although results were not significant for sulphur) (Table 3.2). Pairwise tests revealed that Canopy soils were not significantly different to Control plot soils for any of the tests except pH(Ca), but they were significantly higher than Scalped plots (subsoil) for most nutrients (Table 3.2). +Carbon soils had significantly lower nitrate N than Canopy soils, although ammonium N and organic carbon levels were not different.
Table 3.2: Soil nutrient analysis of Glenthorne Farm soils taken from treatment blocks 16 months after initial treatment application. Treatments were; 3 monthly applications of sugar and sawdust (+Carbon), removal of 5 cm of topsoil (Scalp), location under tree canopies (Canopy) and a Control. Kruskal-Wallis non-parametric tests were used to determine differences. Different letters within each row show significant differences between treatments (P < 0.05). Values are medians, n = 8.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Canopy</th>
<th>+Carbon</th>
<th>Scalp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium N (mg/kg)</td>
<td>9.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nitrate N (mg/kg)</td>
<td>8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorus (mg/kg)</td>
<td>53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium (mg/kg)</td>
<td>569&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>842&lt;sup&gt;a&lt;/sup&gt;</td>
<td>676&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>393&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphur (mg/kg)</td>
<td>8.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Organic Carbon (%)</td>
<td>3.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Conductivity (dS/m)</td>
<td>0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH(Ca)</td>
<td>5.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH(H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>6.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Weeds

General Trends

Exotic weeds were prevalent on all plots, to varying degrees, throughout the study. Although most of the weeds were annual species which died off over summer, the plots often remained covered with their litter until the next growing season began. Broadleaf weeds generally survived longer into the summer than annual grasses. Weed cover peaked in October 2011, five months after herbicide spraying, and again from June to October 2012 (the main growing seasons) (Fig. 3.2). Forty three species of exotic herbaceous weeds were identified within the plots and zero woody species (Appendix 1). Fifteen of these were graminoids (grasses/rushes) and 28 were broadleaf weeds. Most species were found across the site, except for Phalaris aquatica L., Cynodon dactylon (L.) Pers. and Plantago lanceolata L. which were dominant in the northern end of the site but uncommon in the southern end. Avena barbata Pott ex Link and Hordeum leporinum Link appeared to be dominant across the rest of the site that was untouched,
but within the study blocks, *Lolium rigidum* Gaudin became more dominant. This may be due to the timing of vegetation removal on the study plots.

![Figure 3.2](image_url)

*Figure 3.2:* Mean percentage cover for all Glenthorne Farm study plots. Cover was categorised as: Weeds – exotic herbaceous plants; Bare – bare ground; Litter – dead plant matter; and Native – seeded local native species. Plots were cleared in April 2011 and native seed applied in May 2011.

*Treatment Effects*

**CARBON ADDITION**

Plots that had sugar and sawdust added showed significantly lower percentage cover of weeds compared with the Control plots in Spring 2011 and Winter 2012 (Fig. 3.3). Percentage cover of litter was also lower for +Carbon plots in Summer 2012 and Spring 2012. Bare ground cover was significantly higher for all months ($P < 0.001$). +Carbon treatments had a lower number of total weed species and graminoid weed species in Spring 2011 but the same number of broadleaf weed species as the Control treatments (Fig. 3.4).
SCALPING

Scalped plots had lower percentage cover of weeds than the other treatments in all seasons except summer (Fig. 3.3). The percentage cover of litter was lower for Scalped plots in all seasons also ($P < 0.001$), except for Spring 2011 when there was no litter on any plots. Associated with these lower percentage covers, Scalped plots had significantly higher cover of Bare ground at all count dates ($P < 0.001$). The total number of species of weeds growing on Scalped plots was not significantly different to Control plots, however the number of graminoid species was significantly lower in Spring 2011 ($P < 0.001$) and the number of broadleaf weed species was significantly higher over both growing seasons (Spring 2011, $P = 0.005$; Winter 2012, $P = 0.019$; Spring 2012, $P = 0.004$) (Fig. 3.4).

SHADE

Plots that were covered in shade cloth had high levels of weed cover. These were significantly higher than the Control plots in Spring 2012 ($P = 0.014$). This was also the case in December 2011, but the details are not shown. Litter cover and bare ground cover were not significantly different to the Control (Fig. 3.3). Shade plots had significantly lower numbers of total weed species and grass species than Control plots for all seasons, as well as a lower number of broad-leaf weed species in Winter and Spring 2012 (Fig. 3.4).

CANOPY

A few of the plots under tree canopies had quite low exotic cover, but generally weed cover was very high, although not significantly different to Control plots which also had quite high weed cover. However, in Summer 2012, weed cover in canopy plots was significantly lower than Control plots (Fig. 3.3). Bare cover and litter cover were not significantly different to the Control treatments. Canopy plots had the lowest number of total, broadleaf and graminoid weed species of all the treatments over all of the seasons ($P < 0.001$; Fig. 3.4).
Figure 3.3: Box and whisker plots showing the percentage cover of; a) exotic weeds, b) bare ground and c) plant litter in 75 cm x 75 cm experimental plots at Glenthorne Farm. All vegetation was removed from the plots in May 2011. Plots were assigned one of five treatments; 3 monthly applications of sugar and sawdust (Carbon), removal of 5 cm of topsoil (Scalp), covering with shade cloth (Shade), location under tree canopies (Canopy) or no treatment (Control). Maximum whisker length is 1.5 x IQR, additional points are shown as outliers. Kruskal-Wallis non-parametric tests were used to determine differences amongst treatments for each season (***) = P < 0.001; n.s. = not significant). Different letters under boxplots within each season show significant differences between treatments (P < 0.05) using Dunn’s pairwise comparisons with Bonferroni adjustment. Letters in bold and filled boxes highlight treatments that were significantly different to the Control.
Figure 3.4: The number of a) graminoid, b) broadleaf and c) total exotic weed species recorded within 75 cm x 75 cm experimental plots at Glenthorne Farm. Plots were cleared of vegetation in May 2011 and allocated one of 5 treatments; 3 monthly applications of sugar and sawdust (Carbon Addition), removal of 5 cm of topsoil (Scalping), covering with shade cloth (Shade), location under tree canopies (Canopy) and a Control. Maximum whisker length is 1.5 x IQR. The Kruskal-Wallis non-parametric test was used to determine differences amongst treatments for each season (*** = P< 0.001). Different letters under boxplots within each season show significant differences between treatments (P < 0.05) using Dunn’s pairwise comparisons with Bonferroni adjustment. Letters in bold and filled boxes highlight treatments that were significantly different to the Control.
Natives

General Trends

Of the 12 native species planted, ten were recorded in plots during the study, although most species were only found in low numbers. No other native species were present in plots except *Lythrum hyssopifolia* L. and *Juncus bufonius* L., which are common species found worldwide, but may be native to some parts of Australia. These were grouped in with the weeds for analysis to differentiate them from the reintroduced natives. *Burchardia umbellata* and *Dianella revoluta* plants were not recorded throughout the study (Table 3.3). Many *Arthropodium strictum* germinants were recorded in August and October 2011 but did not survive to maturity. Some germinants were also present in Winter and Spring 2012. Only one *Lomandra multiflora* ssp. *dura* and two *Kennedia prostrata* individuals were recorded. These survived the whole study, although no flowering had occurred by October 2012. *Microlaena stipoides*, *Austrostipa mollis*, *Vittadinia gracilis* and *Themeda triandra* occurred at low numbers (<32 individuals across all plots at any count date) throughout the study. The three *Rytidosperma* species were difficult to tell apart without looking at the magnified seeds, so these were pooled together. They were the most common seeded native species in the study, ranging from 162-295 individuals across all plots depending on the season. Percentage native cover was generally quite low with many seeded plots having zero natives growing in them. However, percentage native cover did reach up to 35% in some plots towards the end of the study.

No seeded native species germinated in No Seed plots and these plots were not used in the analysis of native plant establishment. No significant differences were found between low and high density seeded plots so these plots were pooled together for analysis, except for *Rytidosperma* spp. data which was significantly different for some of the count dates and was analysed separately. *Vittadinia gracilis* data was also analysed for treatment differences but none of the other native species had enough data for statistical analysis.
Table 3.3: Local native species planted at Glenthorne Farm in May 2011. Seeds were planted in 75 cm x 75 cm plots, each allocated one of five treatments; 3 monthly applications of sugar and sawdust (Carbon), removal of 5 cm of topsoil (Scalp), covering with shade cloth (Shade), location under tree canopies (Canopy) and a Control. The table lists the total number of plants in all the plots for each treatment, the maximum percentage cover of each species in a single plot and the proportion of plots in which each species was present in October 2011 and October 2012.

<table>
<thead>
<tr>
<th></th>
<th>Total Number of Plants</th>
<th>Maximum % Cover</th>
<th># of Plots with species / Total # Plots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Carbon</td>
<td>Scalp</td>
</tr>
<tr>
<td>Oct 2011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rytidosperma spp.</td>
<td>34</td>
<td>88</td>
<td>105</td>
</tr>
<tr>
<td>Microlaena stipoides</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Austrostipa mollis</td>
<td>1</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Themeda triandra</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Lomandra multiflora</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Austrostipa mollis</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Burchardia umbellata</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Themeda triandra</td>
<td>3</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Lomandra multiflora</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dianella revoluta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vittadinia gracilis</td>
<td>2</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Kennedia prostrata</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>No native species</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Treatment Effects

CARBON ADDITION

The percentage cover of seeded native species was not significantly different in the +Carbon treatment than in the Control treatment. The number of native species ($P = 0.027$) and number of native plants ($P = 0.002$) were significantly higher in Spring 2011, but not in later seasons (Fig. 3.5).

SCALPING

Scalped plots had the highest percentage cover of seeded native species in all seasons (although not statistically significant for Summer 2012; Fig. 3.5). They also had significantly higher numbers of native plants present per seeded plot for all seasons when compared with the Control treatment and a greater number of native species present per plot.

The number of individuals and percentage cover of *Rytidosperma* spp. were generally higher in Scalped plots than the other treatments, although statistical significance, in particular comparisons to Control plots, were not found for all seasons (Fig. 3.6). The numbers and cover of *V. gracilis* in Scalped plots were significantly higher than all other treatments except +Carbon, for all seasons ($P < 0.001$; Fig. 3.7). Although there were not enough occurrences of the other species for statistical analyses, there were more occurrences in Scalped plots than the other treatments for all germinated species except *A. strictum* (Table 3.3).

SHADE

Shaded plots had significantly lower percentage cover ($P = 0.015$), number of plants ($P = 0.030$) and number of species of seeded natives ($P = 0.020$) compared with Control plots in Spring 2012, but not for any other season (Fig. 3.5).

CANOPY

Plots under tree canopies had generally very low or zero seeded native species and there were no statistical differences to Control plots (Fig. 3.5).
Figure 3.5: Box and whisker plots showing the a) percentage cover, b) number of plants per plot and c) number of species of native plants sown from seed at Glenthorne Farm in May 2011. Plots were 75 cm x 75 cm and were cleared of vegetation prior to seeding. Plots were assigned one of five treatments; 3 monthly applications of sugar and sawdust (Carbon Addition), removal of 5 cm of topsoil (Scalp), covering with shade cloth (Shade), location under tree canopies (Canopy) and a Control. Seeds of 12 herbaceous species were planted at two densities (120 and 240 seeds/plot), but results were pooled as there were no significant differences at \( \alpha = 0.05 \). Whiskers extend to the 5th and 95th percentiles. Differences between treatments were all highly significant (***, \( P < 0.001 \)). Different letters under boxplots within each season show significant differences between treatments (\( P < 0.05 \)) using Dunn’s pairwise comparisons with Bonferroni adjustment. Letters in bold and filled boxes highlight treatments that were significantly different to the Control.
Figure 3.6: Box and whisker plots showing the percentage cover (a,b) and number of plants (c,d) per 75 cm x 75 cm plot of wallaby grasses (*Rytidosperma* spp.) during different seasons after planting at Glenthorne Farm in May 2011. Seeds were added to plots at low (35 seeds/plot) and high densities (70 seeds/plot). Plots had all vegetation removed in May 2011 and were assigned one of five treatments: 3 monthly applications of sugar and sawdust (Carbon), removal of 5 cm of topsoil (Scalp), covering with shade cloth (Shade), location under tree canopies (Canopy) and a Control. Whiskers represent the 5-95th percentiles. The Kruskal-Wallis non-parametric test was used to determine differences amongst treatments for each season (** ** = P < 0.001, ** = P < 0.01, * = P < 0.05, n.s. = not significant). Different letters under boxplots within each season show significant differences between treatments (P < 0.05) using Dunn’s pairwise comparisons with Bonferroni adjustment. Letters in bold and filled boxes highlight treatments that were significantly different to the Control.
Figure 3.7: Box and whisker plots showing the percentage cover and number of plants per 75 cm x 75 cm plot of New Holland daisies (*Vittadinia gracilis*) during different seasons after planting at Glenthorne Farm in May 2011. Seeds were added to plots at low (15 seeds/plot) and high densities (30 seeds/plot) but results were pooled as there were no significant differences between seed density treatments at α = 0.05. Plots had all vegetation removed in May 2011 and were assigned one of five treatments; 3 monthly applications of sugar and sawdust (Carbon), removal of 5 cm of topsoil (Scalp), covering with shade cloth (Shade), location under tree canopies (Canopy) and a Control. Whiskers represent the 5-95th percentiles. Kruskal-Wallis non-parametric tests were used to determine differences amongst treatments for each season (*** = P < 0.001). Different letters under boxplots within each season show significant differences between treatments (P < 0.05) using Dunn’s pairwise comparisons with Bonferroni adjustment. Letters in bold and filled boxes highlight treatments that were significantly different to the Control.
DISCUSSION

Carbon Addition

The regular addition of sugar and sawdust as a carbon source lowered the available nitrate in the soil, although results from the soil analysis did not reveal a significant difference to the Control treatments, which may be attributed to low sample sizes and the large variance across the site. The mean nitrate N concentration for +Carbon plots was 3.5 mg/kg and two out of the 8 blocks had less than 1 mg/kg, which is similar to some remnant eucalypt woodlands (Prober et al. 2002b). This study did not aim to provide proof of causation, that sugar and sawdust addition lowers soil nitrate availability, as this has been done elsewhere (Blumenthal 2009; Blumenthal et al. 2003; Eschen et al. 2007; Morghan and Seastedt 1999; Paschke et al. 2000; Prober et al. 2005), but to concentrate on the effects of carbon addition on weeds and native species from a practical viewpoint, whatever the underlying mechanisms may be.

