Comparison of methods for establishing native grasses in pastures dominated by annual weeds

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I. ABSTRACT

Native grasses have potential to improve temperate pastures where introduced perennial grasses are not surviving. They are generally well-adapted to Australia's conditions including low-fertility or acidic soils, sporadic rainfall and high summer temperatures. However, they are difficult to establish from seed because of slow seedling development and vulnerability to competition from weeds, especially fast-growing annuals. Native grass practitioners in the Mount Lofty Ranges of South Australia have successfully established native grasses but their methods were poorly documented. For this thesis, 12 practitioners were interviewed and their native grass establishment methods and the problems surrounding these were documented. From the interviews and a review of the literature, a test of concept area and two field trials were established. The test of concept area was used to determine which species to use in the trials and how and when to sow them. Four native Wallaby grasses (*Rytidosperma* spp. Steud), Kangaroo grass (*Themeda triandra* Forsk.) and Weeping rice grass (*Microlaena stipoides* (Labill.) R. Br. and *Microlaena stipoides* var. Burra) established most successfully. Weed control was least time-consuming when the grasses were sown in rows rather than randomly distributed. Management was also simplified by separating C3 and C4 grasses. Soil solarisation with polyethylene and other plastics was also tested and it was found that solarisation can control annual weeds and seed found in the top 50 mm of soil. The first field trial was at Mylor, SA. It compared 7 weed control methods to determine which method created the most bare ground; an indication for a potential establishment window for native grasses. These methods were: removal of 50 mm of topsoil; soil solarization; soil inversion; till and harrow; herbicide; burning and harrowing. It was found that soil solarisation with polyethylene and topsoil removal were the most effective treatments with about 75% (± 3%) bare ground. There was least bare ground with burning (23% ± 4%) and herbicide (28% ± 4%). Till/harrow, harrow only and topsoil inversion ranged from 46-55% (± 3%) bare

ground. There was no bare ground in the control. Since polyethylene is not recyclable in South Australia, a trial comparing the effectiveness of polyethylene and a fully biodegradable plastic was conducted in the Waite Arboretum, SA. The treatments included no treatment, tillage only and tillage with polyethylene of biodegradable plastic. All treatments except the control were sprayed with herbicide. The biofilm remained intact for 27 days. During this time, the mean daily temperature under the polyethylene (41.7 ± 0.4 °C) was always higher than under the biofilm (39.8 ± 0.3 °C). Both were hotter than the tilled treatment (34.1 ± 0.3 °C) and the control (33.9 ± 0.3 °C). Despite the higher temperature no measurable treatment effect could be detected by the end of the experiment but sown native grasses established well in all treatments with 30-50% native grass cover and very little weed. The lack of treatment effect was likely due to the small plot size, the use of herbicides to control some weeds and high seed bank variability within treatments.

In summary, soil seed bank management is critical to successful native grass establishment. Topsoil removal and soil solarization with low density polyethylene were the most successful weed management methods. Other methods may need 2-3 years of treatment before sowing native grasses which increases the risk of soil erosion and may degrade the soil structure. Sowing the grasses in rows made weed management easier and sowing them thickly provided maximum weed competition. The cost and availability of native grass seed will be a significant barrier to the adoption of native grasses for pasture applications but on-farm seed production areas are one solution to this problem.

II. 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 had 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.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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CHAPTER 1. Introduction and literature review

1.1 Introduction

Australia's extensive temperate grasslands and grassy woodlands were developed for stock grazing from early colonisation (Garden *et al.* 1996; Whalley *et al.* 2005; Whalley *et al.* 1978). There are conflicting views on the original extent of these grassy ecosystems (Gibson-Roy 2018; Hyde 1995) but the maps produced by Lunt *et al.* (1998) provide a good summary (Reseigh *et al.* 2008) (Fig. 1).

Most of these grasslands and grassy woodlands were altered by fertilisers, clovers and grasses from other countries to increase grazing production; known as "pasture improvement" (Firn 2007a; Reed 2014; Whalley *et al.* 2005). Today, Australia has over 35 million ha of improved pasture (ABS 2017). Information on the area under pasture in temperate Australia is scarce and data were not available for New South Wales, South Australia or Tasmania (ABS 2006) but in 2006/7, Victoria had 7.4 million ha under pasture.

By the 1980s pasture grasses from other countries, here called introduced grasses, were not persisting on marginal lands (Archer *et al.* 1993; Kemp and Dowling 1991; Lodge 1994). Marginal lands are used for grazing but are not suited to cultivation because of, for example, low fertility, slope, rocks and/or shallow soils (Whalley *et al.* 2005). This caused a renewed interest in native grasses (Firn 2007b; Garden *et al.* 1996); those that evolved *in situ* or arrived without human assistance (Richardson *et al.* 2000).

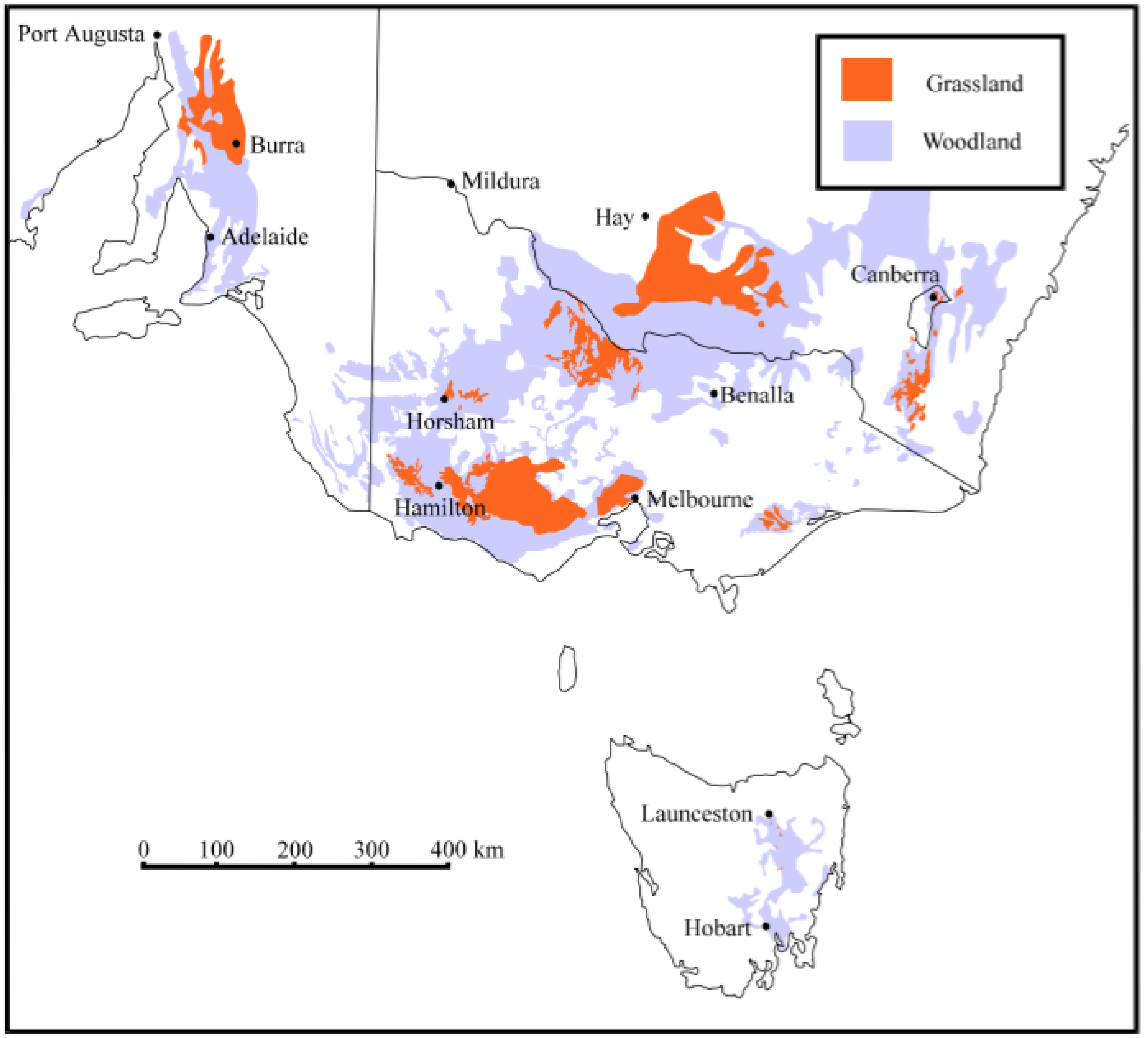

Figure 1. Temperate lowland grasslands and grassy woodland distribution in south-eastern Australia pre-colonisation as proposed by Lunt *et al.* (1998).

For grazing, native grasses were widely considered to be inferior to pasture grasses from other countries (Donald 1970; Whalley *et al.* 1978). However, cultivars and accessions with good persistence and palatability have been identified for the various climatic zones of temperate Australia (Sanford *et al.* 2005; Whalley *et al.* 2005). South Australia has about 440 species of native grass (Jessop *et al.* 2006). Some of these are suitable for grazing because they have good persistence (Waters *et al.* 2005), protein content and palatability (Foster *et al.* 2009). A range of Wallaby grasses (*Rytidosperma* spp.) and Weeping rice grass cultivars (*Microlaena* spp.) have also been selectively bred for pasture production and successfully commercialised (Whalley *et al.* 2005).

Establishing native grasses from seed in a pasture setting is difficult because they have low seedling vigour, making them vulnerable to competition from fast growing annual grasses and broadleaf weeds (Lodge 2000; Semple *et al.* 1999). Control of competition prior to and during establishment is thought to be key for establishment (Lodge 2000; Semple *et al.* 1999). However, establishment methods in degraded pastures are poorly understood and not well documented for pastures in a Mediterranean climate in Australia.

This literature review gives a brief history of pasture improvement with grasses, clovers and fertilisers in temperate Australia. It explains the early prejudices against native grasses and recent research suggesting that native grasses can be persistent and productive and have potential to improve pastures where introduced perennial grasses are failing. Weed control methods used in grassland restoration, horticulture and agriculture are discussed. This leads to the identification of research gaps and project aims for this Masters by Research.

1.2 Pasture improvement in southern Australia

1.2.1 A brief history of pasture improvement

Southern Australia once had extensive temperate grassland and grassy woodlands. These have been largely degraded or destroyed by a combination of overgrazing, the addition of nutrients, cropping and the introduction of grasses, legumes and weeds from other countries (Firn 2007a; Garden *et al.* 1996; Reseigh *et al.* 2008; Whalley *et al.* 2005) . Whalley *et al.* (2005) quote Gardner (1854), who wrote:

…sheep ‘pretty nearly swept the grasses clean out of sight, in the space of 20 years from many a luxuriant and lovely spot’.

A full history of Australian stock grazing is beyond the scope of this review but some of the key events that impacted grassy ecosystems are summarised below.

The antiquity and low fertility of Australian soils underpinned the actions taken to improve stock grazing (Reed 2014). Most of the continent is less than 300 m in elevation (Orians and Milewski 2007). The lack of elevation and nutrient recycling through erosion and/or mountain building events has led to old, highly-weathered soils (Orians and Milewski 2007; Taylor 1983). Concentrations of soil phosphorus (P), iodine (I), cobalt (Co) and selenium (Se) are particularly low compared to other countries (Orians and Milewski 2007). To counter this, superphosphate was used to increase pasture and wheat productivity in Australia from the late 1800s (Cook and Dias 2006). Government subsidies were introduced to increase Australia’s agricultural production for the British Empire and to support an expected exponential rise in population (Cook and Dias 2006).

As superphosphate use was rising, in the early 1900s, Amos Howard of Mount Barker, South Australia discovered the benefits of subterranean clover (*Trifolium subterraneum*) for temperate pastures (Radcliffe 2017). Clovers can fix nitrogen in symbiosis with *Rhizobium* bacteria thus increasing nitrogen input to the soil and to grazing stock (Cook 1951). Amos sold the seed widely (Radcliffe 2017). Increased soil fertility from the combination of superphosphate plus nitrogen fixation (known as “sub and super”) meant that more areas were suitable for sowing introduced grasses to increase pasture production (Cook 1951). Today, in

Australia, over 29 million ha have been sown with subterranean clover (Nichols 2017). Table 1 gives a summary of the key milestones in the sub and super pasture revolution in southern Australia.

Table 1. Selected key pasture improvement milestones from southern Australia.

YEAR	EVENT	AUTHOR
1880s	Botanic gardens and gardening societies are introducing exotic grasses	Cook and Dias (2006)
1918	1.5 million ha of sown pasture	Wilson (1968)
1929 onwards	Commonwealth Plant Introduction Scheme – an alliance of state and federal industries and universities. 2250 grass species from 53,278 accessions were introduced to Australia between 1924-2000.	Cook and Dias (2006); Garden *et al.* (1996)
1939	Australia importing 680,000 t of phosphate rock from Nauru	Reed (2014)
Early 1950s	85% of Australian exports are from primary production. Improved pasture area exceeds native pasture.	Reed (2014)
1965	19 million ha of sown pasture in Australia	Wilson (1968)
1975	Government superphosphate subsidy removed	Kemp and Dowling (1991)
2017	Over 29 million ha of southern Australia sown with subterranean clover	Nichols (2017)

By the 1950s Australian pasture production from native species was overtaken by production from introduced species (Reed 2014). There was a general prejudice against using native grasses for pasture improvement because they were thought not to respond positively to P fertilization (Firn 2007a). This was based on limited and poorly designed studies (Whalley *et al.* 2005) and is explained in more detail in section 1.3.

Pasture improvement was supported by the discipline of "agrostology", which emerged in the UK in the 1830s as the science of cultivated grasses as distinct from agronomy which was the science of cultivated grains (Reed 2014). Agrostologists were recruited to find pasture grasses that, combined with superphosphate and subclover, could further boost pasture production (Reed 2014). They studied ways to correct soil nutrient deficiencies and focused on finding the right species for optimal grazing production (Cook and Dias 2006).