Carbon addition had a measurable effect lowering the percentage cover of weeds on experimental plots at Glenthorne Farm. Although far from eradicated, weed cover was significantly lower compared with Control plots after one year of carbon application. During summer, when most of the weeds had died, the amount of weed litter in +Carbon plots was significantly lower than Controls. Exotic annual grass litter has been shown to inhibit native species (Lenz et al. 2003) and create positive feedback mechanisms that enable exotic annual grasses to remain dominant (Prober et al. 2002b). This reduction in weeds and litter led to the amount of bare ground in the plots being significantly higher than the other treatments (except Scalping) throughout the study. This availability of bare ground or canopy gaps can enhance the initial establishment of native species (Morgan 1998b), and if this availability can be maintained for a year or more, native species may have a much better chance of longer term survival and reproduction.

The number of native species and the number of native plants per plot was significantly higher in +Carbon plots than Control plots in the spring following seeding, but was not
maintained in subsequent seasons. Carbon addition appears to have aided the germination or survival of native seeds in the first few months, perhaps due to the increased bare ground available and decreased weed competition. However, for the rest of the study, the numbers of natives in +Carbon plots was similar to Control plots. This could mean that the mortality rates of the seeded native species were higher in +Carbon plots after initial germination. Higher germination plus higher mortality would combine to create a net effect similar to the Control plots, where few plants were present in spring 2011 but mortality rates appeared lower. The loss of native plants in +Carbon plots could be due to the sugar and sawdust application directly or due to the subsequent physical, biological and chemical changes in the soil environment. Alternatively, the competition from weeds may have remained high enough to affect the survival of the native plants. For carbon addition to work, the correct levels need to be added to balance out the negative and positive effects (Eschen et al. 2006).

The effectiveness of carbon addition for weed control and native revegetation will depend on a number of factors. Firstly, as Blumenthal et al. (2003) states, carbon addition only works where the following conditions are met; 1) native species are suppressed by weeds, 2) weeds are nitrophilic relative to native species, and 3) the decrease in N must be of a duration and magnitude to alter the balance of competition between weeds and natives. Meeting these criteria will therefore depend upon the soil N levels as well as other physical, chemical and biological properties of the soil that can affect nitrate availability. Also the weed and native species in question, in particular their levels of nitrophily and competitiveness at low N levels will also affect the success of this method. The amount of carbon added and the source should be monitored and adjusted as necessary as sugars may leach faster in wetter months and sawdust may break down more slowly in drier months. It may also be helpful to determine an optimum nitrate range in which weeds are likely to be inhibited, but not natives, and regularly testing the soil and adapting the carbon rates as necessary.

Carbon addition is generally used to provide native perennial grasses with an advantage over invasive annual grasses as they are better equipped to deal with low N conditions due to their ability to remobilise and recycle nutrients from senescing tissues, rather
than relying solely on soil nutrients (Morton 1977). However there is evidence that this advantage may not come into effect until perennial grasses are mature. Groves *et al.* (2003) found that Australian perennial grasses such as *Themeda triandra* and *Rytidosperma carphoides* (F.Muell. ex Benth.) Connor & Edgar were unable to compete with exotic grasses at low nutrient levels during the first 13 weeks of growth. James *et al.* (2011) suggest that low N soils do not reduce competition between native perennial grasses and invasive annual grasses during the seedling stage as annuals are still able to grow faster than perennials and pre-emptively take up soil nutrients. Their meta-analysis showed that even at low N, annual grasses were able to maintain higher growth rates and produce more biomass than perennial grasses. Therefore carbon addition may not improve native seedling establishment, which is in accordance with the results from Glenthorne Farm where higher initial germination of natives did not result in increased establishment. However, carbon addition may be of use after the first growing season to create a competitive advantage for mature native perennials against annual grass weeds, improving their long term establishment success.

**Scalping**

Scalping treatments produced a similar but slightly greater effect on weed cover than carbon addition. Weed cover was significantly lower in Scalped plots than all the other treatments over the growing seasons (Spring 2011, Winter 2012 and Spring 2012) and weed litter was significantly lower in Summer 2012. Scalped plots also had the highest percentage cover of bare ground over the whole study period. Thus native seeded species were given more opportunities to establish as bare ground was maintained much longer after clearing compared with Control plots. These results may be partly attributed to a reduction in soil nutrients associated with topsoil removal. Although soil analysis did not show significant differences between treatments and the Control, there were slightly lower levels for ammonium, potassium, phosphorous and carbon in Scalped plots. However the reduction in weed cover in this study is more likely to be due to the reduction of the weed seed bank on scalped plots. There was an observable reduction in the annual grasses that are usually dominant at this site and the number of broadleaf weed species was increased compared to the other treatments, suggesting
that grass seeds were removed with the topsoil, leaving deeper buried, dormant broadleaf weed seeds that were able to become more prominent with the reduction in grass weeds.

Although the low numbers of native plants made it difficult to detect differences between the experimental treatments and the Control, all of the native species that established had greater cover and numbers in Scalped plots than the other treatments. The abundance of natives was not great enough for a revegetation effort to be considered particularly successful but scalping was definitely the most effective treatment in this study. At the end of monitoring, a greater proportion of Scalped plots had natives present and they had higher percentage cover of natives, higher numbers of native plants, and higher numbers of seeded native species present. In particular, *Rytidosperma* spp. and *Vittadinia gracilis* established more successfully on Scalped plots and although analysis was not possible for the other seeded species due to the large number of plots without established native plants, the raw data shows more plants appeared in Scalped plots for *Themeda triandra*, *Austrostipa mollis*, *Kennedia prostrata* and *Microlaena stipoides*.

In this study at Glenthorne Farm, scalping has only been moderately effective at controlling weeds and promoting the establishment of native groundcover from seed, but there is great potential for its use as a revegetation tool. On cleared agricultural land where weeds abound and natives are scarce, scalping can be used to “refresh” the soil and remove some of its history. When weed seed load is high due to years of agricultural use, removing a large proportion of that seed is a vital step in controlling the dominance of weeds. It is unlikely that all viable weed seeds will be removed however, so combining scalping with other methods such as herbicide application, burning, grazing or slashing may greatly improve its effect. Allowing weed germination to occur following scalping, and then removing these weeds before seed set by one of the aforementioned methods, will help to remove more of the weed seed from the soil. In heavily invaded areas, this may need to be done for a number of subsequent seasons, but by taking this time to reduce weed loads prior to planting, a lot of money and effort should be saved on subsequent weed management and lost natives.
Scalping also removes any nutrients that were in the topsoil and reveals the generally lower nutrient subsoil. This can be a benefit to weed control as most annual weeds thrive in high nutrient environments whereas Australian native perennials are generally more competitive in low nutrient soils (Prober et al. 2002b). This is particularly useful in agricultural soils where the soil nutrient levels have often been increased considerably by the addition of fertilisers and other agricultural practices. As discussed above, decreasing the available nutrients in soil can improve the establishment of natives in many cases. However, some subsoils may not be appropriate to scalp. At Glenthorne Farm, the subsoil is thick clay, which is often waterlogged in winter and in summer it is cracked, hard and dry. These conditions may not be suitable for direct seeding and germination of some native species and may have contributed to the low native establishment rates, although scalped plots still had higher native establishment than the other treatments. Other studies have had problems with sandy infertile substrates that inhibited plant establishment when all of the topsoil was removed (Choi and Pavlovic 1998; Kardol et al. 2008). These issues may be minimised by only removing part of the topsoil.

**Shade**

The Shade treatment did not reduce the cover of weeds at Glenthorne Farm. By the end of the study, the percentage cover of weeds growing under shade cloth was significantly higher than in the Control treatments. In particular, the common annual grasses *Lolium rigidum* (ryegrass), *Avena barbata* (wild oats) and *Vulpia bromoides* (silver grass) completely dominated a number of plots and grew a lot taller and survived a lot longer into the dry summer than on open plots. Increased height in seedlings is expected as the plant strives to reach more light. In some cases this may lead to death of the plant or reduced reproductive effort if sufficient light is not reached in time. However there were no indications of this occurring during the study. This may be due to the grasses being annual species which are able to reproduce quickly and die in summer anyway. In fact, due to summers being quite hot and dry in South Australia, the shade actually seemed to extend the life of some of the plants, probably due to reduced temperatures and
reduced evaporation of surface soil moisture in summer under the shade. The weeds were still able to produce large quantities of seed and created a thick mat of litter over the summer. The percentage cover of weeds in Shaded plots (and litter in summer) was close to 100% for most plots throughout the study. It is likely that under extreme levels of shading, these weeds would be inhibited, but under only 70% shade cloth and with sunlight reaching the plots when the sun was low in the sky, the light levels were not sufficiently lowered to cause a negative reaction. However these light levels are comparable with eucalypt open woodlands so similar responses could be expected by these weeds in remnant or revegetated eucalypt woodlands.

The number of species of weeds on Shaded plots compared to Control plots was significantly lower throughout the study. This may have been due to the dominance and increased above ground growth of some of the annual grasses out-competing other species, or the decreased light availability may have inhibited the growth or germination of some species.

Most of the natives that germinated in Shade plots (generally *Rytidosperma* spp.) were thin, grew underneath much taller annual grasses and did not survive. Only two *M. stipoides* and nine *Rytidosperma* spp. plants remained on Shaded plots at the end of the study.

**Canopy**

Plots that were set up under remnant mature eucalypt trees had similar levels of weed cover to Control and Shade plots. Many of the plots had 100% weed cover during growing seasons, made up predominantly of annual grasses. Canopy plots had lower numbers of weed species per plot than the other treatments, with *Lolium rigidum* and *Bromus* spp. dominating. Very few natives were present on Canopy plots. Some *A. strictum* lilies germinated but did not survive and some *Rytidosperma* spp. germinated but only three survived to October 2012.
The effect of Canopy on weed growth was similar to the Shade treatment, with plants growing densely and tall. However there appeared to be a slightly different subset of weed species growing under the tree canopies. This may be due to the long term effects of living under a shaded environment. Less species are able to grow in shaded environments, either by direct inhibition or through competition with more shade tolerant species. This effect is magnified when the shade remains for many years. Some species will die out and others may become dominant and reproduce. At Glenthorne Farm there were slightly higher levels of a number of soil nutrients under canopies compared to open ground, which is commonly the case (Belsky 1994; Prober et al. 2002a; Scholes and Archer 1997). This may also have increased the dominance and abundance of certain weed species that have high nutrient uptake rates. The end result is not necessarily a lower abundance of weeds but lower weed species diversity.

CONCLUSION

In summary, I have not found any evidence to suggest that planting trees before groundcover species provides any benefits in terms of weed inhibition or native plant establishment for Australian grassy woodland revegetation. Although the number of weed species growing under artificial shade and tree canopies in this study was lower, the weed cover was the same or greater than unshaded plots and the weed growth was often thicker and taller. Very few seeded native plants established and there was no natural recruitment of unseeded native species suggesting that the presence of any plant canopy is unlikely to aid in the restoration of native species at Glenthorne Farm or other similar environments. Of course the effect of tree canopy on understorey vegetation will vary greatly with conditions and species, and there are other benefits of tree canopies not discussed here, but for the purpose of revegetation I see no reason to plant tree species before groundcover species unless for financial or logistical reasons. In fact, it is likely to be more beneficial to plant all species together as pre-planting weed removal can be carried out without worrying about previously planted seedlings and the establishment of ground cover can help to control weeds that would otherwise need to be managed when only trees are planted.
Carbon addition and scalping were more effective than the Shade treatments at reducing exotic weed cover. These treatments were able to maintain bare ground for over a year on a site that normally has 100% weed cover. Although native establishment was still fairly low, there is evidence that these treatments may be useful for weed management and improving ground cover revegetation success in certain circumstances.

Although carbon addition did reduce percentage weed cover, there was not an associated increase in the establishment of native species from seed. It may be that the reduction in soil N also inhibits native species and will only provide some benefit for native perennial grasses after the first growing season once they are able to recycle the nutrients in their senescing tissues. Other types of native plants, in particular annuals, may not benefit from carbon addition at all. If applied once the perennial grasses were mature, carbon addition could improve their long term establishment by reducing the growth of weeds, but an alternative form of weed management would be required during the planting stages of revegetation.

Out of the four treatments tested, scalping shows the greatest potential for use in native revegetation. It significantly reduced weeds (particularly annual grasses), maintained the most available bare ground and had the greatest success in establishing native species from seed. This “success” is relative to the other treatments and may not be considered overly successful by revegetation managers due to the relatively high weed cover and low native germination, but there is definitely potential. More research on appropriate seeding rates and germination requirements of native herbaceous species may help improve initial establishment rates. Whilst this method will not be suitable for areas with remnant natives and native soil seed banks due to its destructive nature, it is well suited to the revegetation of retired farmland and other highly altered and cleared landscapes. Scalping not only involves the removal of standing vegetation, it also removes a lot of the soil seed bank and also excess nutrients in the topsoil. The depth of soil removal can be adjusted depending on the conditions of the site in question and the soil either left in mounds (which can reduce water runoff) or removed from the site.
Scalping would probably work best used in conjunction with herbicide application. There will still be weed seeds in the soil following topsoil removal, so by waiting until germination occurs and then applying herbicide, more of the soil seed bank will have been exhausted. Depending on the level of infestation at the site, this may need to be done a few times. At Glenthorne Farm, scalping appeared to greatly reduce the abundance of annual grasses which were usually dominant, but this gave the broadleaf weed seeds a chance to germinate and establish. Therefore a broadleaf herbicide could be used and native grasses planted at the same time to reduce the time needed to wait between scalping and planting. Native forbs could then be planted in subsequent seasons once the broadleaf weeds were reduced.
Chapter 4: Can topsoil removal increase the establishment of native herbaceous species on retired agricultural land?

INTRODUCTION

Since European settlement, Australia’s native woodlands and grasslands have been cleared to make way for cropping and pasture systems to feed a growing human population (Prober and Smith 2009; Yates and Hobbs 1997). In recent decades, research has shown how detrimental this clearing has been, particularly in southern temperate areas, and the importance of trying to protect what remains (Bennett and Ford 1997; Paton et al. 2000; Prober and Smith 2009; Yates and Hobbs 1997). Current research also suggests that to slow down the rate of native faunal extinction, the area of native habitat needs to be increased (Ford and Howe 1980; Szabo et al. 2011; Vesk and Mac Nally 2006). As this conservation awareness spreads, small pockets of agricultural land are being made available by owners for revegetation (Smith 2008). In the past, old-fields were left to regenerate naturally, but there is growing evidence that in Australia, restoration of a native community on ex-agricultural land often does not occur without some help (Cramer et al. 2008; Standish et al. 2008). In particular, inadequate native seed availability, dispersal and unfavourable germination conditions greatly limit the recolonisation potential of old-fields (Standish et al. 2007). Thus, these areas require an input of propagules (revegetation) to achieve restoration.

Although revegetation of trees and shrubs has been broadly studied and practised, our understanding of how to restore native grasslands and the herbaceous understorey of woodlands is still lacking. Agricultural land has generally not only been cleared of trees and shrubs, but has had exotic pasture grasses, crop species and agricultural weeds introduced. It may also have had fertilisers added, the soils tilled and been grazed by livestock. The result being an environment that not only has a different suite of plant species compared with the original ecosystem, but one with altered nutrient and water cycles and different physical, chemical and biological soil properties. All these changes
combine to make restoring the native ecosystem, and providing habitat for native fauna, very challenging.

The dominance of exotic, invasive plant species (herein called "weeds") is commonly the main challenge facing revegetation efforts. Australia has over 2000 species of exotic plants that have naturalised (Hnatiuk et al. 1990) and a number of these have attributes such as high reproductive rates, fast growth and adaptations to disturbance that enable them to invade, dominate and persist in new environments (Adair 1995). Some species will also change the soil conditions around them by processes such as root exudation, litter production and nutrient uptake, which in turn can alter the soil microbial community (Wolfe and Klironomos 2005). When these changes benefit the weeds and inhibit native species, the persistence of the weeds may be greatly improved (Kulmatiski and Beard 2008). Removing these weeds can be quite difficult, especially where they have persisted for a long time, building up a soil seed bank and altering the soil conditions, as the removal of one generation simply makes space for the next generation. For weed removal to be effective, the next generations also need to be removed, and in many instances the soil conditions will also need to be altered to a more natural state that benefits the planted natives rather than the weeds.

One method that tackles both of these issues is topsoil removal (scalping). This method involves scraping off the top layer of soil with a grader or similar equipment to remove the accumulated weed seeds as well as any excess nutrients that may be in the soil. Studies in the United States of America, Europe and Australia in various grassland habitats have shown that topsoil removal can reduce weed cover (Choi and Pavlovic 1998; Kiehl and Pfadenhauer 2007), reduce weed abundance (Gibson-Roy et al. 2010a), improve the establishment of native species (Choi and Pavlovic 1998; Cole et al. 2005) and regionally rare species (Rasran et al. 2007) and increase grassland diversity (Pywell et al. 2007). This method would obviously not be ideal where native species are still present, but in the case of agricultural land that has been significantly cleared and altered, it may be very effective. Although such a destructive method could be considered extreme, it may be necessary in order to reduce the dominant weeds sufficiently for any native herbaceous species planted to stand a chance of survival on
highly invaded sites. In fact, even topsoil removal may not be enough on its own, as there is likely to still be seeds in the remaining soil. Allowing seeds to germinate following topsoil removal and then applying a herbicide may be necessary to deplete the seed banks of weeds in the soil enough for successful native species establishment. This would also reduce the amount of ongoing weed management required which is a lot more difficult to carry out once desired species are present.

The aim of this study was to test and quantify the effectiveness of topsoil removal as a weed control method and its potential for use in broad scale revegetation of native herbaceous species. Using a de-stocked paddock at Glenthorne Farm, South Australia, four treatments were compared; topsoil removal, herbicide application, topsoil removal followed by herbicide application, and a control. Seeds of native grassland species were also sown to investigate the effect of the treatments on native herbaceous revegetation. I hypothesised that:

1) Weed cover on the experimental treatment plots would be reduced compared to the control; and

2) Native species establishment would be greater on the experimental treatment plots compared to the control.

METHODS

Site Description

The study was carried out on Glenthorne Farm near O’Halloran Hill, South Australia (S35°03’37 E138°33’11’), 17km south of the city centre of Adelaide. Glenthorne Farm is 208 ha and has been used for various farming practices since 1838 including cattle, sheep and horse grazing, cropping and research. It is now owned by the University of Adelaide and is predominantly used to graze sheep. It is situated in the southern Mount Lofty Ranges, 200 m above sea level and has an undulating, hilly topography. It is in the temperate zone of southern Australia and experiences hot, dry summers and cool, wet winters with an average annual rainfall of 635mm (BOM, 2013). The vegetation was originally Grey Box (Eucalyptus microcarpa (Maiden) Maiden) grassy woodlands, with a
loam over clay soil (Caetano de Abreu 2009). This study was carried out within a 5 ha section of Glenthorne Farm which was fenced to exclude livestock and rabbits for a Grey Box eucalypt revegetation project in 2009. Other than some mature eucalypt trees, very little native vegetation remains on the site and the property is now covered in exotic pasture grasses and herbaceous weeds.

**Experimental Design**

In May 2012, twelve 30 m x 30 m blocks within the Glenthorne Farm study site were scalped using a grader. Scrapes were approximately 4 m wide, 5-10 cm deep and ran parallel to the hill contour. Topsoil was left on site as 0.5 m high windrows between scalped rows. Blocks of plots were marked out within scalped areas as well as on adjacent un-scalped land. Half of these blocks were sprayed with a glyphosate herbicide 3 weeks after scalping to create four soil treatments; Scalp + Herbicide, Scalp Only, Herbicide Only and a Control. Groups of the four treatment blocks were replicated six times across the site (24 blocks). Within each block, 1 m x 1 m plots were used for seed addition and monitoring. Seed addition treatments were; native forbs and grasses (F), native grasses (G), *Eucalyptus microcarpa* (Grey Box) seed (EU) and no seed (NS). Scalped blocks had 2 x F, 2 x G, 1 x EU and 1 x NS plot per block. Due to limited amount of seed un-scalped blocks had 1 x F and 1 x EU plot. The species and amounts of seed added to each plot are shown in Table 4.1. *Eucalyptus microcarpa* seeds were added to explore the potential for revegetation from seed or natural regeneration of these trees at Glenthorne Farm. The re-establishment of Grey Box trees on Glenthorne Farm was beyond the scope of this study and as none of the sown *E. microcarpa* seeds germinated, this treatment was included in the NS treatment during analysis.

Planting was carried out on the 20 June 2012. The plots that had not been scalped had all vegetation removed from the soil surface with a mattock. Each plot was then hoed and raked to loosen the soil surface. Seed mixes as per Table 4.1 were combined with 250 ml of water and 100 g of sawdust a couple of days before sowing. The mix was hand broadcast onto the plots and then a thin layer of soil raked back over to cover the seeds. Despite windy conditions, the moist sawdust appeared to minimise any seed loss,
however approximately 90 mm of rain fell over the following two days so some seed may have been washed away. For *Calostemma purpureum* R.Br, 20 bulbs as well as three seedlings with a first leaf of 5-10 cm were planted in each Forb plot.

Monitoring of the plots was carried out at the end of each month from August to November 2012. A grid with 25 cm x 25 cm squares was used to estimate the percentage cover of weeds (unseeded plants), natives (seeded species), bare ground and plant litter in each 1 m$^2$ plot. The percentage cover of weeds was further split into graminoid weeds (monocots) and broadleaf weeds (dicots). All plant species were identified where possible and the presence of inflorescences or seeds noted. For each native seeded species, the number of plants, percentage cover, number of flowering plants and number of inflorescences were recorded for each plot. The four *Rytidosperma* species were too difficult to distinguish in the field, so were grouped together. The various plant species are distinguished by their genus in the results and discussion.

Table 4.1: The amount of seed of each species planted per 1 m x 1 m plot at Glenthorne Farm. F plots contained grasses and forbs, G plots contained grasses only, and EU plots contained only *Eucalyptus microcarpa* seed.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Family</th>
<th>Form</th>
<th>Plots</th>
<th>Approx. #/plot</th>
<th>g/plot</th>
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<tr>
<td><em>Calostemma</em></td>
<td><em>purpureum</em></td>
<td>Amaryllidaceae</td>
<td>bulb</td>
<td>F</td>
<td>23</td>
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</tr>
<tr>
<td><em>Vittadinia</em></td>
<td><em>gracilis</em></td>
<td>Compositae</td>
<td>daisy</td>
<td>F</td>
<td>50</td>
<td>0.038</td>
</tr>
<tr>
<td><em>Arthropodium</em></td>
<td><em>strictum</em></td>
<td>Liliaceae</td>
<td>lily</td>
<td>F</td>
<td>50</td>
<td>0.111</td>
</tr>
<tr>
<td><em>Microlaena</em></td>
<td><em>stipoides</em></td>
<td>Gramineae</td>
<td>grass</td>
<td>G, F</td>
<td>50</td>
<td>0.357</td>
</tr>
<tr>
<td><em>Rytidosperma</em></td>
<td><em>geniculatum</em></td>
<td>Gramineae</td>
<td>grass</td>
<td>G, F</td>
<td>100</td>
<td>0.065</td>
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<td><em>pilosum</em></td>
<td>Gramineae</td>
<td>grass</td>
<td>G, F</td>
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<td>0.154</td>
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<td><em>Rytidosperma</em></td>
<td><em>setaceum</em></td>
<td>Gramineae</td>
<td>grass</td>
<td>G, F</td>
<td>100</td>
<td>0.082</td>
</tr>
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<td><em>Rytidosperma</em></td>
<td><em>caespitosum</em></td>
<td>Gramineae</td>
<td>grass</td>
<td>G, F</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>573</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
<td>450</td>
<td>0.746</td>
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<tr>
<td><em>Eucalyptus</em></td>
<td><em>microcarpa</em></td>
<td>Myrtaceae</td>
<td>tree</td>
<td>EU</td>
<td>200</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Analysis

Results were analysed using either ANOVA or Kruskal-Wallis tests depending on whether the data were parametric or not. To determine which treatments were different, Tukey’s HSD (Honest Significant Difference) tests were used after ANOVA and Dunn’s pairwise comparisons with Bonferroni adjustment used following Kruskal-Wallis tests.