Australian agrostologists extensively searched for and studied new pasture grasses through the Commonwealth Plant Introduction Scheme; an alliance of Universities, industry, state and federal government agencies (Cook and Dias 2006). By the 1940s ryegrass (*Lolium* sp. L.), cocksfoot (*Dactylis* sp.L.), phalaris (*Phalaris* spp. L.), Paspalum spp.. L., lucerne (*Medicago sativa* L.) and a range of clovers (*Trifolium* spp. L.) were recommended for extensive grazing (Cook 1951). As Australian climatologists gained a better understanding of Australia's climate, areas of the globe with similar climates were targeted for plant collection (Reed 2014). The Mediterranean was especially suited to southeastern Australian conditions (Reed 2014). Introductions to Australia included 2250 grass species from 53,278 accessions between 1924 and 2000 through the Commonwealth Plant Introduction Scheme (Cook and Dias 2006).

The introduction of the Plant Breeders Rights Acts of 1987 and 1994 and changing Government priorities have meant that the development of new varieties is now usually driven by seed companies (Reed 2014). Government and University grass research tends to focus on genomics and biotechnology, for example to improve drought tolerance and disease resistance (Reed 2014).

1.2.2 Problems with improved pastures

Pastures improved with superphosphate, clovers and introduced grasses increased pastoral production from about 1 sheep per acre on native Rytidosperma sp. pasture to 5 sheep per acre by the 1950s (Stephens and Donald 1959). By the late 1970s however, researchers, graziers and agronomists were noticing that perennial grasses were not persisting and pasture productivity was decreasing (Archer *et al.* 1993; Kemp and Dowling 1991; Whalley *et al.* 2005). In 1993, Archer *et al.* reviewed the evidence to date and concluded that pastures in high rainfall areas (>600 mm average annual rainfall or > 400 mm in Mediterranean climates) “have declined to the extent that botanical composition and production of many pastures is far from a desirable optimum”.

When the perennial grass component of pastures declines, pastures become more vulnerable to weed invasion (Kemp and Dowling 1991). Deeper rooted perennials are often replaced with cool season species, especially introduced annuals and legumes (Whalley *et al.* 2005; Wilson and Simpson 1994). When ground cover is low, annuals germinate and grow quickly with the opening rains, complete their lifecycle and then die through heat and moisture stress in summer (Mitchell *et al.* 2015). This leaves the soil vulnerable to erosion over summer (Mitchell *et al.* 2015). If deep-rooted grasses are lost, salinity can also become a problem if the water table is rises (Dear and Ewing 2008; Kemp and Dowling 1991).

Native grass researchers and promoters suggest that in some conditions the grasses introduced to Australia are not persistent (Reseigh *et al.* 2008; Whalley *et al.* 2005). These conditions usually include sloping sites, shallow soils, soil acidity, low summer rainfall and salinity. Such conditions are found over thousands of hectares of temperate Australia. However, most of the research cited is from workshops and conferences. A search for the peer-reviewed evidence behind the claims has led only to the studies discussed in the following two paragraphs. These studies appear to have underpinned most of the native grass research of the past 20 years.

Johnston (1996) is widely cited in the literature for stating that introduced cultivars were not suited for marginal, sloping lands with unpredictable rainfall and low fertility. No evidence was provided. Kemp and Dowling (1991) studied the botanical composition of pastures improved with introduced perennial grasses over a 95 x 85 km area in central New South Wales covering rainfall from 600 to 1000 mm. Perennial grasses were not found at 20% of the previously improved sites. They represented a higher proportion than annuals only when average annual rainfall was above 900 mm. The annual grass component averaged 36% and was therefore higher than the sown introduced perennials, which averaged just 21% of the pasture composition.

Reeve *et al.* (2000) surveyed 544 livestock producers from 9 high rainfall regions in New South Wales, Victoria, South Australia and Tasmania. The survey suggested that sown species became less productive or disappeared after 6 -10 years. Possible reasons for the decline were thought to be dry seasons, not enough fertiliser, soil acidity and weeds.

In summary, since early settlement, pastures have been improved with fertilisers, grasses and legumes from other countries. This strategy appears to have failed over large areas of marginal lands. While grazing management has also been identified as a key to pasture composition and sustainability it is beyond the scope of the current review. Section 1.3 investigates whether native grasses have the potential to ameliorate these pastures.

1.3 Improvement of degraded pastures by sowing native grasses?

1.3.1 Prejudices against native grasses

Until the 1980s many agronomists and researchers believed that native grasses did not have potential to improve pastures. Many were influenced by Donald (1970) from the Waite Institute, who stated:

> All evidence indicates that our native plants have neither actual nor potential value as artificial sown species….They suffer a serious disability – that they are incapable of high production, or response to high levels of fertility.

He cites a single study of legumes by Begg 1963 to support this claim.

Since the strong emphasis of the time was on increasing pasture biomass, and hence animal production, native grasses were largely passed over in favor of introduced species (Lazenby and Swain 1969). Just 20 of 601 papers published in five pasture-related journals between 1960 and 1970 concerned native grassland species (i.e. in the Papilionaceae and/ or Gramineae families) (Whalley 1970). Lazenby and Swain (1969) decried native pastures because they had few herbs and legumes and because the quality and quantity of feed varied considerably with rainfall and seasonal conditions. Paradoxically, they also recognised that introduced species may not be persistent, especially at low water availability. They stated that in Mediterranean climates perennials do not survive summer drought and pasture improvement should be based on annual species.

Fertiliser subsidies were removed in 1975 (Kemp and Dowling 2000). This combined with drought in the 1980s led many graziers to reconsider the advantages of native grasses as low-input pastures became more economical (Lodge 1996). Native grasses often survived harsh conditions and didn't need high fertiliser inputs (Kemp and Dowling 2000; Lodge 1994; Lodge 1996). Renewed interest in native grasslands from the mining, roads and conservation sectors have also given weight to the calls for research in this area (Lodge 1996).

The early prejudices against native grasses have now been reexamined and errors or biases have been identified (Firn 2007a; Jones 1996; Robinson and Archer 1988). Until the 1990s, the production levels of temperate native grasses and introduced grasses were not assessed on a "like for like" basis (Jones 1996). Established native grasses were usually compared with newly sown and fertilised introduced grasses

(Robinson and Archer 1988). To show how native grasses might have been disadvantaged by this comparison, Jones (1996) compared fertilised native grasses (*Anthosachne scabra* (R. Br.) Nevski*,* *Microlaena stipoides* and *Themeda triandra*) with unfertilised introduced grasses (*Phalaris aquatica* and *Paspalum dilatatum* Poir.). The native grasses *Anthosachne scabra* and *M. stipoide*s had 30% and 27% crude protein whereas the two introduced grasses which had only 8%.

Robinson and Archer (1988) compared the herbage production of selected native grasses with two popular introduced grasses; *Phalaris aquatica* L. and *Festuca arandinacea Schreb.* in sown pure swards that received similar fertilisation and irrigation. They found that the native *Rytidosperma bipartitum* (Link) A. M. Humphries & H.P. Linder had similar relative growth rates (RGR) to the introduced species when averaged over a year. Although it had lower growth rates in cooler months they were higher in warmer months. Over the year, the RGR of native *Poa sieberana* Spreng. was 6% higher than the two introduced species. *Microlaena* had high spring growth rates. *Themeda triandra* had very high summer growth rates when compared to the introduced species.

1.3.2 Potential benefits of native grasses in pastures

The potential benefits of native grasses were well known in the early 1900s. In “The Grasses and Fodder Plants of N.S.W.”, Ernest Breakwell, an agrostologist for the NSW Department of Agriculture defined grasses as “good or bad” for grazing regardless of whether they were native or introduced (Breakwell 1923). Breakwell suggested that *Danthonia* (now known as *Rytidosperma*) and some other native species were “good” because they were persistent under reasonable stocking rates, palatable, nutritious and resistant to extremes of local conditions like drought and frost.

As outlined in Section 1.2, pasture improvement has already significantly altered the composition of our native grasslands and grassy woodlands. It seems unlikely that this process can feasibly be reversed given that there have been changes to soil nutrient status, as well as increased acidity, salinity and erosion (Whalley 1970). Just replacing introduced grasses with natives without addressing these changes may not be successful (Jones 1996). However, using grasses with desirable traits such as drought tolerance, persistent year-round ground cover, and year-long flowering may improve pasture sustainability (Jones 1996; Mitchell *et al.* 2015; Whalley *et al.* 2005).

The decline in the composition and persistence of some introduced pastures combined with an awareness of the potential impacts of global warming on pasture productivity have led researchers, agencies and graziers away from a strict focus on biomass-per-hectare (Firn 2007a; Mitchell *et al.* 2015; Whalley *et al.* 2005). There is a new emphasis on low fertiliser inputs and sustainable swards, with deep-rooted perennials that include both summer and winter active grasses that can maximise water use and year-round soil protection (Mitchell *et al.* 2015; Whalley *et al.* 2005). This is particularly the case for the higher rainfall pastures (above 400mm in a Mediterranean climate) with lower capability for agricultural production. This is land not suited to cultivation but suitable for grazing (Lodge 1994; Whalley *et al.* 2005). Here, native grasses have the potential to provide persistent, productive and palatable grasses for sustainable grazing (Lodge 1994; Sanford *et al.* 2005; Whalley *et al.* 2005).

Climate change is predicted to affect Australia's pastures negatively (Stokes and Howden 2010). A full discussion of this complex topic is beyond the scope of this review, but a summary of important impacts follows. In the southeast of Australia, average annual temperature may increase by 1.5-5 °C by 2100 depending on the emissions scenario (CSIRO 2017). April to October rainfall has already decreased by about 11% since the mid-1990s and winter rainfall will decrease from 2-32% depending on emissions

(CSIRO 2017). Droughts may become more common and last longer (CSIRO 2017) and evaporation rates may increase due to higher temperatures (Crimp *et al.* 2010).

In Mediterranean climates, the growing season may become shorter, with less available soil moisture (Henry *et al.* 2012). An analysis for the Murray-Darling Basin showed that pasture biomass may decline by 8-40% in the drier western regions by 2030 through a combination of lower rainfall and higher evaporation (Crimp *et al.* 2010). Changes to the local climate may also mean that pasture weeds from warmer areas can expand their range into the Murray-Darling-Basin (Crimp *et al.* 2010). Annual pastures might grow only in winter and spring (Mitchell *et al.* 2015). Therefore, it could become more difficult to maintain stocking rates that maximise production and protect the grazing sward for the future (Crimp *et al.* 2010).

The potential benefits of native grasses are summarised in Table 2. It shows that native grasses are climate adapted through evolution in Australian conditions and may be able to survive where exotic species fail. Some species are known to provide good quality fodder and many of these will respond positively to increased fertility. Many researchers have called for the use of native grasses in pasture amelioration (Garden *et al.* 1996; Lodge 1994; Whalley *et al.* 2005) and for further research and development, including plant breeding, to allow Australia's grasses to play an important role in future climate adaptation (Mitchell *et al.* 2015).

Table 2. Potential benefits of native grasses for pasture amelioration and climate adaptation.

Benefits of native grasses	Description	Author
Increased production with increased fertility	Some native grasses increase production with the addition of fertiliser	Jones (1996); Robinson and Archer (1988)
Year-long fodder production	Rytidosperma species grow more slowly in spring but will grow over summer if soil moisture is adequate	Robinson and Archer (1988)
Facultative seeders and sprouters. These include: *Microlaena, Bothriochloa, Dicanthium, Eragrostis, Anthoschne,* and *Rytidosperma.*	Many exotic grasses, especially annuals, have determinate growth and flowering. Many native grasses are facultative seeders and/or sprouters that grow and set seed whenever soil moisture and other conditions are suitable.	Lodge and Whalley (1981); Volaire and Norton (2006)
Evolutionary adaptations to moisture stress and erratic rainfall	When soil moisture is low, many native grasses become dormant and then revive with rainfall	Mitchell *et al.* (2015)
Adaptation to low nutrient conditions	Native grasses are well suited to extensive, low input/low output grazing. This system can have benefits to graziers when introduced species are not persistent and/or fertilisation is not profitable.	Lodge (1994)
Graziers can lower fertiliser inputs without losing perennial grasses		Whalley *et al.* (2005)
Tolerance of harsh soil conditions including acidity and salinity		Doronila *et al.* (2014); Lodge (1996)

1.3.3 Native grasses for improvement of low-input pastures

Once the potential benefits of native grasses for pasture improvement were recognised, native grass research increased. Some of the key research milestones are summarised in Table 3. It outlines the growing interest and involvement with native grasses of a range of bodies including livestock associations, universities, government agencies and commercial entities. A more detailed explanation of key research for Mediterranean climates follows.

Table 3. Some of the key research milestones for native grasses.

Year	Event	Reference
1932	First native grass cultivar, *Danthonia richardsonni*, released by the Waite Institute.	Reed (2014); Cook and Dias (2006)
1986	Wool Research & Development Fund: Project to domesticate valuable native grasses and first native grass conference.	Lodge and Peterson 1987 in Whalley *et al.* (2005)
1987	Plant Varieties Rights legislation allows breeders to make royalties	Lodge (1996)
1996	Native and Low-Input Grasses Network (NLIGN) forms to co-ordinate research on grasses suited to land where introduced grasses were not persistent. Trials were conducted from 1998-2001 at 8 sites in southern Australia.	Whalley *et al.* (2005)
1999	Start of Low Input Grasses in Limiting Environments (LIGULE) project. Aim: to find native grasses useful for 'recharge control in the Murray-Darling Basin'	Johnston *et al.* (1999)
Early 2000s	Domestication of some valuable *Rytidosperma* and *Microlaena* ecotypes	Mitchell (2007)
2008	Rural Solutions SA publishes a Strategy For Broadacre Adoption of Native Grasses in South Australia	Reseigh *et al.* (2008)
2015	Call by CSIRO, Department of Primary Industries, Victoria and University of New England for the domestication of native plants, including grasses for climate change adaptation in pastoral areas	Mitchell *et al.* (2015)

Most native grass pasture research has been conducted in New South Wales and/or Victoria where rainfall patterns differ from those in South Australia and summer rainfall is more likely. An exception was the Native and Low-input Grasses Network (NLIGN), funded by Meat and Livestock Australia Ltd. It was a consortium of primary industry/agricultural research agencies from New South Wales, Victoria, Tasmania, South Australia, and Western Australia and included the University of New England in New South Wales (Norton *et al.* 2005). The project established a “Mediterranean zone” with trial sites at Flaxley in the Adelaide Hills and at Kendenup in south-west WA from 1998-2001.