As native forbs were not sown into all plots due to limited seed availability, results for native forbs were calculated using only those plots where forb seeds were added (F, 36 plots). Data for native grasses were analysed for all seeded plots except the EU ones (F + G, 60 plots) as there were no significant differences for native grasses between the other two seed treatments.

RESULTS

Weed Species

A total of 38 exotic weed species were identified across all plots (Appendix 2), plus two cosmopolitan species which are sometimes considered natives but were included in the weed data for this study, *Juncus bufonius* L. (toad rush) and *Lythrum hyssopifolia* L. (lesser loosestrife). The number of weed species on each plot was only significantly different between treatments for October and November, when Scalp + Herbicide plots had a greater number than Control plots (*P* = 0.01) (Fig. 4.1c). When the number of weed species was split into graminoid (monocotyledonous) and broadleaf (dicotyledonous) species, the differences between treatments were greater. For graminoid weeds, the number of species on each plot was highly significantly different for all months (*P* < 0.003). Control plots had a higher number of graminoid species than the three other treatments in August and October (*P* < 0.001), and the number of graminoid species was also higher than both Scalped treatments in September (*P* = 0.002) and higher than the Scalp Only treatment in November (*P* < 0.001) (Fig. 4.1a). Scalp Only plots generally had the lowest number of graminoid weed species with significantly lower mean species numbers than Herbicide Only as well as Control plots in October and November (*P* < 0.001). The numbers of broadleaf weed species were also significantly different between treatments for all months (*P* < 0.001). Both Scalp
treatments had higher numbers of broadleaf weed species than the Control treatment for all four months (Fig. 4.1b). In addition, Scalp Only plots had higher broadleaf species numbers than the Herbicide Only treatment in August and September and Scalp + Herbicide plots had higher numbers than Herbicide Only in September, October and November. The proportion of weed species on each plot that were graminoid was significantly higher for the Control treatment than both Scalp treatments for August, September and October ($P < 0.001$)(Fig. 4.2). In November, Control and Herbicide Only plots had a greater proportion of graminoid weeds than Scalp Only plots ($P < 0.001$).
Figure 4.1: The mean ± s.e. of a) graminoid weed species, b) broadleaf weed species and c) total weed species within 1 m x 1 m experimental plots on Glenthorne Farm. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Graminoid and broadleaf weed species were recorded for each plot at the end of August, September, October and November 2012. One-way ANOVA was used to determine differences amongst treatments for each month (*** = P < 0.001, ** = P < 0.01, * = P < 0.05, n.s = not significant). Different letters within each month show significant differences between treatments calculated using Tukey post-hoc tests (P < 0.05).
Figure 4.2: The median proportion of a) weed cover and b) weed species made up of graminoid (monocotyledonous) species compared with broadleaf (dicotyledonous) species within 1 m x 1 m experimental plots on Glenthorne Farm. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Percentage cover of graminoid and broadleaf weeds was recorded for each plot at the end of August, September, October and November 2012. Whiskers show the interquartile range. Kruskal-Wallis non-parametric test was used to determine differences amongst treatments for each season (** = P < 0.01; *** = P < 0.001; n.s. = not significant). Different letters under values within each month show significant differences between treatments calculated using Dunn’s pairwise comparisons with Bonferroni adjustment (P < 0.05).
Weed Cover

Scalp Only treatments had higher percentage weed cover than Scalp + Herbicide plots in August ($P < 0.001$) and higher than both Scalp + Herbicide and Control treatments in September ($P = 0.001$; Fig. 4.3c). In October and November there was no significant difference between treatments. Graminoid weed cover was significantly different between treatments in August and November. In August, both Herbicide treatments had lower graminoid cover than the Control plots ($P = 0.004$; Fig. 4.3a). In November, Scalp Only plots had lower graminoid cover than Herbicide Only and Control plots ($P < 0.001$).

Broadleaf weed cover was significantly different for all months except November. Scalp Only had greater broadleaf weed cover than all the other treatments in August ($P < 0.001$) and greater cover than Control plots in September ($P = 0.004$; Fig. 4.3b). In October, Scalp + Herbicide plots had higher percentage cover of broadleaf weeds compared with Control plots ($P = 0.044$). When the proportion of graminoid cover to broadleaf weed cover was compared for each plot, the Control treatment had a significantly higher ratio of graminoid to broadleaf weeds than the three other treatments in August ($P = 0.002$), and higher ratios than both Scalped treatments in October ($P = 0.004$; Fig. 4.2). The results for September were not significant and in November, Control and Herbicide Only plots had higher graminoid ratios than Scalp Only plots ($P = 0.001$).

Scalp Only had lower percentage cover of bare soil than the Control treatments in August, September and October ($P < 0.001$, $P = 0.001$, $P = 0.006$ respectively; Fig. 4.4). Scalp Only also had lower bare cover than Scalp + Herbicide plots in August and September. Percentage bare soil was lower in Scalp + Herbicide plots than Control plots in October and November ($P = 0.028$). The Control treatment had the highest percentage cover of bare soil in all four months.

Exotic plant litter was not present on the plots until October, when Herbicide Only had significantly less litter cover than either of the Scalped treatments ($P < 0.001$). Results were not significant for litter cover in November.
Figure 4.3: Box and whisker plots showing the percentage cover of a) graminoid weeds, b) broadleaf weeds and c) all weeds within 1 m x 1 m experimental plots on Glenthorne Farm. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Graminoid and broadleaf weed species were recorded for each plot at the end of August, September, October and November 2012. Maximum whisker length is 1.5 x IQR with additional points shown as outliers. Kruskal-Wallis tests were used to determine differences amongst treatments for each month (*** = P < 0.001, ** = P < 0.01, * = P < 0.05, n.s = not significant). Different letters under values within each month show significant differences between treatments calculated using Dunn’s pairwise comparisons with Bonferroni adjustment (P < 0.05).
Natives

Percentage cover of native (seeded) plants in each plot was compared for the different weed control treatments. Only plots where seeds were added were used in this analysis. At the end of the study (Nov 2012) the mean percentage cover of native plants ranged from 3% for Control plots to 7.5% for the Scalp + Herbicide treatment. There were marked differences between treatments with the Scalp + Herbicide treatment having higher native cover than the Control treatment for all months ($P \leq 0.008$; Fig. 4.5) and the Scalp Only treatment having higher native cover than Control plots in August ($P = 0.006$).
The percentage cover of native grasses was significantly higher in Scalp + Herbicide plots compared with Control plots in all months (Fig. 4.6). Scalp Only plots also had higher native grass cover than Control plots in August. The mean number of native grass plants in each plot was also higher in both Scalp treatments in all months (Fig. 4.6) Herbicide Only plots had lower numbers of native grasses than Scalp + Herbicide plots in November.

The differences in the number of individuals and percentage cover of native forbs amongst the treatments were highly significant for all months (Fig. 4.7). Both Scalp treatments had higher native forb cover than the Control treatment throughout the study. The forb cover of Scalp Only plots was also higher than Herbicide Only plots in August, September and October. The number of native forb plants in Scalp Only plots
was also higher than Control plots for all months and higher than Herbicide Only plots in August, September and October. Scalp + Herbicide plots had higher forb numbers than Control plots in August, October and November.

*Rytidosperma, Microlaena* and *Vittadinia* retained relatively stable numbers of individuals throughout the study. However, *Arthropodium* and *Calostemma* suffered complete mortality by November (except for one *Calostemma*). All of the native species showed greater cover and abundance in one or both of the Scalp treatments for at least two of the four months studied when compared to the Control (Tables 4.2 & 4.3). *Rytidosperma, Microlaena* and *Vittadinia* plants began to flower in October and the percentage of flowering plants and the number of inflorescences per flowering plant was analysed for November for each of the three species (Table 4.4). For percentage of plants flowering, Scalp + Herbicide plots had higher percentages than Control plots for each genus. They were also higher than Scalp Only plots for the two grasses. The number of inflorescences per flowering plant was also higher for Scalp + Herbicide plots than the Control for *Microlaena* and *Vittadinia*, and higher than Scalp Only plots for *Rytidosperma* and *Microlaena*. 
Figure 4.6: The mean a) percentage cover and b) number of individuals of seeded native grasses within 1 m x 1 m experimental plots on Glenthorne Farm. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Seeds of 4 Rytidosperma species and Microlaena stipoides were planted in June 2012. Percentage cover and number of individuals were recorded for each plot at the end of August, September, October and November 2012. One-way ANOVA was used to determine differences amongst treatments for each month (** = P < 0.01). Different letters under values within each month show significant differences between treatments calculated using Tukey post-hoc tests (P < 0.05). Error bars are ± s.e.
Figure 4.7: The mean a) percentage cover and b) number of individuals of seeded native forbs within 1 m x 1 m experimental plots on Glenthorne Farm. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Seeds of Vittadinia gracilis, Arthropodium strictum and Calostemma purpureum were planted in June 2012. Percentage cover and number of individuals were recorded for each plot at the end of August, September, October and November 2012. Kruskal-Wallis non-parametric tests were used to determine differences amongst treatments for each month (*** = P < 0.001; ** = P < 0.01). Different letters under boxplots within each month show significant differences between treatments (P < 0.05) using Dunn’s pairwise comparisons with Bonferroni adjustment.
Table 4.2: Results of one-way ANOVA comparing the percentage cover and the number of individuals of native grasses grown from seed in 1 m x 1 m experimental plots on Glenthorne Farm, South Australia. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Seeds of 4 *Rytidosperma* species (400 seeds) and *Microlaena stipoides* (50 seeds) were planted in June 2012 and data collected monthly from August to November 2012. Values are means ± standard error, different letters on each row indicate significant differences between treatments using Tukey post-hoc tests (P < 0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Month</th>
<th>F</th>
<th>df</th>
<th>Control (n=6)</th>
<th>Herbicide Only (n=6)</th>
<th>Scalp Only (n=24)</th>
<th>Scalp + Herbicide (n=24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rytidosperma</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spp.</td>
<td># of</td>
<td>Aug</td>
<td>4.491</td>
<td>3</td>
<td>20.5±5.7</td>
<td>32.3±7.0</td>
<td>47.3±4.9</td>
<td>54.6±4.7</td>
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<tr>
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<td>individuals</td>
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<td>3</td>
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<td></td>
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<td>3</td>
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<td>3</td>
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<td>57.1±3.6</td>
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<tr>
<td></td>
<td>% Cover</td>
<td>Aug</td>
<td>5.184</td>
<td>3</td>
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<tr>
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<td>4.402</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Nov</td>
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<td>3.03±0.87</td>
<td>3.36±0.32</td>
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<tr>
<td><em>Microlaena</em></td>
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<td>3</td>
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<tr>
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<td>3</td>
<td>18.7±3.2</td>
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<td>27.3±1.2</td>
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<td>3</td>
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<tr>
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<tr>
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**Table 4.3:** Results of Kruskal-Wallis non-parametric tests comparing the percentage cover and the number of individuals of three native forbs grown from seed in 1 m x 1 m experimental plots on Glenthorne Farm, South Australia. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Seeds of *Vittadinia gracilis* (50 seeds), *Arthropodium strictum* (50 seeds) and *Calostemma purpureum* (23 bulbs) were planted in June 2012 and data collected monthly from August to November 2012. Values are medians with interquartile range in brackets, different letters on each row indicate significant differences between treatments using Dunn’s pairwise comparisons with Bonferroni adjustment (P < 0.05).

<table>
<thead>
<tr>
<th>Species</th>
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<th>df</th>
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<td>6.944</td>
<td>3</td>
<td>0.0^a (0 - 1.75)</td>
<td>0.0^a (0 - 0.25)</td>
<td>2.0^a (0 - 6.75)</td>
<td>4.0^a (0 - 6)</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>12.174</td>
<td>3</td>
<td>0.0^a (0 - 0.5)</td>
<td>1.0^ab (0.75 - 4.25)</td>
<td>6.0^b (2 - 9)</td>
<td>3.0^b (1 - 10.5)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>12.584</td>
<td>3</td>
<td>0.0^ab (0 - 0.75)</td>
<td>1.0^ab (0 - 2.75)</td>
<td>5.5^b (2.25 - 8)</td>
<td>3.0^ab (1 - 10.25)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>13.593</td>
<td>3</td>
<td>0.0^a (0 - 0.75)</td>
<td>1.0^ab (0.75 - 2.75)</td>
<td>5.5^b (3 - 8)</td>
<td>3.5^b (2 - 9.75)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>% Cover</td>
<td>Aug</td>
<td>8.667</td>
<td>3</td>
<td>0.0^a (0 - 0.025)</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0.288)</td>
<td>0.2^a (0 - 0.288)</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>12.195</td>
<td>3</td>
<td>0.0^a (0 - 0)</td>
<td>0.1^ab (0 - 0.2)</td>
<td>0.25^b (0.1 - 0.4)</td>
<td>0.25^b (0.1 - 0.55)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>11.865</td>
<td>3</td>
<td>0.0^a (0 - 0.1)</td>
<td>0.0^ab (0 - 0.45)</td>
<td>0.3^b (0.2 - 1)</td>
<td>0.55^b (0.2 - 2.325)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>13.224</td>
<td>3</td>
<td>0.0^a (0 - 0.1)</td>
<td>0.15^ab (0.075 - 0.775)</td>
<td>0.65^a (0.4 - 1.25)</td>
<td>1.0^b (0.35 - 2.65)</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Arthropodium strictum</em></td>
<td># of individuals</td>
<td>Aug</td>
<td>15.334</td>
<td>3</td>
<td>0.5^a (0 - 4)</td>
<td>2.5^a (0 - 5.25)</td>
<td>12.5^b (5.25 - 17.75)</td>
<td>11.5^b (4.25 - 13)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>15.629</td>
<td>3</td>
<td>1.0^a (0 - 9)</td>
<td>3.0^a (0 - 6.75)</td>
<td>14.0^b (9.25 - 18.25)</td>
<td>9.5^b (5.25 - 12.75)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>9.931</td>
<td>3</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0.25)</td>
<td>1.0^a (0 - 3)</td>
<td>1.0^a (0 - 2)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>0.000</td>
<td>3</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>% Cover</td>
<td>Aug</td>
<td>12.225</td>
<td>3</td>
<td>0.0^a (0 - 0.05)</td>
<td>0.1^ab (0 - 0.125)</td>
<td>0.5^b (0.1 - 0.575)</td>
<td>0.3^ab (0.1 - 0.475)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>14.804</td>
<td>3</td>
<td>0.05^a (0 - 0.225)</td>
<td>0.1^a (0 - 0.225)</td>
<td>0.5^b (0.3 - 0.675)</td>
<td>0.35^ab (0.125 - 0.5)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>9.338</td>
<td>3</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>0.05^a (0 - 0.2)</td>
<td>0.075^a (0 - 0.1)</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>0.000</td>
<td>3</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>0.0^a (0 - 0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Species</td>
<td>Variable</td>
<td>Month</td>
<td>H</td>
<td>df</td>
<td>Control</td>
<td>Herbicide Only</td>
<td>Scalp Only</td>
<td>Scalp + Herbicide</td>
<td>P value</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------</td>
<td>-------</td>
<td>------</td>
<td>----</td>
<td>--------------</td>
<td>----------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>---------</td>
</tr>
<tr>
<td><em>Calostemma purpureum</em></td>
<td># of individuals</td>
<td>Aug</td>
<td>22.108</td>
<td>3</td>
<td>7.0^a (1.5 – 11.5)</td>
<td>7.5^ac (2.75 – 15.5)</td>
<td>19.0^b (19 – 23.25)</td>
<td>18.5^bc (17 – 22.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>21.475</td>
<td>3</td>
<td>3.0^a (0.75 – 9.25)</td>
<td>6.5^a (1.5 – 11.5)</td>
<td>17.0^b (14.5 – 19.75)</td>
<td>13.5^ab (10 – 16.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>21.435</td>
<td>3</td>
<td>0.5^a (0 – 2)</td>
<td>0.0^a (0 – 2.5)</td>
<td>8.0^b (6.25 – 11)</td>
<td>6.0^ab (2 – 8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>4.118</td>
<td>3</td>
<td>0.0^a (0 – 0)</td>
<td>0.0^a (0 – 0)</td>
<td>0.0^a (0 – 0)</td>
<td></td>
<td>0.249</td>
</tr>
<tr>
<td>% Cover</td>
<td></td>
<td>Aug</td>
<td>23.521</td>
<td>3</td>
<td>0.2^a (0.075 – 0.35)</td>
<td>0.35^a (0.1 – 0.65)</td>
<td>1.0^a (1 – 1)</td>
<td>0.95^b (0.825 – 1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sep</td>
<td>22.973</td>
<td>3</td>
<td>0.15^a (0 – 0.425)</td>
<td>0.3^a (0.075 – 0.5)</td>
<td>0.7^b (0.7 – 0.95)</td>
<td>0.5^ab (0.5 – 0.775)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oct</td>
<td>21.422</td>
<td>3</td>
<td>0.0^a (0 – 0.125)</td>
<td>0.0^a (0 – 0.125)</td>
<td>0.45^b (0.325 – 0.5)</td>
<td>0.3^ab (0.1 – 0.475)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nov</td>
<td>4.118</td>
<td>3</td>
<td>0.0^a (0 – 0)</td>
<td>0.0^a (0 – 0)</td>
<td>0.0^a (0 – 0)</td>
<td></td>
<td>0.249</td>
</tr>
</tbody>
</table>
Table 4.4: Results of Kruskal-Wallis non-parametric tests comparing the percentage of plants flowering and the number of inflorescences per flowering plant of three native species grown from seed in 1 m x 1 m experimental plots on Glenthorne Farm, South Australia. Existing vegetation was removed from the plots in May 2012 using four different methods; manual removal with a mattock (Control), glyphosate application (Herbicide), topsoil removal using a grader (Scalp) and topsoil removal followed by glyphosate application 3 weeks later (Scalp + Herbicide). Seeds were sown in June 2012 and the numbers of inflorescences per plant were counted in November 2012. Values are medians with interquartile range in brackets. Different letters on each row indicate significant differences between treatments using Dunn’s pairwise comparisons with Bonferroni adjustment (P < 0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>H</th>
<th>df</th>
<th>Control</th>
<th>Herbicide Only</th>
<th>Scalp Only</th>
<th>Scalp + Herbicide</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rytidosperma</em> spp.</td>
<td>% of plants flowering</td>
<td>22.870</td>
<td>3</td>
<td>21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 – 26)</td>
<td>(7.5 – 76)</td>
<td>(17 – 57)</td>
<td>(57 – 80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td># inflorescences per flowering plant</td>
<td>11.352</td>
<td>3</td>
<td>1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 – 2.6)</td>
<td>(0.75 – 2.57)</td>
<td>(1.6 – 2.4)</td>
<td>(2.3 – 3.1)</td>
<td></td>
</tr>
<tr>
<td><em>Microlaena stipoides</em></td>
<td>% of plants flowering</td>
<td>21.223</td>
<td>3</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 - 1.1)</td>
<td>(0 – 16)</td>
<td>(0 – 6.4)</td>
<td>(8.4 - 20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td># inflorescences per flowering plant</td>
<td>19.550</td>
<td>3</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 – 0.25)</td>
<td>(0 – 1.5)</td>
<td>(0 – 1.0)</td>
<td>(1 – 1.5)</td>
<td></td>
</tr>
<tr>
<td><em>Vittadinia gracilis</em></td>
<td>% of plants flowering</td>
<td>8.004</td>
<td>3</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 – 17)</td>
<td>(0 – 100)</td>
<td>(45 – 85)</td>
<td>(13 – 100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td># inflorescences per flowering plant</td>
<td>10.855</td>
<td>3</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0 – 0.25)</td>
<td>(0 – 8.85)</td>
<td>(1.13 – 4)</td>
<td>(0.75 – 11.2)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Effects of Treatments on Weeds

The treatments in this study did not have a significant effect on the total number of weed species or percentage cover of weeds per plot. In fact the Control plots generally had lower weed cover and more bare ground than the treatment plots, which opposes my original hypothesis that all of the treatments would reduce the weed cover compared to the Control. However, the treatments did affect the species composition of weeds, particularly the proportion of broadleaf weeds to annual grasses. The proportion of weed species in each plot that were graminoid (mostly annual grasses) compared to broadleaf was significantly higher in Control plots than both Scalp treatments in all months (except Scalp + Herbicide in November). The proportion of weed cover made up by graminoids was also significantly higher in Control plots than all treatments in August, both Scalp treatments in October and Scalp Only in November.

The dominant plants growing at the Glenthorne Farm site were annual grasses such as *Avena barbata* Pott ex Link (wild oats), *Lolium rigidum* Gaudin (annual ryegrass) and *Hordeum leporinum* Link (barley grass). It appears that removing the topsoil has greatly decreased the abundance of the annual grass weeds and their seeds, but these have been replaced by broadleaf weeds such as Salvation Jane (*Echium plantagineum* L.), clovers (*Trifolium* spp.), thistles (Asteraceae family) and soursobs (*Oxalis pes-caprae* L.). Some of these species (e.g. *E. plantagineum*) have a rosette growth form that has a large surface area on the soil compared with grasses, which may have contributed to the high percentage cover results on scalped plots. The seeds or bulbs of these species appear to have persisted in the subsoil seed bank and topsoil removal provided the disturbance necessary to expose the seeds and allow them to grow without competing against the normally dominant annual grasses. In comparison, Control plots were soon recolonised by annual grasses, probably from the soil seed bank and perhaps root stocks that were not removed during site preparation. Scalping may also have removed excess nitrogen, allelopaths, pathogens or mycorrhizae that promote the dominance of annual grasses, thus allowing broadleaf weeds to become dominant. The Herbicide Only plots had significantly different levels of graminoid cover to Control plots in August, suggesting
that the herbicide spray provided a short window of reduced annual grass dominance, probably due to the death of root stocks as well as aboveground biomass being removed. However the surface soil seed bank was still present and this enabled the annual grasses to re-establish by September when there were no significant differences in graminoid cover between treatments.

These results suggest that although topsoil removal may be effective in removing a significant proportion of weeds and their propagules and altering the dominant species, if there are any viable weed seeds left in the soil, weed cover can still be very high within a few months. In this case, the scalping may not have been to an appropriate depth to remove enough of the soil seed bank. The cracking clay soils found on Glenthorne Farm may allow seeds to quickly penetrate deep into the soil profile, meaning there are more viable seeds beneath the topsoil layer than may be found at other sites. It may be beneficial to do a detailed study of the soil seed bank to determine to which depth topsoil removal will be most effective, keeping in mind that the subsoil at certain depths may not be conducive to revegetation (Choi and Pavlovic 1998; Cole et al. 2005). The Scalp + Herbicide treatment was designed with this issue in mind as a way of removing some of this seed by allowing it to germinate and then spraying the resulting weed seedlings with herbicide to further exhaust the soil seed bank. Percentage weed cover was significantly lower on Scalp + Herbicide plots compared with Scalp Only plots in August and September, suggesting the herbicide application did improve weed control in the beginning. However, weed cover was not significantly different to the Control and no differences were detected between any treatments in October and November. There may not have been a long enough interval between scalping and spraying for a large number of seeds to germinate (only 3 weeks), or there were simply too many weed seeds still left in the soil for it to make much difference. Gibson-Roy (2010) had success using a similar method (scalp then spray one month later) in Victorian grasslands. He found weed growth was limited to <1 plant per square metre after four months. There is potential for this method to be effective, but depending on the site, it may be necessary to repeat herbicide application a number of times to reduce the accumulated seed bank to a manageable level.
Effects of Treatments on Native Species

Despite the lack of a reduction in weed cover on scalped plots, there was a significant improvement in native species establishment compared to Control plots, confirming my second hypothesis. The Scalp Only treatment was very effective at improving native species establishment, with increased native grass number, forb cover and forb number in all months. All of the native species showed higher numbers and cover in at least one of the study months when compared to the Control treatment. Scalp + Herbicide treatments had slightly better results again, with the number and cover of native grasses and native forbs higher in Scalp + Herbicide plots than Control plots in all months (except forb numbers in September). All of the native sown species except for Arthropodium showed increased cover and number for at least one of the sampling times. Scalp + Herbicide plots also had a higher percentage of native plants that were flowering and a greater number of inflorescences per flowering plant.

It is difficult to pinpoint why there was such a marked increase in the establishment of native species on scalped compared with un-scalped plots, when the cover of weeds was actually higher. It could be due to changes in the soil microenvironment that improved germination of the native species. The exposed clay subsoils may have retained more water or maintained different temperatures than the topsoil remaining on un-scalped plots. The germination of some Australian native forbs and grasses are known to require certain moisture levels and temperatures (Cook 2007; Maze et al. 1993; Willis and Groves 1991) which may have been more suitable in the scalped plots. For example, Rytidosperma caespitosum is known to require good soil moisture to germinate (Lenz and Facelli 2005; Maze et al. 1993), however over 135mm of rain fell in the two weeks following seed sowing at Glenthorne Farm (Bureau of Meteorology 2014a), so soil moisture was unlikely to be a limiting factor. Lenz and Facelli (2005) also found that R. caespitosum did not germinate when seeds were planted in stands of Avena barbata, even when water was added and A. barbata was clipped. This may be due to an accumulation of root exudates in the soil produced by this weed that inhibits the growth of other plants. Avena barbata is also common at Glenthorne Farm and it may be that scalping the soil removed not only weed seeds but also any inhibitory chemical compounds that had built up in the topsoil. Inhibitory allelochemicals have been found
in weed species present at Glenthorne Farm, or similar species, including *Avena fatua* L. (Fragasso et al. 2012), *Lolium rigidum* (Moore et al. 2010), *Phalaris aquatica* L. (Adams et al. 2010; Leigh et al. 1995) and *Vulpia* spp. (An et al. 2001). However, none of these studies tested the effects of the allelochemicals on native Australian species. This could be an added and as yet unexplored benefit of topsoil removal on native species establishment in weed infested areas.