The purpose of the NLIGN research was to evaluate perennial grasses that were suitable for pastures on land classes IV, V and VI where fertiliser input would be low and nitrogen would be supplied by a legume (Norton *et al.* 2005). The study was unique for including both native and introduced species. Grasses were assessed for palatability, persistence and recruitment.

Sanford *et al.* (2005) summarised the results and reported on the superior lines. In the Mediterranean zone, natives had lower herbage production than the introduced grasses tested but higher survival and recruitment rates. They also showed very good growth over summer when green feed is commonly low in Mediterranean climates. It is important to note that horticultural methods were used to establish the grasses (i.e. tube stock and weed mat). No legumes were included and trials were not grazed (Norton *et al.* 2005). The authors stress that results might have been different under these conditions (Norton *et al.* 2005).

1.3.4 Barriers to adoption of native grasses for pastures

Barriers to using native grasses include the price, quality and availability of seed (Cuneo *et al.* 2018; Dear and Ewing 2008; Reseigh *et al.* 2008). Some native grasses are poor seed producers and seed dormancy also poses problems because it can reduce germination rates (Cole and Johnston 2006; Lodge and Whalley 1981). This makes native grass seed very expensive and difficult for broadacre adoption (Reseigh *et al.* 2008). Native grass seed tends to be used mainly in industries like mining or roadside restoration where budgets are often much larger than for pasture renovation (Mitchell *et al.* 2015).

Other challenges of working with largely undomesticated plants include seed shattering, sequential ripening, fluffy seed structures and long awns (Cole and Johnston 2006; Mitchell *et al.* 2015). With sequential ripening and seed shattering, seeds drop from the grass as they ripen, making a single harvest difficult (Shapter *et al.* 2013). Fluffy seed structures and long awns prevent the use of conventional seeders and harvesters because the seed doesn't move smoothly through the machine (Reseigh *et al.* 2008). These features may contribute to their ability to survive in a harsh environment (Lodge and Whalley 1981; Mitchell *et al.* 2015) but they make it difficult to use them in conventional agriculture (Reseigh *et al.* 2008).

This Masters research project will address the following research gaps:
Methods for establishing native grasses in degraded pastures are not well understood and further research is needed if they are to compete with introduced grasses (Firn 2007a; Garden *et al.* 1996; Reseigh *et al.* 2008; Whalley *et al.* 2005). A key problem is the low seedling vigour of native grasses, making them easily out-competed by faster growing weeds (Cole *et al.* 2017; Semple *et al.* 1999). This is discussed in more detail in section 1.4.

1.4 The soil seed bank

Native grasses have low seedling vigour and are easily out-competed by weeds, especially fast-growing annuals (section 1.4). Competition for light, water and nutrients at the seedling stage is most detrimental to their survival (Barrett and Wilson 1981). Therefore, their establishment depends on sufficient control of weed growth, which often means control of the soil seed bank to prevent later re-establishment (Lodge

2000; Morris and Gibson-Roy 2017; Semple *et al.* 1999). This section outlines the main weeds of annual pastures and describes the soil seed bank.

1.4.1 Weeds in annual pastures

As outlined in chapter 1.2, when introduced perennial grasses fail, pastures can become dominated by annual grasses and legumes, especially clover (Kemp and Dowling 1991). Little is known about the composition of pastures in high rainfall areas (Wilson and Simpson 1994), especially those in South Australia.

A survey of an 95 x 85 km area of the central tablelands of New South Wales, identified subterranean clover as the most common legume, with white clover more common above 700 mm rainfall (Kemp and Dowling 1991). On average legumes comprised 42% of the pastures. Annual grasses averaged 36% of the pasture; most abundant were brome (*Bromus diandrus* Roth.), vulpia (*Vulpia bromoides* (L.) Gray & *V. myuros* (L.) C.C.Gmel. and annual rye grass (*Lolium rigidum* Gaudin). Degraded native grasslands can be invaded by these grasses as well as by *Avena* spp. L. (e.g. wild oats) and *Hordeum* spp. L. (barley grasses) (Prober *et al.* 2004).

On former agricultural land in Western Australia, annual grasses were dominant and wild oats (*Avena barbata*) was particularly persistent (Standish *et al.* 2008). Annual weeds that germinate and grow before native grasses emerge provided the most competition for native vegetation (Cole and Johnston 2006).

1.4.2 The soil seed bank

The soil seed bank is the “ungerminated but viable seed that lies in the soil” (Park and Allaby 2017) and some authors also include seeds in the litter layer (Cole *et al.* 2016; Standish *et al.* 2008). It is comprised of seed that has fallen from the standing vegetation (Scott and Morgan 2012) and seed that has arrived via other means such as animals and wind (Standish *et al.* 2007). Some seeds have self-burying awns (Sindel *et al.* 1993; Smith *et al.* 1999) , while others may be buried by stock trampling and burrowing animals. The composition of soil seed banks varies with seasons, locations and with extreme events like fire and floods (Yates *et al.* 1994).

Some seeds are dormant for a period to protect them against germination when conditions are not ideal (Flematti *et al.* 2015). For example, the dormancy period can vary from 8-9 weeks for annual rye grass (*Lolium rigidum*) to up to 12 months for Kangaroo grass (Sindel *et al.* 1993). Many grassland species are thought to have low seed persistence in the soil (Morgan 1998). Conversely, *Austrostipa compressa* ((R. Br) S.W.L. Jacobs & J. Everett) is a "fire ephemeral" that grows and sets seed with fire and then is outcompeted by other vegetation. Seed lies dormant in the soil until the next fire (Smith *et al.* 1999). In Western Australia, the soil seed bank still contained over 100 seeds m^{-2} of *Austrostipa compressa* 45 years after the last fire the (Smith *et al.* 1999).

There can also be other types of propagules in the soil, for example bulbs and buds. Some authors have extended the definition and called it the "seed/bud bank" (Morris and Gibson-Roy 2017; Morris and De Barse 2013). For the purposes of this paper, the term "soil seed bank" includes seeds and other propagules.

Little is known about the soil seed bank in annual pastures and abandoned agricultural land (Scott and Morgan 2012). Temperate Australian grassland studies have shown that soil seed banks are species poor and dominated by introduced annual species – mostly monocots and legumes (Lunt 1990; Morgan 1998; Morgan 2001; Standish *et al.* 2007) - even when the standing vegetation is dominated by natives (Fisher *et* *al.* 2009). These findings may be biased by methodology (Lunt 1990; Plue *et al.* 2017). For instance, seed banks are often studied by growing them out in a greenhouse (known as the seedling emergence method) (BGoSA 2016; Lunt 1990; Plue *et al.* 2017). In a Swedish grassland study comparing greenhouse methods with man-made disturbance gaps in grasslands, Plue *et al.* (2017) found biases related to the methodology used. Twenty-four species emerged in the greenhouse that were not found in the disturbance gaps and conversely, 28 species from the disturbance gaps were absent in the greenhouse test. In general, the greenhouse method encouraged more seeds to germinate. This is probably because soil moisture is not limiting and conditions are more stable, but it was not suitable for some plants with special germination requirements. Another reason for the difference in results between methodologies was the significant difference in the volume of soil sampled for the greenhouse study versus the disturbance gaps. The disturbance gaps had 10 times the soil volume.

The soil seed bank of a long-grazed native *Themeda triandra* grassland near Melbourne was dominated by three introduced annual grasses and forbs (*Vulpia bromoides*, *Romulea rosea* L. Eckl., *Aira cupaniana* Guss.) comprising 81% of germinants (Lunt 1990). *Vulpia bromoides* was most abundant (61% of the seed bank). A study at different soil depths of a former grazing property near Mount Bold in the Mount Lofty Ranges, showed that 78% of seedlings were from the top 50 mm of soil and 19% were from 50-100 mm (BGoSA 2016). In total, 97% of seedlings came from the top 100 mm of soil. In general, more dicotyledons than monocotyledons emerged.

1.4.3 Annual grasses in the seed bank

Weed control methods useful in agriculture, horticulture and restoration either remove, control or rely on competition with the soil seed bank. It is thought that annual grasses have a relatively short persistence in the seed bank (Hashem 2018; Peltzer 2017; Peltzer 2018). Table 4 summarises the seed dormancy, longevity and control methods for three common annual grasses. It shows that a high percentage of seed germinates in the season after the seed falls and that the seed bank of these annual grasses is not very persistent; about 2-3 years. Legumes (Bell *et al.* 1993) and annual broadleaf weeds have a more persistent seed bank (Prober *et al.* 2004).

Combining weed management methods (known as integrated weed management) to maximise weed removal is often recommended (Peltzer 2017). For example, a mass germination of weeds can be triggered by fire and smoke and then killed with herbicide to provide an establishment window for the desired species (Dixon *et al.* 2009).

Weed control methods that might be useful for pasture renovation are discussed in the following section.

1.5 Methods for establishing sown native grasses

Slow seedling growth has been a considerable barrier to the use of native grasses in agriculture and pasture establishment from seed is poorly understood (Firn 2007a; Norton *et al.* 2005; Semple *et al.* 1999). Due to their low seedling vigour, native grasses are easily out-competed by introduced grasses and weeds, especially fast growing annuals emerging from the litter and soil seed bank (Cole and Johnston 2006; Mitchell *et al.* 2015; Semple *et al.* 1999). Annual grasses and broadleaf weeds germinate and grow quickly and are more competitive for resources such as light and moisture than native grass seedlings (Cole *et al.* 2017).

This section summarises native grass establishment research for grazing and for grassland restoration (due to the sparse research in native grass establishment from seed in old pastures). It also explores other weed control methods used in agriculture, horticulture and grassy ecosystem restoration that may be useful in pasture renovations.

Table 4. Common introduced annual grasses; seed dormancy, longevity and control strategies.

Botanical name	Common name	Longevity in soil seed bank	Seed emergence	Prevention/control	Source
Bromus diandrus	Great brome/Ripgut brome	6-24 months	85-90% of seed germinates in the autumn after seed fall if rainfall is adequate	Prevent seed fall and delay crop planting until after germination of *Bromus*	(Hashem 2018)
Lolium rigidum	Annual ryegrass	Less than 1% carryover from season to season	Winter to spring emergence related to rainfall	Burning residues, inversion ploughing to bury seed 100 mm deep, shallow harrow in autumn, herbicides, preventing seed set	(Peltzer 2017)
Avena fatua		75% of seed is depleted within 12 months of seed fall. Seedbank can be depleted within 3-5 years.	40% emerge with opening rains. 10-30 % emerge later with adequate rainfall. Most emerge from top 5-7.5 mm of soil	Shallow harrowing in autumn, herbicides, preventing seed set. Winter fallow and summer crop.	(Peltzer 2018)

1.5.1 Methods for pasture renovation

Native grasses have potential to improve degraded pastures on land of low productive capacity in temperate Australia (chapter 1.3), however establishment methods are poorly understood (Firn 2007a; Lodge 2000; Semple *et al.* 1999). Bare ground is needed to create a germination window for native species (Gibson-Roy *et al.* 2010) because they tend to be slow-growing above ground in the early stages of development (Dear and Ewing 2008; Lodge 2000; Reed *et al.* 2008).

Semple *et al.* (1999) conducted six sowing experiments from 1993-1995 in central west New South Wales that compared the emergence and establishment from seed of both native and exotic pasture grasses. Seedbed preparations were also tested. Establishment of cool season native grasses was largely unsuccessful due to weed competition and low soil moisture over summer. Summer-active native grasses were more successful and five recommendations were made for their establishment. These were:

1. select a low fertility site (this was not defined);
2. good control of the soil weed bank – at least 18 months' control was recommended but methods were not outlined;
3. high sowing rates to help suppress weeds e.g. 200 seeds per metre of sowing row;
4. a tilled seed bed, and;
5. above average rainfall in November and January (in NSW).

Lodge (2000) tested the growth rate of two *Rytidosperma* species (*R. richardsonii* and *R. linkii*) against subterranean clover (*Trifolium subterraneum* var *brachycalycinum* cv. Clare) and annual ryegrass (*Lolium rigidum* cv. Wimmera) in a pot experiment. Both annual ryegrass and subterranean clover competed strongly with *Rytidosperma* because of their more rapid growth rate. They recommended controlling these species for 1-2 years before sowing *Rytidosperma* species but did not explain how this could be done effectively (Lodge 2000).

Since methods for establishing native grasses in pastures are not well understood, the methods used in grassland or grassy woodland restoration are reviewed in the section that follows.

1.5.2 Methods for grassland restoration

According to Nicholas and Marshall (2015), today grasslands are "one of the most studied ecosystems in Australia" and there is a good understanding of the barriers and challenges to restoration. Restoration involves taking actions that lead to the recovery of an ecosystem that has been altered in a negative way (SERA 2017). The degree of restoration needed depends on the level of site degradation and the desired outcome (Prober *et al.* 2005). There is a continuum of intervention from simply removing threatening processes (e.g. weed invasions) to full plant community restoration (SERA 2017).

Fertilised soils often favour introduced grasses (Cole *et al.* 2016). Organic carbon addition to the soil has been investigated for its ability to reduce soil available N and P (Jonasson *et al.* 1996; Morris and De Barse 2013; Prober *et al.* 2005) so that weeds cannot grow as strongly. Soil microorganisms have a higher N and P requirement per unit biomass than plants and can compete with plants for it (Jonasson *et al.* 1996). The addition of organic carbon to the soil can increase microbial growth, and therefore N and P use. This increases competition with plants as the microbes scavenge the soil for N (Jonasson *et al.* 1996) in a process known as net immobilization (Harte and Kinzig 1993). A potential flaw of the studies reviewed below is that once the added organic carbon is depleted, the microorganisms will die and release nutrients back into the soil (Fig. 2 from Harte and Kinzig (1993)).