The difference in native species establishment between scalped and un-scalped plots may also be due to the difference in the proportion of broadleaf versus annual grass weeds found between treatments. Annual grasses may be more competitive or more capable of inhibiting native herbaceous species than broadleaf weeds, so where the dominance had changed from grass weeds to broadleaf weeds, natives were able to grow larger or were more likely to survive. I was unable to find any studies in the literature that compared the competitive abilities of different functional groups of weeds on Australian native species, so this is another area that requires further study. There is evidence that the phenology of competing plants is important. Cleland et al. (2013) showed that planting native species with similar seasonality to common exotics, reduced invasion by exotic species. At Glenthorne Farm, the broadleaf weeds tended to grow and flower later in the season than the annual grasses and were more likely to match the phenology of the perennial native species which were planted. Thus the planted natives may have been more successful when competing with broadleaf species than with annual grasses that actively grow earlier in the season.

When the two Scalping treatments are compared, *Rytidosperma* had consistently higher results in the Scalp + Herbicide treatment, whilst the lilies (*Arthropodium* and *Calostemma*) were more abundant in the Scalp Only treatment. The increase in *Rytidosperma* establishment may have been due to the additional herbicide application which reduced the cover of weeds in August and September compared with the Scalp Only treatment. Thus the *Rytidosperma* seedlings had more space and resources to grow in the crucial early phase of development which allowed them to be more competitive once the weed cover increased in October and November. Both Firn et al. (2010) and Stevens and Fehmi (2011) found that having just a three week lag between native grass
germination and the germination of a competitive exotic grass, tipped the balance in favour of the native grasses.

Why the lilies survived longer in the Scalp Only treatment is not known. The Scalp Only treatment had the lowest percentage of bare ground cover in the first few months of the study so it could be that the lilies actually require some protection from physical elements as seedlings, which was provided by the weed vegetation. However, few of these liliaceous forbs survived beyond November, so establishment was not improved. Seeing as no lilies survived in a previous experiment at the same site (Chapter 3), it would appear that there is some biological, physical or chemical element at Glenthorne Farm that is not conducive to the survival of these species, and this was only partially alleviated on Scalp Only plots.

The percentage of native plants flowering and number of inflorescences per plant were also increased for *Rytidosperma*, *Microlaena* and *Vittadinia* in Scalp + Herbicide plots, although not in Scalp Only plots. This may also be due to the reduction in weeds in August and September which allowed the natives to accrue more resources and thus spend more on reproduction. Although the plots were not monitored for the second generation, Scalp + Herbicide plots did have the largest number of propagules produced and thus the greatest likelihood of the native population increasing in subsequent years.

CONCLUSION

On sites such as Glenthorne Farm, removing 150 years of agricultural alteration to re-establish native vegetation is no easy task. Weeds are a major hurdle to overcome and I hypothesised that topsoil removal would reduce weeds and therefore increase the establishment of natives. Despite only short-term reductions or increases in weed cover, there was significant improvement in the numbers of native plants establishing on scalped plots. This suggests that topsoil removal provides other advantages to native revegetation, perhaps by removing excess nutrients, allelopathic chemicals or soil pathogens that usually favour the dominant weed species. It may not be necessary (or possible) to completely eradicate weeds from heavily infested revegetation sites, but
scalping can alter the types of weeds and reduce competition, offering a more level playing field for native seedlings, especially when using direct seeding methods. Taking a little longer to exhaust more of the soil weed seed bank using herbicides or hand-pulling following scalping, may increase the benefits considerably. Repeated removal of weeds prior to them setting seed over several years may be required to reduce accumulated soil seed banks to levels that provide reduced competition to native plants. A greater reduction in weed seeds will allow more time for native seedlings to grow and access resources before having to compete with weeds. This should also reduce the amount of weed management required following planting and increase the chances of natives producing seed and increasing restored populations.
Chapter 5: Summary and recommendations for groundcover revegetation

My study aimed to explore a number of pre-planting treatments and methods to see whether they could be used to improve the control of weeds on retired farming land and aid in the establishment of native ground cover species. This information could then be used to create a list of guidelines for re-establishing endangered Grey Box grassy woodlands on Glenthorne Farm, as well as more generally for the revegetation of native herbaceous understorey to other cleared and weed invaded land.

SUMMARY OF TREATMENT RESULTS

Carbon Addition

In the literature, Carbon Addition has mainly been explored as a method to improve the natural reestablishment of native plants in old-fields (Kardol et al. 2008; Paschke et al. 2000), American prairies (Averett et al. 2004; Blumenthal et al. 2003; Kirkpatrick and Lubetkin 2011; Mitchell and Bakker 2011) and Australian weed invaded remnants (Prober et al. 2005). For Glenthorne Farm, where there has been no natural regeneration, Carbon Addition was combined with revegetation of native grasses and forbs by direct seeding. Glasshouse experimentation showed that Carbon Addition had the desired inhibitory effect on four common invasive annual grasses, with each species showing a decrease in biomass of over 60% after ten weeks when compared with control treatments (Chapter 2). This effect was also shown when tested in the field at Glenthorne Farm, with percentage weed cover and litter cover reduced, resulting in significantly higher levels of bare ground than the control plots throughout the 18 month study (Chapter 3). Although weed cover was reduced compared to the control treatment, weed cover was still relatively high at the end of the study with Carbon Addition plots having a median of 78% cover of weeds. This suggests that even with regular 3 monthly applications of sawdust and sugar, weeds remain an issue for restoration managers.
The increase in bare ground available for native plant germination in Carbon Addition plots did not result in an increase in native plant cover in this study. The reasons for this are not clear but could be due to changes in the soil such as a reduction in soil nitrate or an increase in osmotic potential that inhibited native seedling growth. As well as reducing the soil nitrate as desired, sugar application increases the osmotic potential of the soil, making it more difficult for roots to absorb water (Kirkpatrick and Lubetkin 2011), which may have impaired the survival of native seedlings by creating drought-like conditions. Also the added sawdust was slow to break down and may have smothered some of the emerging seedlings. Despite best efforts to distribute the sawdust evenly, there were often clumps up to 2 cm deep which could take months to break down depending on the weather conditions. The total number of *Rytidosperma* spp., *Arthropodium strictum* and *Austrostipa mollis* plants in Carbon Addition plots, however, was higher than the Control plots in October 2011, but they experienced much higher mortality rates, such that these plots had similar or lower total numbers of plants to the Control plots a year later in October 2012. Thus carbon addition appears to affect the survival of native plants, rather than germination. Based on these findings, carbon addition on its own does not substantially improve the establishment of native grasses and forbs, although applying different levels of carbon may provide different results.

The benefits of Carbon Addition may not pertain to young native seedlings, but only to mature perennial plants. Carbon addition is supposed to benefit native perennial species by providing a low nitrogen environment that gives them an advantage over exotic annuals that are strongly competitive in high resource environments due to their rapid resource uptake. Slower growing perennial species are more competitive in low nitrogen environments as they are able to recycle nutrients from their senescing tissues and do not rely as strongly on the availability of soil nutrients (Morton 1977; Prober *et al.* 2002b). However this strategy of recycling nutrients internally is not effective until the second growing season. During the first growing season, young perennial seedlings still rely on soil nutrients to grow, so will still experience strong competition from faster growing annuals in low nutrient environments (James *et al.* 2011). This may explain the lack of successful establishment of native groundcover species at Glenthorne Farm. Carbon addition may still be a useful tool for reducing weed growth and improving the
survival of mature perennial native species, but may not be suitable for revegetation by direct seeding.

**Topsoil Removal**

Topsoil removal was overall the most effective treatment for reducing weeds and promoting native species establishment. Even when tested in the glasshouse where the effect of the reduced soil seed bank was not a factor, three species of annual grass weeds showed a significant reduction in biomass when grown in subsoil compared with plants grown in topsoil (Chapter 2). This is likely to be due to a reduction in soil fertility in deeper soils compared with topsoils. However, care needs to be taken when considering topsoil removal that the subsoil still contains adequate nutrients and appropriate soil conditions for native species growth.

Removing the topsoil can inhibit weeds to some extent by removing excess nutrients (Allison and Ausden 2004; Perry et al. 2010; Tallowin and Smith 2001) but the main purpose of topsoil removal in this context, was to deplete the soil seed bank so that less weeds germinated. Chapter 3 showed that topsoil removal can significantly reduce the cover of weeds allowing more bare ground to be available in which native grasses and forbs can germinate. However, weeds were still prevalent with a median of 70% weed cover on Topsoil Removal plots in October 2012. Whilst this was significantly lower than the Control plots, it is arguably a lot higher than might be considered ideal for a successful restoration project. This issue was more evident in the second field study at Glenthorne Farm (Chapter 4) where weed cover was not significantly lower in Topsoil Removal plots than Control plots except in September 2012. Even when an application of herbicide was included to kill the first round of germinating weeds following topsoil removal, these results were not improved. Issues with the methodology may account for some of these results. There was large variation within treatments for some variables including percentage weed cover, so the study may have benefitted from having larger sample sizes to improve the power to detect significant differences between treatments. Another factor was the depth of topsoil that was removed. Around 5-10 cm of soil was removed but there were still high numbers of weed seeds germinating, suggesting that
at this field site, topsoil removal may need to be deeper. The differences between the
two field experiments, which used similar methods and were carried out at the same
site but in subsequent years, also give an indication of the annual variation. Differences
in rainfall timing, rainfall amounts and temperatures may have altered the germination
conditions between the two years. For example, although annual rainfall in both 2011
and 2012 was around 700 mm, the rainfall in June, immediately following planting, was
quite different between the two years. In 2011, planting in May was followed by a drier
than average June with 55.4 mm of rainfall (Fig. 5.1). In 2012, planting for the second
field experiment was followed by above average June rainfall of 179.8 mm. The very wet
and early winter in 2012 may have caused a mass germination event of certain weed
species, caused certain weeds to grow a lot larger or to germinate earlier than usual. If
the seeds of the affected species were stored in the subsoil seed bank, rather than in the
topsoil, this may explain the lack of weed cover reduction found in the scalping
treatments in Chapter 4 compared to Chapter 3.

![Graph showing monthly rainfall for 2011 and 2012](image)

**Figure 5.1:** Total monthly rainfall for 2011 and 2012 and the long term mean rainfall at Happy Valley
Reservoir (near Glenthorne Farm). Data from the Australian Bureau of Meteorology (www.bom.gov.au,
accessed 17/11/2014.)

The results for percentage weed cover also do not show that there was an effect on
some weed species, in particular the dominant annual grasses. This was shown in the
difference between the proportion of graminoid to broadleaf weed species, which was
reduced for topsoil removal treatments in both studies, and the proportion of graminoid
weed cover which was reduced in the second field study (Chapter 4). Unfortunately, the space left by the reduction in grasses, was taken up by broadleaf weeds, whose seed were likely lying dormant in the deeper soil, whilst the topsoil removal had removed a large proportion of the annual grass seeds. A number of broadleaf weed species have been shown to have persistent seed banks with seeds still able to germinate many years after burial (Burnside et al. 1996; Sheppard and Smyth 2002).

Despite these mixed results for the effectiveness of Topsoil Removal in controlling weeds, there was a significant improvement in the number and cover of native species in both field studies. In the Scalp + Herbicide plots (Chapter 4) the percentage of native plants flowering and the number of inflorescences produced per plant were also significantly higher, meaning this treatment was more likely to show an increase in native plant numbers in subsequent years through natural recruitment. Why the native species were able to establish more successfully in Topsoil Removal plots without significant reductions of weed cover is difficult to say but it may have to do with the change in dominance of certain groups of weeds. The normally dominant annual grass weeds may be more competitively inhibitive to the native seedlings than the broadleaf weeds that replaced them. Grasses have been found to be stronger competitors than forbs, particularly during emergence and early establishment (Goldberg et al. 2001; Gomez-Aparicio 2009; Pywell et al. 2003). Percentage cover of weeds is unlikely to represent the relative intensity of competitive interactions, particularly those occurring below ground. Native plants in Topsoil Removal plots may have experienced less competition to those in Control plots, despite an apparently similar level of weed cover. Other reasons why native plant establishment was improved could include; lower nutrient levels in the subsoil which reduced the competitiveness of the weeds, removal of allelopathic chemicals or pathogens which may have built up in the topsoil, or the germination conditions (e.g. soil temperature, light availability) may have been altered in a way that favoured the germination of the species planted. Although a lot more research is still required, topsoil removal, and in particular topsoil removal followed by herbicide application, shows great potential for broad scale revegetation applications. Some small adjustments in the methods such as altering the depth of topsoil removed
and the timing and number of herbicide applications could provide significant improvement in the control of weeds.