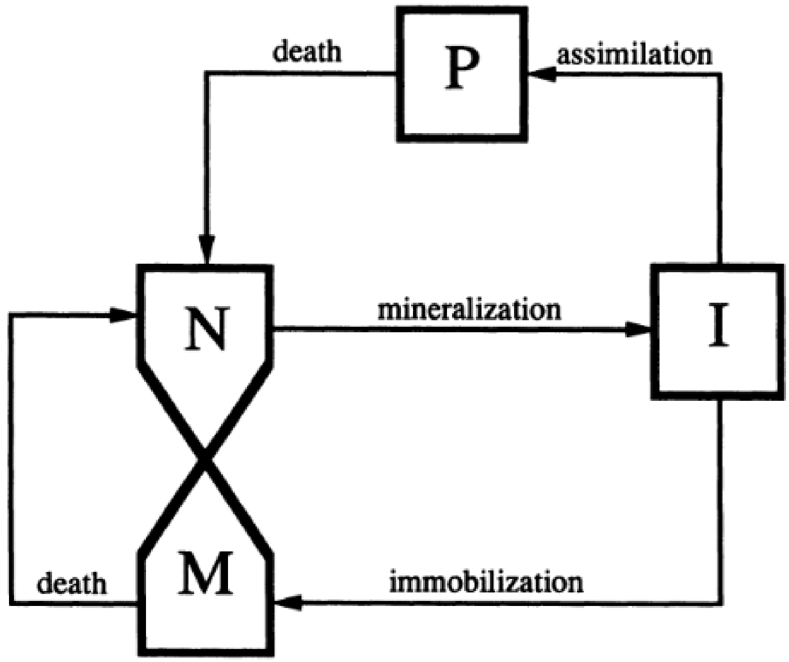

Figure 2. The flow of nutrients among plants (P), microorganisms (M), dead organic matter (N) and inorganic nutrient (I). Mineralization is the conversion of organic nutrients into inorganic, plant available nutrients. From Harte and Kinzig (1993).

In an experiment to test the effect of organic carbon addition on biomass production, Jonasson *et al.* (1996) found that 500 g sugar m^{-2} added to a Swedish grass-shrubland soil reduced the soil extractable N and P by 3-4 fold after one growing season. Herb and grass biomass production fell from a mean of 114 g m^{-2} to 43 g m^{-2}.

In two remnant woodlands, Prober *et al.* (2005) compared burning and sugar addition of 500 g m^{-2} added every 3 months to reduce soil nitrogen with the aim of encouraging *Themeda australis* (syn. T. *triandra)* establishment. Spring burns with added *T. australis* seed improved establishment and reduced soil nitrate in some treatments (Prober *et al.* 2005). Sugar addition reduced soil nitrate to levels found at the reference site. Subsequent studies have also found that sugar addition reduces soil nitrate concentrations (Cole *et al.* 2016; Morris and De Barse 2013).

However, in other studies sugar addition had little or no effect on native grass establishment. In two degraded grassy box gum woodlands, Cole *et al.* (2017) compared native grass establishment methods using combinations of added sugar and seed bank depletion through spring burning and grazing. Results varied across sites and seasons but there was no recruitment of C3 native grasses due to competition from introduced annual weeds (Cole *et al.* 2017). They concluded that better methods for controlling weeds were needed so that C3 native grasses could establish.

Brown *et al.* (2017) compared weed control methods for native grass establishment. They tested topsoil removal and herbicide (glyphosate) either alone or in combination with sugar or sawdust to temporarily reduce soil nitrate. One hundred mm of topsoil was removed from the “scalped” plots to reduce weed propagules and excess soil nutrients. A mixture of summer and winter active native grass species, locally collected, were hand sown after treatment. Removal of the topsoil gave the best results with 29 native grasses m^2 compared with < 2 native grasses for the herbicide/sugar and herbicide/sawdust treatments.

1.5.3 Methods for native grassland restoration

Grassland reconstruction occurs on highly degraded sites where few if any desirable species are remaining, for example former cropping land (Gibson-Roy *et al.* 2010). A mix of grasses and forbs including warm and cool season species are sown together, which makes selective herbicide use after sowing very difficult (Morris and Gibson-Roy 2017). This means that the soil weed bank must be very strongly controlled prior to sowing because herbicide options are few (Gibson-Roy *et al.* 2010; Morris and Gibson-Roy 2017).

The Grassy Groundcover Research Project (GGRP), a collaboration between the University of Melbourne and Greening Australia, has been studying and applying methods for grassland restoration since 2005 (Morris and Gibson-Roy 2017). Restoration work has been undertaken on over 100 sites in Victoria and New South Wales.

Topsoil removal

In temperate Australia, topsoil removal has been the most successful grassland reconstruction method (Brown *et al.* 2017; Gibson-Roy 2014; Morris and Gibson-Roy 2017). Also known as scalping, it has also been used in Europe (Diaz *et al.* 2008; Klimkowska *et al.* 2010) and California (Holl *et al.* 2014) to remove the majority of weed propagules prior to native habitat reconstruction. Where topsoil removal has been successful, removal depths (by excavator) vary from 100 mm (Brown *et al.* 2017; Buisson *et al.* 2006) to 400 mm (Klimkowska *et al.* 2010) followed by native seed addition. The optimum scalping depth removes the majority of the soil seed bank and reduces available P to levels similar to an indigenous reference site (Gibson-Roy *et al.* 2010; Morris and Gibson-Roy 2017).

Problems with topsoil removal include:

- increased risk of erosion (Brown *et al.* 2017);
- removal of a large portion of soil organic carbon (Geissen *et al.* 2013);
- changes in soil structure and hydrology (Brown *et al.* 2017; Geissen *et al.* 2013; Holl *et al.* 2014);
- cost (Jaunatre *et al.* 2014; Klimkowska *et al.* 2010), and;
- disposal or an alternative use for significant volumes of topsoil (Brown *et al.* 2017).

The effect of topsoil removal on soil ecology is not well understood. Diaz *et al.* (2008) found that topsoil removal in a British lowland heath did not suppress ericoid mycorrhizal fungi colonisation. Vergeer *et al.* (2006) however found that, in heathland vegetation, the top 50 mm of soil had the most arbuscular mycorrhizal fungi (AMF). This decreased significantly with depth. Thirty months after topsoil removal

(150-200 mm), AMF abundance was very low. Earthworms were negatively affected by topsoil removal and had not recolonised after more than 10 years at a site in the Netherlands (Geissen *et al.* 2013).

Gibson-Roy *et al.* (2010) compared chemical fallow, burning, cultivation, soil inversion, and topsoil removal for weed control. Soil inversion involved stripping the top 100 mm of soil and then the next 100 mm of soil (stored separately) and then putting the top 100 mm on the bottom and the bottom 100 mm on the top. No native species were sown. Complete removal of the top 100 mm of soil controlled the weed and bud bank most effectively. All plots were 1 x 1 m. The scalped plots averaged 2370 exotic plants prior to treatment and just 0.2 plants after 4 months. Cultivation and soil inversion were not as effective as scalping but were thought to be useful methods when scalping was not an option.

On the Cumberland Plain in NSW, barriers to restoration included elevated soil nutrient availability and a seed bank dominated by weedy species (Morris and De Barse 2013). Plant canopy composition and abundance were measured for 33 months after topsoil removal. A seed mix of native grasses and forbs was added two different times. Scalping (to a depth of 150 – 200 mm) gave the best result with native species making up >90% of plant abundance after 33 months.

Fire and smoke

Fire and smoke can be used to trigger germination of the seed bank (Dixon *et al.* 2009) and/or to kill seed near the soil surface (Cole *et al.* 2016). Changes to the seed environment after a fire include more light with a different spectrum, more temperature extremes due to biomass removal, altered soil nutrients, fluctuating water content and reduced competition (Nelson *et al.* 2012; Waters *et al.* 2013).

One hundred and seventy species from 37 families have shown an enhanced germination to smoke compounds (Adkins and Peters 2001). Pyran-butenolide molecules, known as karrikins, are thought to be largely responsible for this (Dixon *et al.* 2009; Nelson *et al.* 2012; Waters *et al.* 2013). They are unstable at high temperatures and so probably form in cooler parts of the fire where they collect in smoke, condense and then bind to the soil close to the site of formation (Flematti *et al.* 2015). The soil binding is due to sticky combustion products (Dixon *et al.* 2009).

On the other hand, smoke derived from high lignin-rich plant materials, leaf and leaf litter chemicals can inhibit germination (Nelson *et al.* 2012; Papenfus *et al.* 2015). At least 10 inhibitory compounds have been identified (Nelson *et al.* 2012) including, for example, trimethylbutenolide (Papenfus *et al.* 2015).

In New South Wales, annual grasses were reduced through spring burning that limited seed reaching the soil and removed surface seed (Cole *et al.* 2016; Prober *et al.* 2004; Prober *et al.* 2005). Annual grasses were reduced in abundance by 50-70% after burning in two consecutive springs (Prober *et al.* 2004) but spring burning can also induce germination of broad-leaf annuals and increase their abundance (Prober *et al.* 2007; Prober *et al.* 2005). In a study on the Cumberland Plain, fire and slashing with low levels of sugar addition (0.5 kg/m^2) every 3-5 months reduced exotic plant abundance and increased native species by about 5/m^2 compared to the control (Morris *et al.* 2016).

1.5.4 Other methods for weed management

Soil solarisation

In areas with a Mediterranean climate, the soil seed bank can be diminished by covering soil at field capacity with a clear polyethylene sheet for 30-50 days in the hottest summer period (Horowitz *et al.* 1983; Sauerborn *et al.* 1989). This method is referred to in the literature as solarisation, tarping or plastic mulching (Horowitz *et al.* 1983; Stapleton and Devay 1986). The principle of solarisation is that solar radiation passes through the clear sheet, heating the soil beneath, which can lead to the death of weed seeds and pathogens (Marshall *et al.* 2013).

Soil solarisation has been used on thousands of acres in hot, dry regions (Katan *et al.* 2010; Shennan *et al.* 2018; Stapleton *et al.* 2005; Vidotto *et al.* 2013). Soil solarisation was originally developed through extension work with farmers near the Jordan River in Israel in the mid 1970s (Katan *et al.* 2010) and has been studied and adopted in Israel (Chen and Katan 1980; Cohen *et al.* 2008; Horowitz *et al.* 1983; Katan *et al.* 2010) California (Stapleton 2000), Syria (Linke 1994; Sauerborn *et al.* 1989), Turkey (Oz 2018) and Italy (Pannacci *et al.* 2017; Vidotto *et al.* 2013). The International Centre for Agricultural Research in Dry Areas (ICARDA) and the University of California at Davis have been notable leaders in solarisation research (Katan *et al.* 2010). There is very little Australian literature on this topic.

Interest in soil solarisation has grown since chemicals for soil sterilisation (e.g. methyl bromide) were restricted due to environmental concerns (Katan *et al.* 2010; Shennan *et al.* 2018; Vidotto *et al.* 2013). The rise in organic agriculture is also driving interest in non-chemical methods for controlling weeds and pathogens (Katan *et al.* 2010; Stapleton *et al.* 2005).

Soil solarisation is effective against a range of weed seeds (Linke 1994; Sauerborn *et al.* 1989) and is used in fruit and vegetable horticulture (Katan *et al.* 2010), in orchards (Katan *et al.* 2010), in agriculture for controlling weeds and pathogens (Sauerborn *et al.* 1989; Stapleton 2000) and for native vegetation restoration (Cohen *et al.* 2008; Holl *et al.* 2014; Katan *et al.* 2010; Marushia and Allen 2011). The four keys to successful soil solarisation as outlined by Vidotto *et al.* (2013) are:

A. soil temperature and heat duration
B. soil moisture
C. seed morphology
D. seed dormancy

A. Soil temperature and heat duration

Solarisation can lead to maximum soil temperatures of approximately 45-57°C at 50 mm depth (Horowitz *et al.* 1983; Linke 1994; Sauerborn *et al.* 1989) but temperatures vary daily with depth and the ambient temperature (Horowitz *et al.* 1983; Linke 1994; Stapleton *et al.* 2005) (Fig. 3). The top 50 mm of soil reaches the highest temperatures but only for short periods during the day, which means it takes 4-7 weeks to kill weed seeds effectively (Stapleton *et al.* 2005).

Marshall *et al.* (2013) developed a predictive model for soil temperatures during solarisation to optimise the number of days for soil treatment. Their parameters included site longitude, latitude, soil bulk density, soil moisture content, soil organic matter, soil texture and ambient temperature. They found that the depth of the air gap between the plastic and the soil led to errors in the model because it could create an unpredictable insulating effect. Their field measurements at Davis, California, showed a maximum difference in temperature between covered and uncovered soil of 11% at 50 mm depth. Linke (1994) argued that, although the difference in temperature between covered and uncovered plots was only 6°C, the soil surface of the uncovered plots dried out very quickly while the covered plots remained moist, creating quite a different (i.e. hot and moist) environment for seeds.

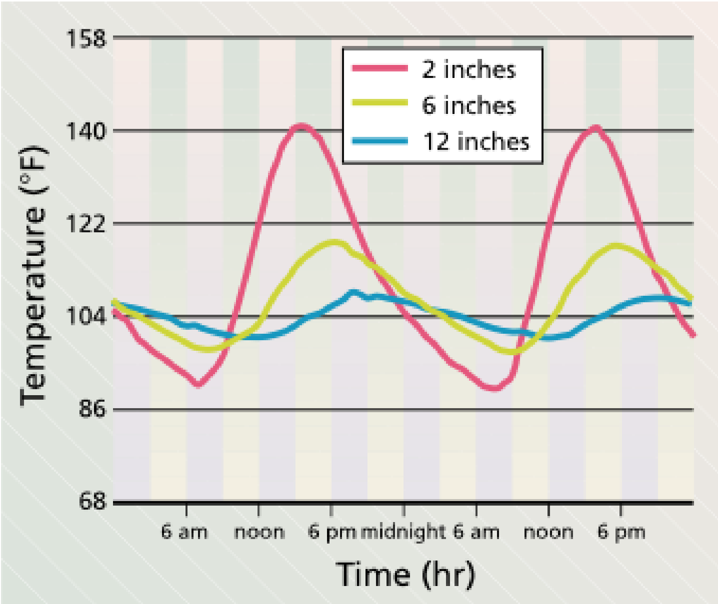

Figure 3. Soil solarisation temperatures reached at 50 mm, 100 mm and 300 mm depths during the day in San Joaquin Valley, California (Stapleton *et al.* 2005).

B. Soil moisture

Soil moisture should be at field capacity prior to applying the plastic sheets to maximise heat transference to the soil and for seed imbibition, but further irrigation is not necessary because there is very little evaporation as long as the sheet is properly sealed at the edges and does not develop any holes (Elmore *et al.* 1997; Horowitz *et al.* 1983; Marshall *et al.* 2013; Oz 2018). Marshall *et al.* (2013) found that there was no significant difference in soil moisture content before and after 29 days of solarisation.

C. Seed morphology

Vidotto *et al.* (2013) mixed the seed of 6 species with soil and exposed them to varying temperatures and found that larger seeds were more heat tolerant. This could be due to protective structures surrounding the seeds and differences in seed moisture content related to the thickness and width of the seed (Vidotto *et al.* 2013).

In addition to the 4 keys to success outlined by Vidotto *et al.* (2013) and Sauerborn *et al.* (1989), Linke (1994) noted that the effectiveness of soil solarisation on the weed seed bank also depends on the life cycle of the species being controlled. He found that the soil seed bank of annual weeds was well controlled, however, perennial plants with rhizomes, deep roots or storage organs were poorly controlled. The effect of soil solarisation on a range of species is summarised (Table 5).

Degraded pastures often have a high percentage of annual grasses and weeds (Mitchell *et al.* 2015), suggesting that soil solarisation is worth testing as a method for reducing the soil seed bank so that native grasses can establish. It may need to be combined with an appropriate herbicide for hard-seeded plants like clover, which can germinate after solarisation (Linke 1994).

The main disadvantages of soil solarisation are labour for laying out the plastic, the plastic waste it creates and the loss of land from production for 4-6 weeks while the soil is being treated (Katan *et al.* 2010; Stapleton *et al.* 2005). It is also an expensive method when compared with herbicide or burning.

Table 5. Summary of the effect of soil solarisation on a range of species including the duration of solarisation and maximum temperature attained. An asterix denotes families (but not necessarily species) present at the Mylor trial site. Maximum soil temperature at 50 mm soil depth was 50 - 60° C.

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
Amaranthaceae	*Amaranthus retroflexus* L. Name check: same	Field trial	Annual	Multiple records MLR, N Yorke and eastern parts of NSW and Vic	40	Linke (1994), Syria
	Amaranthus spp.	Field trial	Summer annual	Genus present in Australia	2-4 weeks	Horowitz *et al.* (1983), Israel
Brassicaceae	*Sinapis arvensis* L. Name check: same	Field trial	Annual	Multiple records for MLR	20	Linke 1994 Syria
Carophyllaceae	*Vaccaria pyramidata* Medik	Field trial	Annual	Present in MLR, western Yorke Peninsula and Riverland	40	Linke 1994 Syria
Euphorbiaceae	*Croton tinctoria* L. (now *Chrozophora tinctoria* (L.) A. Juss.	Field trial	Summer Annual	Population North of Adelaide and in the southern Flinders Ranges	40	Linke 1994 Syria

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
Apiaceae	*Scandix pectin-veneris* L.	Field trial	Annual	45 records near Adelaide	40	Linke 1994 Syria
*Asteraceae	*Carthamus flavescens* Willd.	Field trial	Annual	Species not found on APNI other Carthamus species well recorded for SE Australia, especially MLR.	40	Linke 1994 Syria
	Lactuca orientalis Boiss.	Field trial	Annual	Lactuca genus is common in SE Australia, esp MLR/Fleurieu.	40	Linke 1994 Syria
	Lactuca serriola L.	Field trial	Annual	See above	40	Linke 1994 Syria
	Xanthium pensylvanicum Wallr.	Field trial	Unknown	Other members of this genus present in Australia.	3 weeks	Egley (1983)Mississippi, USA
Boraginaceae	*Heliotropium* sp.	Field trial	Annual - summer	Genus is found all over Australia.	40	Linke 1994 Syria
Caryophyllaceae	*Spergula fallax* (Lowe) Krause	Field trial	Annual	Species not found genus found in MLR.	40	Linke 1994 Syria
Chenopodiaceae	*Chenopodium album* L.	Field trial		Common in south-eastern Australia, especially MLR, north Yorke.		Linke 1994, Syria
Euphorbiaceae	*Euphorbia peplus* L.	Field trial	Annual - Summer	Naturalised and common in southern Australia	40	Linke 1994 Syria
*Geraniaceae	*Erodium aegyptiacum* Boiss.	Field trial	Annual	Species not found Erodium genus common in southern Australia.	40	Linke 1994 Syria
Lamiaceae	Lamium amplexicaule L.	Field trial	Annual – winter weed	Common across MLR to southern Flinders. Common eastern margins Aus.		Horowitz 1983 Israel

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
Linaceae	*Linum* sp.	Field trial	Annual	Genus very common in southern Australia.	40	Linke 1994 Syria
Orobanchaceae	*Orobanche aegyptiaca* Pers.	A .Field trial B. Field trial	Annual B. Reduced by 71% in faba bean crop and 87% in lentil crop	There are other *Orobanche* spp. in Southern Australia eg. *O. minor*, *O cernua.*	A. 40 B. 40	A. Linke 1994, Syria B. Sauerborn *et al.* (1989), Syria
Papaveraceae	*Papaver rhoeas* L.	Field trial	Annual	Records for the S/L and Fleurieu but not common elsewhere	40	Linke 1994, Syria
	Fumaria Judaica Boiss.	Field trial	Annual	Other members of this genus found in Australia	42	Horowitz 1983 Israel
*Poaceae	*Phalaris brachystachys* L.k.	A. Field trial B. Field trial	Annual	Not found.	A. 40 B. 20 or more	A. Linke 1994, Syria B. Sauerborn 1989,Syria
*Polygonaceae	*Polygonum aviculare* L.	Field trial	Annual	Common is southern Australian	40	Linke 1994, Syria
*Portulacaceae	*Portulaca oleracea* L.	Field trial	Summer annual Weed (native in parts of WA & NT)	Distribution Australia-wide. MLR, KI, Southern Flinders, most of northern SA	28	Horowitz 1983 Israel
Ranunculaceae	*Adonis aestivalis* L. (DC) Riedl	Field trial	Annual	Common MLR, Fleurieu, Yorke, southern Flinders. Patchy: w coast of Eyre, Nullabor, WA	40	Linke 1994, Syria
	Ranunculus repens L.	Field trial	Annual	Mostly Fleurieu and SL	40	Linke 1994, Syria

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
Rubiaceae	*Galium tricornutum* Dandy	Field trial	Annual	North Yorke, Adelaide, MLR, incl Fleurieu	40	Linke 1994, Syria
Scrophulariaceae	*Linaria chalepensis* (L.) Mill.	Field trial	Annual	Linaria genus common in south-eastern Aus. Annuals?	40	Linke 1994, Syria

Low control

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
Convolvulaceae	*Convolvulus arvensis* L.	Field trial	Summer Deep roots or storage organs	Found throughout eastern and southern Australia incl NL, SL, Fleurieu and KI	40	Linke 1994, Syria
Liliaceae	Liliaceae sp.	Field trial	Deep roots or storage organs	Genus present in Australia	40	Linke 1994, Syria
Poaceae	*Cynodon dactylon* (L.) Pers.	Field trial The dominant summer weed	Summer Rhizomes to depth of 150 mm or more	Present in most parts of Australia, especially eastern and southern parts	40	Linke 1994, Syria Elmore *et al.* (1997) found that the seed was controlled, California
Iridaceae	*Gladiolus aleppicus* Boiss.	Field trial	Deep roots or storage organs	Other members of Gladiolus in Australia	40	Linke 1994, Syria
Lilliaceae	*Ornithogalum narbonense* L.	Field trial		Other members of genus found in Australia	40	Linke 1994, Syria
	Daucus sp.	Field trial	Summer weed	Species in this genus are present in Australia	40	Linke 1994, Syria
Cyperaceae	*Cyperus rotundus* L.	Field trial	Deep roots and storage organs	Common along eastern Australia and in MLR/Fleurieu	3 weeks	Egley 1983, Mississippi, USA

Family	Species	Experimental method	Annual/perennial in study region	Present in SA, SL?	Duration of solarisation (days)	Study
*Fabaceae	Coronilla scorpiodes L.	A.Field trial B. Field trial	Seed dependent species	Other species in this genus are present in Australia	A. 40 B. 40	A. Linke 1994, Syria B. Sauerborn 1989, Syria
Lilliaceae	*Muscari comosum* (L.) Mill.	Field trial Population more than doubled	A small bulb weed. Emerging from depths of 0-300 mm.	About 15 records in the Adelaide/SL/Fleurieu region	50	Linke 1994, Syria

Cover cropping

Cover cropping is the practice of sowing an annual crop, usually a cereal, at the same time as sowing a perennial pasture (Moyer *et al.* 1995; Swan *et al.* 2014). It is also known as undersowing, companion cropping or nurse cropping (McCormick *et al.* 2014). In south-eastern Australia, cover crops are sown for both financial and practical reasons (McCormick *et al.* 2014). Farmers use it to provide an income while the perennial pasture is establishing and because they believe it will prevent soil erosion, provide more stable temperatures and help to compete with annual weeds (Moyer *et al.* 1995; Swan *et al.* 2014; Waddington and Bittman 1983). Wheat and barley are the most common cover crops in temperate Australia (McCormick *et al.* 2014; Swan *et al.* 2014). They can be harvested as cereal or silage or left in place as a mulch (McCormick *et al.* 2014).

Cover crops are annuals with more rapid growth rates than the perennial pasture species. They can compete strongly for light and soil moisture leading to death and/or low seed set of the pasture species in the first year (Waddington and Bittman 1983). This can leave the pasture vulnerable to weed invasion in the following year (Moyer *et al.* 1995; Swan *et al.* 2014) . In a review for the mixed farming zones of south-eastern Australia with 450-600 mm rainfall, McCormick *et al.* (2014) found little evidence to support the widespread use of cover cropping. Pastures established better without cover cropping and had higher productivity over the lifespan of the pasture (McCormick *et al.* 2014; Swan *et al.* 2014; Waddington and Bittman 1983). Swan *et al.* (2014) recommended that grasses should not be sown with a cover crop for these reasons.

McCormick *et al.* (2014) recommended that if cover crops are used, they should be used according to the findings of an earlier review by Santhirasegaram and Black (1967), which were:

- not be sown in the same row as the pasture crop;

- not have high vigour, and;
- be removed before they limit soil moisture to the pasture crop (e.g. by cutting or grazing).

Santhirasegaram and Black (1967) also recommended a sowing rate reduced from the norm (which varies with country, region and species sown). However, Waddington and Bittman (1983) used cover crops at 50% of the local sowing rate for wheat and still found a detrimental effect on perennial pasture establishment.

1.6 Knowledge gaps

Methods for controlling weeds so that native grasses can establish in annual dominated pastures are poorly understood. In grassland reconstruction, topsoil removal effectively controls the soil weed bank and also removes excess N and P so that native species, including grasses, can establish (Brown *et al.* 2017; Gibson-Roy *et al.* 2010; Morris and Gibson-Roy 2017). However, this may not be a desirable treatment for pasture amelioration on marginal lands. This is because they are often sloping, have skeletal soils and/or high erosion risks (see Ch 4). In farming systems, there is also reluctance to remove topsoil and therefore organic carbon and other important soil properties. Alternative native grass establishment methods are needed (Cole *et al.* 2017; Firn 2007a; Semple *et al.* 1999).

Anecdotally practitioners are successfully establishing native grasses in the Mount Lofty Ranges without using scalping. These methods have not been formally documented. A survey of practitioners to learn about these methods will form part of the Masters research.

It is unclear which native pasture species will grow well at the experimental sites at Mylor in the Adelaide Hills or at the Waite Arboretum in Adelaide. It is also not known whether these species will need irrigation in their first summer, will grow well together or should be sown separately. Ideal sowing rates are also not known. These questions will be explored in a test of concept area prior to field trials.

This Masters study aims to investigate the knowledge gap surrounding ways to manage the seed bank. This will include comparing, adapting and perhaps combining methods known to be effective for controlling weeds in agriculture, horticulture and grassland restoration. Methods for using the differences in life cycle between introduced annuals and native perennials (Prober *et al.* 2004), and between summer and winter active grasses will also be explored.

A further knowledge gap concerns whether or not cover crops might be useful for suppressing weeds and providing protection to the emerging native grasses and the soil. Despite its potential disadvantages, cover cropping is a widespread practice in south-eastern Australia (McCormick *et al.* 2014). In this study native grass practitioners were asked if they are using cover crops and, if so, how this is done. Cover crops will also be used in a test of concept area.

1.7 The research question

To better understand how to establish native grasses in annual pastures this research project will address the following question:

Which methods are suitable for establishing native grasses in annual-dominated pastures?

1.8 Aims of this study

Little is known about whether native grasses can be successfully established in pastures dominated by annual species. The aim of this study is to:

Find the most effective methods of weed control for native grass establishment; drawn from horticulture, agriculture and grassland restoration.

The following objectives support this aim:

- interview native grass practitioners to document their establishment methods, failures and challenges
- investigate the effectiveness of various methods for controlling weeds so that native grasses can establish

1.9 Significance to the discipline

Perennial native grasses can minimise weed invasion, prevent soil erosion, control the water table (and hence soil salinity) and extend the grazing season (Mitchell *et al.* 2015; Whalley *et al.* 2005). But on thousands of acres of pasture across temperate Australia, they were replaced with grasses introduced from other countries. Often these introduced species have not been able to persist in Australian conditions, (i.e surviving and reproducing from one season to the next) (Waters *et al.* 2005), especially when soil fertility is low (Kemp and Dowling 1991).

This project is significant to the grazing industry because, under harsh conditions, native grasses may have superior persistence over introduced species. They have potential to form a more sustainable sward on land that is sloping, has shallow soils, subsurface rock or low fertility (Mitchell *et al.* 2015; Waters *et al.*

2005) (Whalley *et al.* 2005). Where perennial grasses have been lost, reseeding is the main method for re-establishment (Whalley *et al.* 2005) but reliable establishment methods for native grasses are needed (Firn 2007a; Sanford *et al.* 2005; Semple *et al.* 1999).

This study is also significant for grassland restoration practitioners. It explores other methods that might prove useful if topsoil removal is not an option due to site constraints.

Chapter 2: Interviews with native grass practitioners

Chapter 3: Test of concept research

Chapter 4: Mylor 2018 Trial

Chapter 5: Waite Biofilm Trial

Chapter 6: Conclusions and future research

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CHAPTER 2. Interviews with native grass practitioners

2.1 Background

Most Australian native grass research has been conducted in New South Wales and Victoria. There is little published native grass research from the Australian Mediterranean climatic zone, especially in the area of pasture renovation with native grasses. A national study of grasses useful in limiting environments included a Mediterranean climatic zone trial site at Flaxley Research Station in the Mount Lofty Ranges (Sanford *et al.* 2005). The results from the Flaxley trial indicated that some native grasses had potential to improve pastures where introduced grasses were not surviving. But, the trial grasses were grown from tube stock which is not practical for pastures (Sanford *et al.* 2005).