**Tree Canopies**

The presence of shade or mature trees did not inhibit exotic weeds or improve native species establishment. In the glasshouse, 75% shade did not affect the total or shoot biomasses of four annual grass weeds (Chapter 2). It did reduce the dry root biomass of two species, but the main effect was a significant increase in plant height for all of the species. This was also observed in the field study (Chapter 3) where the annual grasses under the shade cloths and under the tree canopies were tall and abundant and generally dominated the plots. The few native plants that were found were unable to reach the light due to the tall dense weed grasses and eventually died. Although other weed species may be inhibited by low light levels, these studies show that common agricultural weeds such as *Lolium rigidum*, *Bromus* spp., *Avena barbata*, *Hordeum leporinum* and *Vulpia bromoides* will grow well under shade in the short term.

When considering the effects of a tree on the surrounding understorey, there are many other factors besides shade to take into consideration. There are often increased nutrients in the soil under tree canopies (Belsky 1994; Belsky et al. 1993; Jackson and Ash 2001; Nichols 2005; Prober et al. 2002a) but there can also be thick layers of litter which prevent successful germination. Trees can also be strong competitors for water and nutrients due to their extensive root systems (Caldwell and Richards 1986). Trees have commonly been used to assist restoration efforts under the belief that they will compete successfully with exotic weeds whilst creating favourable conditions for native groundcover establishment (Nichols et al. 2010). However, the effect of trees is likely to be a lot more species specific than simply affecting weeds one way and natives another. The effect of trees can also vary from year to year and from site to site depending on water and nutrient availability (Belsky et al. 1993; Gea-Izquierdo et al. 2009), making their influence on understorey plants difficult to predict.

The experiments at Glenthorne Farm do not show the direct effects of shade or tree canopy on native groundcover species as very few natives were able to germinate and
survive, but this is likely due to the density of annual grass weeds growing on these plots. Other studies have shown that tree canopies can alter the species composition of groundcover plants, with some species being more prevalent in the open (e.g. C4 grasses such as *Themeda triandra*) and others more commonly found under trees (e.g. *Rytidosperma caespitosum, Microlaena stipoides*) (Gibbs *et al.* 1999, Prober *et al.* 2002). Therefore when revegetating with trees and herbaceous species, incorporating a range of species that prefer different growing conditions will make use of these different niches and strategically planting certain species in different areas according to their preferences where known, could improve the outcomes.

**Planting Density**

Planting density was another factor that was included in the design of the two field studies at Glenthorne Farm, however there were generally no significant differences between the two seeding densities (428 seeds/m² and 214 seeds/m²) in Chapter 3 and the grass only (450 seeds/m²) and grass and forb (573 seeds/m²) treatments in Chapter 4. The germination rate seems to have been low enough that increasing the seed density did not have a measurable effect on weed control or native plant growth. Although there were slightly more native plants germinating in the treatments with more seeds as would be expected, it was not proportional to the increase in seeds. Any future studies or revegetation efforts using these or similar native groundcover species would probably be more successful if higher seed densities were used due to the low germination rates. A greater understanding of germination requirements and seed viability of native species would also be valuable. Although not always practical, different species may respond better by being planted at different times of the year depending on their germination cues.

Due to the high levels of seed required for direct seeding and the low germination rates that often occur, direct seeding may not be the best method for establishing native groundcover. However, the other alternative of using individual seedlings can be very costly and difficult to produce the density of plants required. During this study, another method was trialled at Glenthorne Farm called seedling islands. The idea was to grow plants in the nursery to seedling stage, but instead of growing them in individual pots,
they were grown together in large trays and then the trays planted together as a slab, similar to the idea of rolling out pre-grown turf to create a lawn. Twenty mesh trays (68 cm x 42 cm x 7.6 cm deep) were lined with hessian and filled with potting soil. Ten seeds each from 11 native groundcover species were sown into each tray (Table 5.1) and watered regularly in a glasshouse for six months. In the spring the trays were transported to Glenthorne Farm and ten 70 cm x 90 cm holes 7.5 cm deep were prepared in the ground that could fit two trays each. With the help of the hessian lining and a wooden board, the entire contents of the trays were carefully transferred into the holes, making a seedling island. The seedling islands and a radius of around 1 m of surrounding soil were regularly weeded for 15 months.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microlaena stipoides</em></td>
<td>C3 Grass</td>
</tr>
<tr>
<td><em>Rytidosperma geniculata</em></td>
<td>C3 Grass</td>
</tr>
<tr>
<td><em>Rytidosperma setacea</em></td>
<td>C3 Grass</td>
</tr>
<tr>
<td><em>Rytidosperma pilosa</em></td>
<td>C3 Grass</td>
</tr>
<tr>
<td><em>Austrostipa mollis</em></td>
<td>C3 Grass</td>
</tr>
<tr>
<td><em>Lomandra multiflora ssp. dura</em></td>
<td>Rush</td>
</tr>
<tr>
<td><em>Dianella revoluta</em></td>
<td>Lily</td>
</tr>
<tr>
<td><em>Arthropodium strictum</em></td>
<td>Lily</td>
</tr>
<tr>
<td><em>Burchardia umbellata</em></td>
<td>Lily</td>
</tr>
<tr>
<td><em>Vittadinia gracilis</em></td>
<td>Daisy</td>
</tr>
<tr>
<td><em>Kennedia prostrata</em></td>
<td>Legume</td>
</tr>
</tbody>
</table>

Table 5.1: A list of the Australian native species planted in trays in the nursery to create seedling islands.

Not all species germinated but at the time of planting in September 2011, there was on average 45 plants per seedling island (2 trays planted together, 0.571m²; Table 5.2). These were mainly the grasses (particularly *Rytidosperma* spp., which were unable to be identified to species level) as well as *Arthropodium strictum* and *Vittadinia gracilis*. As in both field trials, for some reason the *A. strictum* seedlings did not survive past October. However the average cover of the native plants had increased by 66% in December 2011 (Table 5.2) and almost all of the plants were flowering or setting seed. One year later,
many new natives were germinating in the weeded area around the islands from the seed dropped the previous season. On average there were 16.2 mature plants and 123 new seedlings per seedling island in December 2012. Of these, 97.2% of the mature plants were producing seed and 27.3% of the new seedlings had inflorescences present. Growing the plants in a nursery appeared to increase the size of the plants and number of seeds produced by *Rytidosperma* spp. and *V. gracilis* compared to those planted by direct seeding in the field trials (pers. obs.). The large number of seeds and new seedlings produced show there is great potential for the seedling islands to continue to grow in diameter each year. Regular weeding is likely to be necessary to maintain these results if weeds are still prevalent, but only small areas need to be done at a time. The potting soil transferred with the seedlings provides a relatively weed free base so that it is mainly the perimeter that requires weed removal. Having the plants well established and at high density before planting should also increase their resistance to weed incursion (Carter and Blair 2012). Even a year after weed removal had ceased, native plants in the seedling islands were observed to be surviving at Glenthorne Farm.

Table 5.2: The average plant cover and average number of native plants in each seedling island (n=10) at the time of planting at Glenthorne Farm (Sept 2011), at the end of the first growing season (Dec 2011) and the end of the second growing season (Dec 2012). Plants were grown in 0.286 m² trays in a nursery for six months prior to planting, with two trays planted together to make one seedling island, giving an area of 0.571 m². Ten seeds each of eleven native herbaceous species were sown per tray, the species with living individuals at the time of counting are shown in the last column.

<table>
<thead>
<tr>
<th>Date</th>
<th>Cover (m²)</th>
<th># Plants</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 2011</td>
<td>0.216</td>
<td>45</td>
<td><em>Rytidosperma</em> spp., <em>A. strictum</em>, <em>M. stipoides</em>, <em>A. mollis</em>, <em>V. gracilis</em></td>
</tr>
<tr>
<td>Dec 2011</td>
<td>0.360</td>
<td>31.8</td>
<td><em>Rytidosperma</em> spp., <em>M. stipoides</em>, <em>V. gracilis</em></td>
</tr>
<tr>
<td>Dec 2012</td>
<td>0.456</td>
<td>16.2 + 123 seedlings</td>
<td><em>Rytidosperma</em> spp., <em>M. stipoides</em>, <em>V. gracilis</em></td>
</tr>
</tbody>
</table>

Although in this trial, the number of native species that germinated was not improved by planting in the nursery, with more information on germination requirements and further trials, this method may become useful for propagating those species that are difficult to establish. It would also be beneficial to use this seedling island technique
where only small amounts of native seed are available. Direct seeding may only be effective for certain species with high seed availability and high germination rates. Seedling islands may provide an alternative method to establish other species with limited seed availability, more specific germination requirements or that are more vulnerable in the early stages of growing, thus greatly increasing the biodiversity of revegetation projects.

**Study Limitations**

As with most studies, there was a compromise between the scientifically ideal methods and what time and resources would allow, making some limitations on data interpretation. The experimental plots in Chapters 3 and 4 were only one metre squared or less due to limits in the amount of native seed available and for monitoring efficiency. It is difficult to know to what extent these results can then be extrapolated to much broader scale revegetation projects. Weed control may be more effective when done on a larger scale as the incursion of seeds and vegetative growth around the edges by neighbouring weeds would be reduced. But the application of weed control methods such as carbon addition and scalping may be less efficient over a broader area and the inherent patchiness of the landscape may make results more variable. The variation across the field site was larger than expected and having larger sample sizes may have improved the power of the data analysis to find treatment effects. Although the study site at Glenthorne Farm was fenced to exclude large herbivores, one hare was observed within the fenceline and some evidence of grazing was found within study plots.

**RECOMMENDATIONS**

The aim of this thesis was to test and explore a range of methods that could help improve weed control and increase the establishment success of native groundcover revegetation on previously farmed land. The studies described in this thesis have provided some insights into the potential uses and limitations of these methods. The
following is a list of some recommendations and suggestions for future native groundcover revegetation efforts.

- Assessment of the site that is to be revegetated is important, making sure factors such as soil type, climate, weed species, previous farming practices, soil fertility, previous native vegetation and any current native vegetation are taken into consideration when planning revegetation projects.
- A combination of methods is likely to be required. For example, topsoil removal can be combined with herbicide spraying to remove more weeds from the soil seed bank, or direct seeding can be combined with seedling islands to increase native species diversity.
- Depending on the size of the site and the availability of resources, starting weed control and revegetation in one section, and then expanding the area in subsequent years may improve success rates. This could be particularly useful if native seed is not available in large quantities as seed can be collected from the revegetation areas for use on the rest of the site. If funding is limited, weed control should be restricted to a smaller area that can be treated and planted effectively. This will provide a more efficient use of resources rather than poorly controlling weeds over a larger area resulting in low rates of successful native establishment.
- For effective weed control on highly weed dominated agricultural land, topsoil should be removed to a suitable depth, then apply herbicide (or burn or slash where appropriate) to kill any weeds germinating from the remaining soil seed bank. For the best results, weed control should be continued for at least a year following topsoil removal and before planting, to ensure the weed seed bank is exhausted. The extra time spent removing weeds prior to planting will be a much more efficient use of resources than trying to remove the weeds once natives are present.
- After extensive weed control on a site, it makes sense to plant the overstorey and understorey at the same time. Planting the understorey species at the same time will help prevent further weed incursions by providing more ground cover. Despite previous beliefs, there is no good evidence that planting the overstorey will promote the establishment of the understorey in Australian woodlands (Munro et al. 2009; Nichols 2005; Nichols et al. 2010) and the experiments at Glenthorne Farm indicate that an established overstorey and shading do not inhibit common weed species (Chapter 3).
- Plant a high diversity of native species including plants that grow well under tree canopies and those that prefer open areas to make use of the different niches that will develop.

- Carbon addition can be used to improve the competitive ability of native perennials where annual weeds are taking advantage of nitrogen enriched soils. However, it is best used once the native plants have matured.

- Planting native species that are able to maintain low nitrogen feedbacks may also be useful for maintaining low nitrogen soils in the long term (e.g. *Themeda triandra*, Prober et al. 2005).

- For sites like Glenthorne Farm where broadleaf weeds are likely to become a problem following topsoil removal, planting only native grasses in the first year and continuing to spray with a broadleaf herbicide may be a solution. Native forbs can then be planted once the broadleaf weeds are under control.

- Planting native species that have plenty of available seed, germinate easily and grow quickly to establish native cover immediately after weed control may help to reduce initial weed incursion. Other species, particularly those that are rare or difficult to grow, can be added later or in smaller numbers as seed, seedlings or seedling islands.