For pastures, the native grasses would have to be established from seed, but investigation of establishment techniques are lacking (Firn 2007). The literature suggests that, in general, native grasses have slow seedling growth and are therefore easily outcompeted by weeds growing from seed or other propagules in the soil seed bank (Chivers and Raulings 2009). Native grass establishment field trials in central New South Wales led to the following recommendations for warm season native grasses (Semple *et al.* 1999):

- Choose a low-fertility site
- Control weeds for at least 18 months before sowing
- Sow thickly to compete with weeds
- Use a tilled seed bed
- Choose a season with above average rainfall

Numerous revegetation practitioners in the greater Adelaide and Mount Lofty Ranges region have experience with native grass establishment but these methods were largely undocumented and were lacking field trial validation. Many practitioners were willing to share their knowledge and also to learn from the experience of others. In order to gain an understanding of native grass establishment methods being used in the Adelaide Plains/Mount Lofty Ranges region, ethics approval from the University of Adelaide was sought to conduct face-to-face interviews in 2018. See Appendix A for the Ethics approval.

Twelve experienced practitioners agreed to participate in interviews of 60-90 minutes in duration. The purpose of the interviews was to identify native grass establishment methods that could be compared in field trials for their effectiveness. Practitioners were asked about the barriers to native grass establishment, particularly the impacts of weeds, and their weed control methods.

2.2 Selection of interviewees

The Native Grass Resources Group Inc. (NGRG) is a non-profit entity in South Australia helping people to recognise, use and protect native grasses. The NGRG membership includes many well-known native grass practitioners in the region and they were happy to help with contacts. Fifteen practitioners were invited to take part in interviews and twelve accepted. Without exception, they were keen to help and generously shared knowledge, problems and research ideas.

2.3 Interview methods

Participants received a detailed information sheet, the interview questions and a consent form prior to the interview (Appendix B). Each interview was conducted face-to-face with the practitioner at the location of their choosing; often in a paddock. After 45 minutes, interviewees were asked if they wanted to finish the interview. All wanted to keep talking, so most interviews lasted for 60-90 minutes. Detailed notes were taken at the time of the interview and were later typed and sent to the participant for checking.

2.4 Interview questions

Each interviewee was asked the following questions:

a. What impact does the soil weed bank have on the establishment of native grasses?

b. Which weeds are the most problematic for the establishment of native grasses?

c. How long do you think the propagules of weeds identified above remain viable in the soil and why do you think so?

d. Which weeds that might affect native grass establishment are easy to control, why and what methods are being used?

e. Which weeds that affect native grass establishment are hard to control, why and what methods have been tried?

f. Are there any weeds that facilitate native grass establishment?

g. What is the most successful time of year to sow Weeping rice grass (*Microlaena stipoides*), Kneed Wallaby grass (*Rytidosperma geniculatum*), Red-leg (*Bothriochloa macra*) and Kangaroo grass (*Themeda triandra*)?

h. Which methods do you think are the most effective for reduction of the soil weed bank?

i. Please describe the methods including their main strengths and weaknesses

j. Are these methods site specific and, if so, what criteria are used for deciding which method to use?

k. Have you ever tried smoke compounds, microwaves or solarisation to reduce soil weed propagules?

l. What was the result?

m. Have you used topsoil removal to control soil weed propagules?

    YES: What was the result?

    NO: is it a method you would consider using

        If no: what are your concerns?

2.5 Summary of interview responses

A summary of the interview responses follows:

a. What impact does the soil weed bank have on the establishment of native grasses?

Sown perennial native grasses have low seedling vigour and are easily overwhelmed by introduced weeds germinating from the soil seed bank. They are outcompeted for light and other resources by sheer numbers.

b. Which weeds are the most problematic for the establishment of native grasses?

Introduced annual grasses (e.g. *Avena* spp., *Bromus* spp. *Vulpia* spp.) because they are abundant and fast growing. Also, introduced perennial grasses (e.g. *Phalaris aquatica*, *Lolium* spp., *Holcus lanatus*, *Pentameris pallida*) because they occupy the same niche and reduce the options for control with herbicides.

c. How long do you think the propagules of weeds identified above remain viable in the soil and why do you think so?

Most didn't know for sure but felt that it was highly variable depending on site/conditions/rainfall/weed species etc. Some gave anecdotes about species that, through soil disturbance, came up even 20 years after they were last seen.

d. Which weeds that might affect native grass establishment are easy to control and how?

Annual broadleafs are easiest to control because there are selective herbicides that can kill them without killing the native grasses.

There are also some selective herbicides for annual grasses (e.g. Matavan 90 for wild oats).

e. Which weeds that affect native grass establishment are hard to control, why and what methods have been tried?

Perennial grasses are hard to control because they occupy the same niche as perennial natives and there are therefore no selective herbicides to use. Many said that they would prefer avoiding sites where perennial introduced grasses were present if grassland restoration was the goal*. If avoidance was not possible, topsoil removal would be a preferred site preparation method. If topsoil removal is not possible, 3 years of site preparation is probably needed. This would involve either killing the vegetation with herbicides until the seed bank was greatly diminished or using a cereal cover crop to reduce the risk of erosion and managing the midrow weeds with herbicides.

Note: in grazing systems, the presence of these perennial grasses can be desirable.

f. Are there any weeds/introduced species that facilitate native grass establishment?

Many thought that cover crops had a role to play in stabilising the soil and providing weed competition while perennial native grasses were establishing. But, one person said that cover crops diminished the success of native grass establishment.

g. What is the most successful time of year to sow Weeping rice grass (*Microlaena stipoides*), Kneeded Wallaby grass (*Rytidosperma geniculatum*), Red-leg (*Bothriochloa macra*) and Kangaroo grass (*Themeda triandra*)?

C3 species	Season	Notes
Microlaena stipoides	Autumn	
Rytidosperma spp.	Autumn	
C4 species		
Bothriochloa macra	Spring is ideal but if sowing C3s do all together in Autumn to save cost	
Themeda triandra	Spring is ideal but if sowing C3s do all together in Autumn to save cost	Many used an alternative method of cutting *T. triandra* hay when it has ripe seed then spreading it over the establishment site (usually January). The following spring, the standing vegetation on the site is sprayed and, once dry, burned to germinate the *Themeda* seed.

Areas with non-wetting sands were seen as a problem. In autumn the soils are often not wet enough until it is too cold for seed to germinate. Spring sowing of these sites is preferred but a dry summer will kill the seedlings without irrigation.

Two people mentioned that it is important to use a high sowing rate to compete with germinating weeds. 50 kg/ ha (species unknown) was used for one project.

h. Which methods do you think are the most effective for reduction of the soil weed bank?

Interviewees fell into 3 camps:

1. advocates of topsoil removal (i.e. removing about 50 -100 mm of topsoil to reduce the soil weed bank)

2. people who believe topsoil removal works but haven't used it themselves due to cost and so used alternative methods (see next question)
3. people who disagree with topsoil removal in principal and used alternative methods (see next question)

Most said that if a quick and efficient result was needed, the initial budget was large enough and the site was suited to it; topsoil removal was the most effective method. Those opposed to topsoil removal recommended a longer site preparation period – up to 3 years which generally involved combinations of:

- Burning
- Cover cropping
- Chemical fallow for up to 3 years
- Harrowing of top 50 mm to stimulate germination

It was also recognised that these methods might lead to ongoing management costs because weeds would continue to emerge from the soil seed bank after native grasses are sown. For this reason, it was suggested to start with one grass species (either C3 or C4) until the sward is free of weeds before adding other species if a species-rich composition was desired.

All mentioned that grasslands are disturbance-based ecosystems that need long-term management to maintain the quality of the sward. Without management (e.g. grazing, burning or slashing) grassy ecosystems accumulate dead leaf matter known as thatch. Thatch is a fire hazard but also diminishes the ecosystem's quality and biodiversity by smothering herbs and forbs. Future management methods should be considered alongside the site preparation methods.

i. Please describe the methods including their main strengths and weaknesses.

All practitioners said methods are site-specific and depend on characteristics like annual average rainfall, vegetation composition, weeds present, slope, aspect etc.

Method 1. Burn > spray germinants with glyphosate > sow to cover crop in Autumn > sow natives either with cover crop in Autumn or into rolled cover crop in spring (or could spray spring germinants + cover crop and sow into this)

Strengths	Weaknesses
Relatively cheap, uses common machinery, kills surface seed, smoke encourages weed germination, cover crop competes with weeds and gives protection for natives, erosion control	Risk of wildfire and not suited to all situations/sites Cover crop can compete with natives for resources

Method 2. Chemical fallow – use knock-down herbicides continuously for 3 years until almost nothing germinates from the soil seed bank.

Strengths	Weaknesses
Low cost of chemicals Depletion of the soil weed bank	Erosion risk through bare soil, loss of soil structure, weeds blowing or carried in from other areas, long preparation time Potential build up of chemical residues

Method 3. Tillage to bury seed > spray germinants > harrow only top 30-50 mm > spray germinating weeds > repeat until germinable weed seed largely exhausted > sow natives with or without a cover crop

Strengths	Weaknesses
Rapid reduction of soil weedbank, potentially faster than 3 year chemical fallow, uses common machinery	Risk of erosion, potential damage to soil structure, not effective for weeds that germinate or invade from deeper than 50 mm

Other methods that were suggested but had not been tried were:

- Pine oil or corn starch spray to suppress the soil weed bank
- Gibberelic acid to stimulate germination of the soil weed bank
- Application of nitrogen to break weed seed dormancy

j. Are these methods site specific and, if so, what criteria are used for deciding which method to use?

Yes, all methods depend on rainfall, slope, aspect, weeds present, landholder goals (eg restoration or grazing) and timeline, budget, future management methods.

k. Have you ever tried smoke compounds, microwaves or solarisation to reduce soil weed propagules?

No one had tried aerosol smoke. Some had tried smoke water for seed germination without much success.

Microwaves: no

Soil solarisation: only one person had tried this and found it effective but impractical.

l. Have you used topsoil removal to control soil weed propagules?

Five practitioners had used topsoil removal and all had seen projects where topsoil removal was used successfully. Those with the most experience believe it is the only effective approach if monocots and dicots will be sown together in the restoration. The cost of topsoil removal must be weighed against the ongoing management issues caused by not removing most of the soil weed bank before sowing.

Those opposed to topsoil removal cited cost, the problems of shallow/stony soils, the issue of what to do with the spoil, removal of the "living soil" and erosion as their main concerns. "There must be a better way" was a familiar comment.

2.6 Summary and conclusions

All practitioners agreed with the published information that perennial native grasses have slow seedling establishment, making them vulnerable to weed competition. Fast growing annual weeds can easily outcompete native grasses especially for light and soil moisture, which can be a scarce resource over summer in our Mediterranean climate. Introduced perennial grasses are also a problem because they compete strongly in the same niche and reduce the available herbicide options. The main challenge is to manage the soil seed bank sufficiently to allow native grasses to emerge and grow. The methods used to do so should take into account site-specific details like: the weeds present, soil type and depth, slope aspect and rainfall. The available budget will also dictate which methods are chosen.

The information from the interviews and the trial site conditions at Mylor were evaluated to decide which methods might be effective there. A further consideration was the time constraint for the Masters by Research.

Test of Concept and trial sites that were free from introduced perennial grasses were selected. From the interviews, methods were selected that a) suited the site and b) could be evaluated within one or two years using field trials. These were:

1) Cover cropping
2) Topsoil removal plus herbicide
3) Cultivate plus shallow tillage plus herbicide
4) Soil inversion plus herbicide

Cover cropping was investigated in a Test of Concept area (Chapter 3) and methods 2-4 were evaluated in randomised block trials (Chapter 4).

Acknowledgements

Warmest thanks to the native grass practitioners for whole-heartedly and generously sharing their expertise, including (in alphabetical order): Andrew Fairney, CEO of Seeding Natives Inc., Chris Penfold, Dragos Moise/Department for Environment and Water, Glenn Christie/Succession Ecology Inc., John Stafford, Robert Myers/Seeding Natives Inc., Shaun Kennedy, Specialist – Vegetation Services, SA Water and Tim Zwierson/Ecotypic Pty Ltd, Wayne Brown/Environments by Design. Some interviewees wished to remain anonymous. The Human Research Ethics Officer, Amy Lehman, was a great help with negotiating the complexities of Ethics approval.

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Appendix A – Ethics approval

THE UNIVERSITY of ADELAIDE

RESEARCH SERVICES
OFFICE OF RESEARCH ETHICS, COMPLIANCE AND INTEGRITY
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CRICOS Provider Number 00123M

Our reference 32791

14 March 2018

Professor Petra Marschner
School of Agriculture, Food & Wine-WT

Dear Professor Marschner

ETHICS APPROVAL No:	H-2018-040
PROJECT TITLE:	Survey of native grass practitioners

Thank you for the emails and amended ethics application provided by Marne Durnin on the 09.03.2018 and 13.03.2018 requesting an amendment to the allow participants to indicate on the consent form whether they would like to be identified in publications.

The ethics amendment for the above project has been reviewed by the Executive, Human Research Ethics Committee and is deemed to meet the requirements of the *National Statement on Ethical Conduct in Human Research (2007)* involving no more than low risk for research participants.

You are authorised to commence your research on:	06/03/2018
The ethics expiry date for this project is:	31/03/2021

NAMED INVESTIGATORS:

Chief Investigator:	Professor Petra Marschner
Student - Postgraduate Masters by Research:	Mrs Marne Marie Durnin
Associate Investigator:	Professor Ralph Whalley

CONDITIONS OF APPROVAL: The revised application provided 5.03.2018 has been approved.

Ethics approval is granted for three years and is subject to satisfactory annual reporting. The form titled Annual Report on Project Status is to be used when reporting annual progress and project completion and can be downloaded at http://www.adelaide.edu.au/research-services/oreci/human/reporting/. Prior to expiry, ethics approval may be extended for a further period.

Participants in the study are to be given a copy of the information sheet and the signed consent form to retain. It is also a condition of approval that you immediately report anything which might warrant review of ethical approval including:

- serious or unexpected adverse effects on participants,
- previously unforeseen events which might affect continued ethical acceptability of the project,
- proposed changes to the protocol or project investigators; and
- the project is discontinued before the expected date of completion.

Yours sincerely,

Professor Paul Delfabbro
Convenor

The University of Adelaide

Appendix B – Participant Information Sheet and consent form

Date:

Soil weed bank reduction research

PARTICIPANT INFORMATION SHEET

V8: 16 March 2018

PROJECT TITLE: Survey of native grass practitioners
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2018-040
PRINCIPAL INVESTIGATOR: Dr. Petra Marschner
Co-INVESTIGATOR: Dr. Ralph (Wal) Whalley
STUDENT INVESTIGATOR: Marne Durnin

Dear

You are invited to participate in interviews with native grass practitioners as described below. This information is for you to keep and refer to if needed.

What is the interview about?
We are doing field experiments of different methods for reducing the soil weed bank. This includes microwaves, solarisation, topsoil removal and smoke compounds. By interviewing experienced native grass practitioners, we hope to document other methods currently being used in the Mount Lofty Ranges and their effectiveness. Two currently effective methods (if any) will be included in the field trials. A full list of interview questions is attached.

Who is undertaking the project?
This project is being undertaken by Marne Durnin as part of a Masters by Research degree. For full disclosure of memberships and affiliations: Marne is a member of Trees For Life, Green Web and the Friends of Mylor Conservation Park. She is a current member and former Chair of the Native Grass Resources Group and is a current Board member of Seeding Natives Incorporated.

Why am I being invited to participate?
You are being asked to participate because you have specialist knowledge and experience with establishing native grasses on weed dominated sites.

What will I be asked to do?
If you choose to participate in this study, I will ask you the open-ended questions included with this document. If you agree, the interview will be audio recorded for future analysis and should take about 40-50 minutes. If you do not want to be recorded, your answers will be taken by hand. You can also show me any sites that demonstrate successes or failures if you want to.

How much time will the project take?
40- 50 minutes plus any site visits

Are there any risks associated with participating in this project?
There are no foreseeable risks associated with participation in this project. We will not release any identifying information to other parties or in our publications or presentations.

What are the benefits of the research project?
There is no personal benefit from participation in this study. Your information will be used to search for improved ways to reduce the soil seed bank so that native species can establish more effectively and to reduce future management inputs. These results will be shared with you from the earliest stages (if wanted).

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study at any time throughout the process.

What will happen to my information?
With your consent, information and photographs gathered during this research project will be analysed by Professor Petra Marschner and Marne Durnin and the aggregated results (but not your name or any identifying details) will be shared with native grass practitioners through various presentations and publications. It will also form part of Marne's Masters thesis. Information will be stored for a period of 5 years. At your request, you and/or your company will be acknowledged for your participation in this research (see consent form attached).

Research funding
This research is supported by the Native Grass Resources Group, the Department of Environment, Water and Natural Resources and the Commonwealth Government of Australia. The University of Adelaide has provided a Masters by Research Scholarship.

Who do I contact if I have questions about the project?
If you would like further information about this project please contact

Marne Durnin, Masters by Research student
Tel: 08 8363 5937 or by email: marne.durnin@adelaide.edu.au
Dr. Petra Marschner
Tel: 8313 7379 petra.marschner@adelaide.edu.au

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at the University of Adelaide (approval number H-2018-040). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult Dr. Petra Marschner. If you wish to speak with an independent person regarding a concern or complaint, the University's policy on research involving human participants, or your rights as a participant, please contact the Human Research Ethics Committee's Secretariat on:
Phone: +61 8 8313 6028
Email: hrec@adelaide.edu.au

Post: Level 4, Rundle Mall Plaza, 50 Rundle Mall, ADELAIDE SA 5000
Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

What if I don't want to answer some questions or decide later to withdraw the information I have provided?
You have the right to withhold (i.e. not report) information. If you provide information and later realise that you do not want this information to be published by Marne, you can ask her to remove the relevant data from her results and discussion.

If I want to participate, what do I do?
If you would like to participate in this research project, please let Marne know. She will either come to your location or interview you by phone if preferred. When you meet, she will ask you to sign the Consent to Participate form, which is attached.

Yours sincerely,

Marne Durnin

Research questions

n. What impact does the soil weed bank have on the establishment of native grasses?
o. Which weeds are the most problematic for the establishment of native grasses?
p. How have you tried to control emerging weeds?
q. How long do you think the propagules of weeds identified above remain viable in the soil and why do you think so?
r. Which weeds that might affect native grass establishment are easy to control, why and what methods are being used?
s. Which weeds that affect native grass establishment are hard to control, why and what methods have been tried?
t. Are there any weeds that facilitate native grass establishment?
u. What is the most successful time of year to sow Weeping rice grass (*Microlaena stipoides*), Kneeded Wallaby grass (*Rytidosperma geniculatum*), Red-leg (*Bothriochloa macra*) and Kangaroo grass (*Themeda triandra*)?
v. Which methods do you think are the most effective for reduction of the soil weed bank?
w. Please describe the methods including their main strengths and weaknesses
x. Are these methods site specific and, if so, what criteria are used for deciding which method to use?
y. Have you ever tried smoke compounds, microwaves or solarisation to reduce soil weed propagules?
z. What was the result?
aa. Have you used topsoil removal to control soil weed propagules?
    YES: What was the result?
    NO: is it a method you would consider using
        If no: what are your concerns?

SURVEY CONSENT FORM

Marne Durnin will bring and collect a signed form from you before the survey commences.

- ❑ I have read the attached Participant Information Sheet and agree to take part in the following research project:

Title:	**Survey of native grass practitioners**
Ethics Approval Number:	H-2018-040

- ❑ I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker, Marne Durnin. My consent is given freely.
- ❑ I understand the purpose of the research project and it has been explained that involvement may not be of any direct benefit to me but that I will be kept informed of progress if requested.
- ❑ I have been informed that, while I will not be named in the published materials, it may not be possible to guarantee my anonymity given the nature of the study and/or small number of participants involved (for example, someone may guess which comments you made).
- ❑ I wish/do not wish to be acknowledged in Marne's thesis for my participation in this research. The acknowledgement should read: ______________________________
- ❑ I understand that I am free to withdraw from the project at any time.
- ❑ I agree that photos or films, taken by the researcher, of the field site may be published in her thesis or presentations.
- ❑ I agree to have my interview recorded by iPad/iPhone.
- ❑ I am aware that I should keep a copy of this Consent Form, when completed, and the attached Information Sheet.

Participant to complete:

Name: ____________________________ Signature: ________________________________ Date:______________

Researcher to complete:

I have described the nature of the research to____

(print name of participant)

and in my opinion, they understood the explanation.

Signature: Position:

CHAPTER 3. Test of Concept results

Introduction

Methods for establishing Australian native grasses from seed in annual dominated pastures are poorly understood (Firn 2007; Semple *et al.* 1999). Therefore, many aspects of the proposed field trials were unclear and untested. In early 2018, a Test of Concept Area (TCA) was established to determine which native Australian pasture grasses might perform well at the experimental site at Mylor and to understand which methods were suitable for native grass establishment.

An additional use for the TCA was seed production. Native grass pasture seed supply can be very unreliable and expensive. When available, native grass seed ranges in price from $255/kg for Weeping rice grass (*Microlaena stipoides)* to $1,100/kg for Kangaroo grass (*Themeda triandra*) (Native Seeds Pty 2020). Seed from the various experiments in the TCA was used for the Waite Arboretum trial and a 2019 trial at Mylor (not part of this thesis).

The TCA was used to filter ideas and test techniques before using them in the more time-consuming and expensive replicated field trials. A range of native grass species and/or accessions were evaluated, as were various methods for weed control and sowing. This was done using a series of unreplicated test plots of about 25 m^{-2}. A summary of the research questions and findings from the TCA follows.
The TCA was located in a paddock adjacent to the Mylor field trial site. It had the same soil type, aspect and weeds. The weeds were predominantly bromes (*Bromus* spp. L.Sp.Pl.), wild oats (*Avena fatua* L.) and silver grass (*Vulpia bromoides* (L.) Gray). Several clover species (*Trifolium* spp. L.) were present on both sites. The predominant broadleaf weed was Cape weed (*Arctotheca calendula* (L.) Levyns).

Can native grasses be established when grazers are present?

Kangaroos and rabbits were attracted to the native perennial grasses, especially in summer, and would repeatedly graze them to the ground which killed many plants. It was impossible to continue the TCA without controlling rabbit and kangaroo grazing. It became clear that control of grazing pressure will be an important first consideration for others wanting to establish native grasses. With generous support from The Native Grass Resources Group, grazing proof exclosures were built around the TCA (~210 m^2) and trial sites (~450 m^2).

Which native grass species were suitable for the trials at Mylor and the Waite Arboretum?

The soil at the experimental site is shallow, acidic, sandy and hydrophobic in summer. The site is also subject to extremes of heat and cold (e.g. from -7 °C to 45 °C during the experimental period). Native grasses were selected for testing based on the following criteria:

i. seed was available, and;

ii. the species or accession was known to be suitable for grazing (Foster *et al.* 2009), and;

iii. it was either locally indigenous or already growing in the vicinity of the trial property at Mylor.

Some additional accessions were tested from a trial of native grasses in a similar environment nearby at Flaxley Research Station (Whalley *et al.* 2005). Some of the accessions grown successfully there were made available by the Australian Pastures Genebank (APG). The species tested and the results are shown in Table1.

Table 1. The most successful native grass species grown in the TCA at Mylor.

Species	Seed source	Results	Establishment notes
C4 grasses			
Themeda triandra	Locally collected	Slow to establish but very hardy to local conditions including low temperatures (e.g. -7°C) and heat (including a 47° C day). Does not like to be slashed below about 100 mm in summer which often kills the plant. Thatch build up starts to reduce plant vigour and seed production after 2-3 years with no grazing.	Germination requires temperatures above 20° C and good soil moisture. Best time to sow seed is August/September. Even with the best quality seed, germination rates can be low (40-60%). Mature plants need about 500 mm by 500 mm space each. Best weed control is gained by planting in rows with 1 seed per about 100 mm, 500 mm between rows. Tolerant of heat (up to ~45 °C and cold to at least -7 °C. In erosion prone areas: can be sown in wider rows with millet in the inter-row as a cover crop. The millet is later slashed to provide a mulch to help retain moisture. Long-lived. Grows well with *Microlaena stipoides*.
	APG SA44882	Most plants killed by frost in winter 2018.	Sown from tube stock.
	APG SA45032	Most plants killed by frost in winter 2018.	Sown from tubestock.
	Devils elbow, near Crafers, SA	This is a small form, culms to a height of 300-400 mm.	Not as cold tolerant as the locally collected form.
Bothriochloa macra	Unknown. Tube stock from State Flora at Belair, SA.	Can be difficult to establish but very persistent once established. Needs irrigation over the first summer.	Rhizomatous and good for erosion control. Growth is negligible from about May until days are regularly above 20° C – usually in late September. Probably not ideal for grazing at Mylor as plants run to seed very early in the season producing a high stem to leaf ratio.

C3 grasses			
Rytidosperma caespitosum	Seeding Natives Inc. seed production area, Mount Pleasant SA.	Easy to establish from seed in a weed free site. Sow in a shallow trench at 10 – 20 mm depth after the autumn rains. Thatch build up starts to reduce plant vigour and seed production after 2-3 years with no grazing.	Grows well from seed in a weed free site. Emergence is later than M. stipoides – about June. Sowing seed thickly (Fig. 1) in a trench of 10 - 20 mm depth helps with weed control and suppression. Competes with *Themeda triandra* – possible allelopathy?
Rytidosperma caespitosum var. Trangie	APG 44786	Good vigour, excellent seed production.	Same as above.
Rytidosperma fulvum	Unknown. Parent plants were found in a garden at the Waite Arboretum.	A rare Wallaby grass with a larger tussock and broader leaves that most other Wallaby grasses in the region. Easy to establish from seed in a weed free site. Sow in a shallow trench at 10 – 20 mm depth after the autumn rains. Drought and frost tolerant.	Suitability for grazing unknown at this stage.
Rytidosperma geniculatum	Seeding Native Inc. seed production area, Mount Pleasant SA.	Easy to establish from seed in a weed free site. Sow in a shallow trench at 10 – 20 mm depth after the autumn rains. Thatch build up starts to reduce plant vigour and seed production after 2-3 years with no grazing.	The tussock is small but very persistent. High seed production.
Microlaena stipoides	Locally collected seed and Seeding Native Inc. seed production area, Mount Pleasant SA.	A very hardy species that spreads well from seed. Easy to establish from seed in a weed free site after the autumn rains. Sow in a shallow trench at 10 – 20 mm depth after the autumn rains or scatter and rake to cover.	Tolerant of drought and frost (to -7° C). High local variability; from rhizomatous, matt-forming forms to tussocky fine-leafed forms. Sowing seed thickly (Fig. 1) helps with weed suppression.
M. stipoides var. Burra	APG 84357		
Anthosachne kingiana sbsp. *multiflora*	Native Seeds Pty	Sold as *Anthosachne scabra* (R. Br) Nevski but is more likely to be *A. kingiana* subsp. *multiflora*	Sown in autumn but no germination until late in a wet spring. Grew and produced seed despite summer dry conditions.

Figure 1. A sowing rate for *Microlaena stipoides* and *Rytidosperma* spp. that leads to a thick sward capable of suppressing weeds.

Which cover crops grow well at Mylor?

The soil at the experimental site is a shallow, acidic sandy loam. It erodes easily either by wind or water and is hydrophobic when dry. Many native grass practitioners stated that cover crops might be useful both for weed and erosion control while native grasses are establishing (see Chapter 2). Dr. Jason Able, Head of the Department of Agricultural Science at the University of Adelaide, an experience cereal breeder, was asked to recommended cereal cover crops that would be short in stature, annual, a low weed risk and would minimise competition to native grasses. He recommended durum wheat (*Triticum durum* var. Aurora), bread wheat (*Triticum aestivum* var. Mace) and barley (*Hordeum vulgare* var. Compass). Coopers Farm Supplies at Mt. Torrens, SA also recommended Forrester forage oats (*Avena sativa* var. Forrester).