**CONCLUSION**

This thesis has explored the following factors that may limit the success of native groundcover establishment at Glenthorne Farm and how they may be mitigated;

1) Shading or other impacts of planted overstorey trees,

2) Competition by introduced exotic weed species,

3) Increased soil nutrients, in particular nitrogen, which could favour the growth of weed species over native species,

4) Low germination or high mortality rates of native seedlings.

Shading can increase the dominance of some weed species, particularly annual grasses, and did not improve the establishment of native species at Glenthorne Farm. If trees are established first, then establishing a native understorey may be more difficult as shading and increased fertility under trees can promote dense growth of some annual grass weeds. Weeds are very abundant at Glenthorne Farm, including in the soil seed bank.
Topsoil removal was able to improve the establishment of native groundcover species even when weed cover was not significantly reduced. Removing a deeper layer of topsoil and following up with herbicide applications until the soil seed bank is almost exhausted is likely to improve weed control and therefore increase the success of native species establishment and reduce ongoing weed management. Carbon addition reduced soil nitrate and weed cover moderately, but did not improve the establishment of native seedlings. This method may be more effective once planted natives are more mature to help keep weeds under control.

This study has shown that groundcover revegetation can be difficult with many factors to consider, issues to address and limited research to guide us. However re-establishing native groundcover is becoming increasingly important as more ecological communities like Grey Box grassy woodlands become threatened. As revegetation practitioners have become more capable and confident with our tree and shrub revegetation, it is time to concentrate on the herbaceous understorey so that we can create more complete, functional and complex native ecosystems that will hopefully be more resilient to future disturbances and provide better quality habitat for native fauna.
### APPENDIX 1

**Table 6.1**: List of all identified weed species found on study plots at Glenthorne Farm. 272 plots, each 75 x 75 cm, were monitored eight times each between August 2011 and October 2012, making 2176 Plot Counts. This table shows the percentage of plot counts where each species was present, and the percentage of plot counts where the species was one of the two most dominant weed species in the plot at that time (determined by greatest % cover).

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
<th>% Plot Counts - Present</th>
<th>% Plot Counts - Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lolium rigidum</em></td>
<td>Annual grass</td>
<td>68.7</td>
<td>42.96</td>
</tr>
<tr>
<td><em>Phalaris aquatica</em></td>
<td>Perennial grass</td>
<td>32.4</td>
<td>24.79</td>
</tr>
<tr>
<td><em>Avena barbata</em></td>
<td>Annual grass</td>
<td>31.8</td>
<td>11.66</td>
</tr>
<tr>
<td><em>Lythrum hyssopifolia</em></td>
<td>Broadleaf</td>
<td>29.3</td>
<td>8.40</td>
</tr>
<tr>
<td><em>Vulpia bromoides</em></td>
<td>Annual grass</td>
<td>28.9</td>
<td>8.56</td>
</tr>
<tr>
<td><em>Bromus hordeaceus</em></td>
<td>Annual grass</td>
<td>27.1</td>
<td>4.36</td>
</tr>
<tr>
<td><em>Oxalis pes-caprae</em></td>
<td>Broadleaf</td>
<td>22.8</td>
<td>8.46</td>
</tr>
<tr>
<td><em>Echium plantagineum</em></td>
<td>Broadleaf</td>
<td>21.7</td>
<td>8.72</td>
</tr>
<tr>
<td><em>Sonchus oleraceus</em></td>
<td>Broadleaf</td>
<td>20.5</td>
<td>5.67</td>
</tr>
<tr>
<td><em>Rumex obtusifolius</em></td>
<td>Broadleaf</td>
<td>19.1</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Juncus bufonius</em></td>
<td>Rush/Sedge</td>
<td>15.7</td>
<td>1.68</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>Broadleaf</td>
<td>13.7</td>
<td>10.77</td>
</tr>
<tr>
<td><em>Bromus diandrus</em></td>
<td>Annual grass</td>
<td>13.1</td>
<td>3.20</td>
</tr>
<tr>
<td><em>Lactuca serriola</em></td>
<td>Broadleaf</td>
<td>13.1</td>
<td>4.83</td>
</tr>
<tr>
<td><em>Conyza bonariensis</em></td>
<td>Broadleaf</td>
<td>12.8</td>
<td>5.57</td>
</tr>
<tr>
<td><em>Panicum capillare</em></td>
<td>Annual grass</td>
<td>12.1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Oxalis corniculata</em></td>
<td>Broadleaf</td>
<td>10.3</td>
<td>2.15</td>
</tr>
<tr>
<td><em>Trifolium subterraneum</em></td>
<td>Broadleaf</td>
<td>9.8</td>
<td>2.89</td>
</tr>
<tr>
<td><em>Poa annua</em></td>
<td>Annual grass</td>
<td>8.1</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Kickxia elatine ssp. crinita</em></td>
<td>Broadleaf</td>
<td>7.4</td>
<td>2.84</td>
</tr>
<tr>
<td><em>Polygonum aviculare</em></td>
<td>Broadleaf</td>
<td>6.9</td>
<td>1.63</td>
</tr>
<tr>
<td><em>Hordeum leporinum</em></td>
<td>Annual grass</td>
<td>5.3</td>
<td>0.58</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>Perennial grass</td>
<td>5.3</td>
<td>3.26</td>
</tr>
<tr>
<td><em>Bromus alopecuros</em></td>
<td>Annual grass</td>
<td>4.1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Hirschfeldia incana</em></td>
<td>Broadleaf</td>
<td>3.6</td>
<td>2.63</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>Broadleaf</td>
<td>3.3</td>
<td>0.21</td>
</tr>
<tr>
<td><em>Bromus sp.</em></td>
<td>Annual grass</td>
<td>3.2</td>
<td>1.31</td>
</tr>
<tr>
<td><em>Arctotheca calendula</em></td>
<td>Broadleaf</td>
<td>2.9</td>
<td>0.53</td>
</tr>
<tr>
<td><em>Trifolium campestre</em></td>
<td>Broadleaf</td>
<td>2.5</td>
<td>0.63</td>
</tr>
<tr>
<td><em>Cerastium glomeratum</em></td>
<td>Broadleaf</td>
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<td>0.53</td>
</tr>
<tr>
<td><em>Medicago polymorpha</em></td>
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</tr>
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<td><em>Vicia sativa ssp sativa</em></td>
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<td>0.26</td>
</tr>
<tr>
<td><em>Malva linnaei</em></td>
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<td>1.6</td>
<td>0.89</td>
</tr>
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<td>Species</td>
<td>Category</td>
<td>ECI</td>
<td>LPI</td>
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<tr>
<td>------------------------------</td>
<td>-------------------</td>
<td>------</td>
<td>------</td>
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<td><em>Hypochoeris radicata</em></td>
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<td>0.05</td>
</tr>
<tr>
<td><em>Helminthotheca echioides</em></td>
<td>Broadleaf</td>
<td>0.7</td>
<td>0.05</td>
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<tr>
<td><em>Paspalum dilatatum</em></td>
<td>Perennial grass</td>
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<td>4.94</td>
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<tr>
<td><em>Vaccaria hispanica</em></td>
<td>Broadleaf</td>
<td>0.3</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Isolepis setacea</em></td>
<td>Rush/Sedge</td>
<td>0.3</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Silybum marianum</em></td>
<td>Broadleaf</td>
<td>0.3</td>
<td>0.05</td>
</tr>
<tr>
<td><em>Pseudognaphalium luteoalbum</em></td>
<td>Broadleaf</td>
<td>0.2</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Trifolium arvense</em></td>
<td>Broadleaf</td>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Briza minor</em></td>
<td>Annual grass</td>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Crassula sp.</em></td>
<td>Broadleaf</td>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Sonchus asper</em></td>
<td>Broadleaf</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>
## Appendix 2

**Table 6.2:** List of all identified weed species found on study plots at Glenthorne Farm. There were 96 1 m x 1 m plots and each was monitored four times (Aug, Sep, Oct and Nov 2012) making 384 Plot Counts. The plots were split into four pre-planting weed control treatments; a control, herbicide spray only, topsoil removal (Scalp Only) and topsoil removal followed by herbicide spray 3 weeks later (Scalp+Herbicide). Values are the percentage of Plot Counts where each species was present for each treatment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant Type</th>
<th>Control</th>
<th>Herbicide Only</th>
<th>Scalp Only</th>
<th>Scalp + Herbicide</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena barbata</em></td>
<td>Annual grass</td>
<td>81.3</td>
<td>22.9</td>
<td>9.7</td>
<td>19.4</td>
</tr>
<tr>
<td><em>Briza minor</em></td>
<td>Annual grass</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Bromus alopecuus</em></td>
<td>Annual grass</td>
<td>2.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Bromus diandrus</em></td>
<td>Annual grass</td>
<td>14.6</td>
<td>29.2</td>
<td>3.5</td>
<td>9.7</td>
</tr>
<tr>
<td><em>Bromus hordeaceus</em></td>
<td>Annual grass</td>
<td>41.7</td>
<td>6.3</td>
<td>6.3</td>
<td>4.9</td>
</tr>
<tr>
<td><em>Bromus sp.</em></td>
<td>Annual grass</td>
<td>16.7</td>
<td>20.8</td>
<td>13.9</td>
<td>11.8</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>Perennial grass</td>
<td>4.2</td>
<td>0.0</td>
<td>2.1</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Hordeum leporinum</em></td>
<td>Annual grass</td>
<td>35.4</td>
<td>10.4</td>
<td>1.4</td>
<td>6.3</td>
</tr>
<tr>
<td><em>Isolepis setacea</em></td>
<td>Rush/Sedge</td>
<td>2.1</td>
<td>2.1</td>
<td>2.8</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Juncus bufonius</em></td>
<td>Rush/Sedge</td>
<td>37.5</td>
<td>41.7</td>
<td>49.3</td>
<td>50.7</td>
</tr>
<tr>
<td><em>Lolium rigidum</em></td>
<td>Annual grass</td>
<td>64.6</td>
<td>45.8</td>
<td>38.2</td>
<td>22.9</td>
</tr>
<tr>
<td><em>Panicum capillare</em></td>
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<td>18.8</td>
<td>43.8</td>
<td>12.5</td>
<td>41.7</td>
</tr>
<tr>
<td><em>Phalaris aquatica</em></td>
<td>Perennial grass</td>
<td>2.1</td>
<td>0.0</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Poa annua</em></td>
<td>Annual grass</td>
<td>16.7</td>
<td>10.4</td>
<td>22.9</td>
<td>22.9</td>
</tr>
<tr>
<td><em>Vulpia bromoides</em></td>
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<td>12.5</td>
<td>8.3</td>
<td>3.5</td>
<td>9.7</td>
</tr>
<tr>
<td><em>Arctotheca calendula</em></td>
<td>Broadleaf</td>
<td>2.1</td>
<td>0.0</td>
<td>39.6</td>
<td>13.2</td>
</tr>
<tr>
<td><em>Cerastium glomeratum</em></td>
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<td>0.0</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Ceniza bonariensis</em></td>
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<td>0.0</td>
<td>8.3</td>
<td>7.6</td>
<td>14.6</td>
</tr>
<tr>
<td><em>Crossula sp.</em></td>
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<td>0.0</td>
<td>2.8</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Echium plantagineum</em></td>
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<td>10.4</td>
<td>18.8</td>
<td>76.4</td>
<td>65.3</td>
</tr>
<tr>
<td><em>Helminthotheca echoides</em></td>
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<td>2.1</td>
<td>6.9</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Hirschfeldia incana</em></td>
<td>Broadleaf</td>
<td>2.1</td>
<td>4.2</td>
<td>0.7</td>
<td>6.3</td>
</tr>
<tr>
<td><em>Kickxia elatine ssp. crinita</em></td>
<td>Broadleaf</td>
<td>4.2</td>
<td>29.2</td>
<td>9.0</td>
<td>33.3</td>
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<tr>
<td><em>Lactuca serriola</em></td>
<td>Broadleaf</td>
<td>14.6</td>
<td>45.8</td>
<td>22.2</td>
<td>24.3</td>
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<tr>
<td><em>Lythrum hyssopifolia</em></td>
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<td>31.3</td>
<td>37.5</td>
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<tr>
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<td>33.3</td>
<td>28.5</td>
<td>38.2</td>
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<td>0.0</td>
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<td>Type</td>
<td>Percent 1</td>
<td>Percent 2</td>
<td>Percent 3</td>
<td>Percent 4</td>
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<td>-----------</td>
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<td>Pseudognaphalium luteoalbum</td>
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<td>0.0</td>
<td>2.1</td>
<td>4.9</td>
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<td>29.2</td>
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<tr>
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</tr>
<tr>
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<td>2.1</td>
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<tr>
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<tr>
<td>Vaccaria hispanica</td>
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<td>2.1</td>
<td>4.2</td>
<td>19.4</td>
<td>20.1</td>
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