In autumn 2019, the four cereal cover crops were sown in 300 mm or 400 mm rows with Wallaby grass (*Rytidosperma caespitosum* (Gaudich) Connor & Edgar) in the midrow. Seed predation by birds, especially magpies, was a severe problem. The birds did not remove native grass seed but removed the entire cereal cover crop twice. Cover crops then had to be resown and the area netted to reduce bird predation. This showed that any cover crops used in trials at Mylor would also have to be netted. It also implied that cover cropping might be a problematic method for native grass establishment in pastures more generally if seed predation by birds is high.

In addition, the cereals competed with the native grass for soil moisture as soils dried in early summer. The cereals were slashed to reduce their vigour and to provide a mulch for the native grasses. Wallaby grass survival and plant biomass was higher in the rows spaced at 400 mm between cover crops than the rows spaced at 300 mm. Wallaby grass survival was highest with durum and bread wheat as the cover crop whereas the barley and oats cover crops were too competitive leading to poor native grass establishment.

Summer growing White French millet *(Panicum milleaceum* L.) provided useful protection, weed competition and mulch for establishing C4 Kangaroo grass. The Kangaroo grass was sown at 800 mm row spacings and the millet in the midrow. This would be difficult to do with commercial seeders but a small manual seeder worked well in the TCA (Fig. 2).

Figure 2. A hand seeder used to sow *Panicum milleaceum* and *Themeda triandra* (with awns removed).

Does soil solarisation control weeds well enough for native grasses to establish?

It was hypothesised from the literature that solarisation with clear, low density polyethylene would control annual weeds well enough for native grasses to establish. This was tested using 50 µm low density polyethylene. The plastic sheets were applied to soil at field capacity for 30-50 days at the hottest time of the year (i.e January to mid-February). Rain was too unpredictable for soil wetting, so irrigation was needed. This meant the trial site would also need irrigation.

Solarisation worked well for establishment of Wallaby grass (*Rytidosperma caespitosum*), Weeping rice grass (*Microlaena stipoides*) and Kangaroo grass (*Themeda triandra*). Annual grasses like silver grass (*Vulpia bromoides* (L.) Gray) and bromes (*Bromus* spp. L.) were well controlled. However, some weeds were not controlled. These were either hard-seeded species like subterranean clover (*Trifolium subterraneum* L.) or species with propagules below the main solarisation zone of about 50 mm like Sorrell (e.g. *Rumex acetosa* L.). Native grass establishment was highest when the weeds that were poorly controlled by solarisation were killed either by manual removal or herbicide.

Although solarisation with low density polyethylene was effective for native grass establishment, it cannot be recycled in South Australia. To test whether it could be used for more than one season, the same sheets were used in two consecutive years. They were degraded by UV exposure after the first season's use and split in the second year either at application or soon afterwards. When the plastic split, heat was released and solarisation was less effective for controlling weeds. These problems led to the field trial comparing polyethylene with biodegradable plastic (Ch. 5).

How should native grasses be sown for the trials?

Different sowing methods for native grasses were compared: scattered or in rows. Weed control was more efficient when they were sown in rows (Fig. 3) because a) weeds in the midrow could be managed more quickly and b) it was easier to distinguish the native grasses from the weeds.

For *Rytidosperma caespitosum* and *Microlaena stipoides*, row spacings of 200, 300 and 400 mm were tested because these would be common spacings for many seeders. There was too much competition between adjacent rows in the 200 mm spacing and too much space for weeds to establish in the 400 mm rows. The 300 mm spacing was a good balance between competition and weed exclusion.

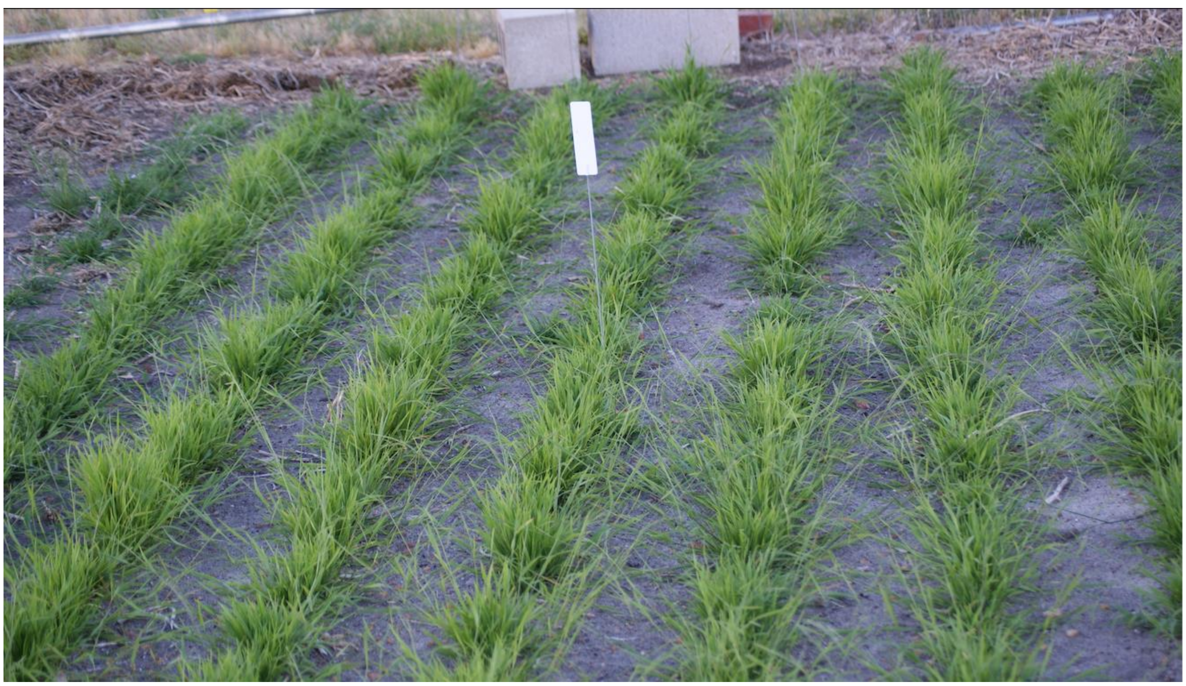

Figure 3. Six month old *Microlaena stipoides* plants growing in rows spaced at 300 mm to maximise weed control efficiency.

It is difficult to sow most native pasture grasses with conventional seeders because their awns and appendages cause them to stick together and to clog the seeding mechanisms. Native grasses are therefore often sown using special seeders that distribute the seed randomly. Sowing the seed in rows as shown in Fig. 3 would require pelletisation of the seed, which adds additional cost and handling.

Should C3 and C4 grasses be sown together or separately?

Both at Mylor and in the Waite Arboretum, C3 grasses were more difficult to establish than C4 grasses because the seed bank is dominated by cool season annual weeds. They compete for light, nutrients and soil moisture when the C3 grasses are establishing. This was consistent with Semples' (1999) findings. As suggested by Chivers and Raulings (2009), weed control was best and maintenance was least when starting with a single species of native grass. Once weeds were very well controlled (e.g. about 2 years after solarisation) it was possible to oversow C4 species into the C3 sward.

How much weed control is needed?

Once native grasses established thickly, weeds were few. The best native grass establishment was with 100% weed control, a high sowing rate and the optimum row spacing for the species. With less weed control and/or poor establishment (i.e. through competition, drought or poor seed quality) weeds continued to be a problem and often increased in the following years.

Do native grasses need irrigation for establishment?

At times of soil moisture stress (especially in January and February) irrigation in the first year of establishment meant that more plants survived and had higher biomass, leading to better weed suppression. However, irrigation can also trigger weed germination.

Summary and conclusions

In summary, the Test of Concept area was an important tool for filtering ideas relatively quickly and at low cost compared to a randomised trial. It showed that native grasses cannot establish when there is regular grazing pressure from kangaroos and/or rabbits. It also demonstrated the benefits of planting native grasses in agricultural rows to increase weeding efficiency. In general, the most successful method was to start with a thick sowing of a single species (either C3 or C4) to provide maximum native grass competition for weeds. Once this sward is well-established and weed-free, other species (C3 or C4) can be added to it.

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Contribution to the Paper	Research (literature and practitioner interviews) to determine which methods to include in the trial, trial design, trial set up and maintenance, data collection, data analysis, first draft of manuscript.		
Overall percentage (%)	75		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	8 March 2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Assistance with literature research, trial design, literature and editing of manuscript.		
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A comparison of seven methods for controlling the soil seed bank

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Abstract

Perennial native grasses have the potential to improve pastures dominated by annual grasses and broadleaf weeds. However, these native pasture grasses can be difficult to establish due to their slow seedling growth which makes them vulnerable against competition from rapidly growing annuals. Good control of the soil seed bank is needed to provide an effective establishment window of bare ground to seed into. In this field study, seven methods of seed bank management were compared in a randomised block trial. These were: 1) herbicide only, 2) harrow, 3) till/harrow, 4) soil solarisation, 5) topsoil removal, 6) soil inversion and 7) burning. In all methods, herbicide was used to increase the effectiveness of weed control and to kill weeds emerging after spring rains. Soil solarisation and topsoil removal were equally effective with about 75% bare ground by the sowing time for C3 native grasses. Till/harrow, harrow and topsoil inversion were only partially successful with 46-55% bare ground respectively while burning (23% bare ground) and herbicide alone (28% bare ground) had the least bare ground at sowing time. It is concluded that soil solarisation and topsoil removal combined with spraying of the first emerging weeds are suitable options for native grass establishment.

Keywords: pasture renovation, soil seed bank, native perennial grasses

Introduction

Annual weed seeds dominate the soil seed bank beneath pastures dominated by annual species (Friend *et al.* 1997) like bromes (*Bromus* spp. L.), wild oats (*Avena fatua* L.), and Cape weed (*Arctotheca calendula* (L.) Levyns). This population of viable propagules in the soil and litter forms a substantial soil weed bank (Lodge 2001). Seeds enter the seed bank through a variety of means including self-burying (Smith *et al.* 1999), animal trampling and burial by insects (Standish *et al.* 2007). These propagules can germinate and grow quickly, making it difficult for seedlings of slow-growing perennial native grasses to establish (Cole and Lunt 2005; Mitchell *et al.* 2014). Therefore, the soil weed seed bank needs to be controlled to create a window for their establishment (Cole and Lunt 2005).

Some perennial native grasses have potential for amelioration of temperate pastures where fertilisation is limited and annual plants are dominating (Archer *et al.* 1993; Whalley *et al.* 2005). In a three-year assessment over eight sites across temperate Australia, the Native and Low-input Grasses Network (NLIGN) identified native grasses that provide good quality fodder and have potential to persist in harsh conditions (Sanford *et al.* 2005). In the high rainfall Mediterranean climate zone (i.e. > 900 mm annual average rainfall), native grasses had greater survival and recruitment than introduced pasture grasses.

Effective methods for native grass pasture establishment in the higher rainfall areas (i.e. above 600 mm annual average rainfall) of southern Australia are lacking (Lodge 2000; Semple *et al.* 1999). In south-eastern Australia, topsoil removal (scalping) has been the most successful method of controlling the soil weed bank for grassland restoration (Brown *et al.* 2017; Morris and Gibson-Roy 2017). The depth of topsoil removed is site-specific (often 100 mm or more) depending on the weeds present, land use history, nutrient levels and soil types (Gibson-Roy and Delpratt 2015). Topsoil scalping removes much of the weed seed/propagule bank and also lowers soil nutrients, especially phosphorus and nitrogen, which fuel the growth of weeds (Gibson-Roy and Delpratt 2015). However, it is unsuitable for shallow soils or sites with a high risk of erosion (Brown *et al.* 2017). At these sites, other methods for weed seed bank management are required.

There are a number of methods commonly used to control weed seed germination in agriculture and/or horticulture. Mouldboard ploughing inverts the soil and buries weed seeds, reducing weed germination (Cole and Johnston 2006). Solarisation of the soil by covering it with a plastic sheet in summer is a successful weed control method used mainly in horticulture on thousands of hectares in Mediterranean zones (Katan *et al.* 2010; Stapleton *et al.* 2005). The principle is that soil under the plastic sheet reaches higher temperatures than uncovered soil, killing seeds and any seedlings that germinate under the plastic (Linke 1994; Marshall *et al.* 2013). Some organic farmers in New Zealand use a 'false seedbed' to reduce the soil seed bank prior to conventional pasture establishment (Merfield 2013). The seed bed is prepared through tillage to bury weed seed. Weed seeds at or close to the soil surface germinate and are then destroyed with harrowing of the top 50 mm of soil prior to sowing the desired crop (Merfield 2013). In another method, standing grass is killed and then burned to stimulate the seed bank. The emerging weeds are then killed before seed sowing (Merfield 2013).

It has been suggested that herbicide (e.g. glyphosate) could be used to increase the effectiveness of other pre-sowing weed treatments (Gibson-Roy *et al.* 2010). For example, it has been found that hard seeds are often not killed and may be stimulated by soil solarisation (Linke 1994). Therefore, follow up with a suitable herbicide could increase treatment effectiveness.

The aim of this study in a high rainfall region (> 900 mm average annual rainfall) in South Australia, was to compare seven methods of seedbed preparation. They were: 1) herbicide, 2) harrow, 3) till/harrow, 4) soil solarisation, 5) topsoil removal, 6) soil inversion and 7) burning. In all methods, weeds emerging after a wet spring were sprayed with Roundup Biactive™. The area of bare ground at the sowing time for C3 native grasses (after the autumn rains) was used to determine whether there was a potential germination window for native grasses. The hypothesis was that topsoil removal would be the most effective weed control method and that tilling/harrow would be more effective than harrow.

Materials and Methods

The experiment was conducted at Winderlup, near Mylor (-35.038040, 138.722950) in the southern Mount Lofty Ranges of South Australia, approximately 30 km south-east of Adelaide, from January 2018 to May 2019. The area has a Mediterranean climate with hot dry summers and cool wet winters. The altitude is about 320 m with an east-facing aspect. Annual rainfall recorded at the nearby Bureau of Meteorology station at Verdun in 2018 was 733.8 mm with most rain falling in the cooler months between April and September (BOM 2019).

Monthly rainfall from Verdun station (3 km away) and monthly temperature, taken as an average between Mt. Lofty station (7.4 km away) and Mt. Barker station (8.7 km away), are given in Figure 1. It shows that during the trial period, rainfall was well below the average of the previous 10 years in early spring 2018 but was nearly double this average in November 2018. From January 2019 to the end of the experiment, rainfall was also below average. Mean daily temperatures were above average for most of the trial period.

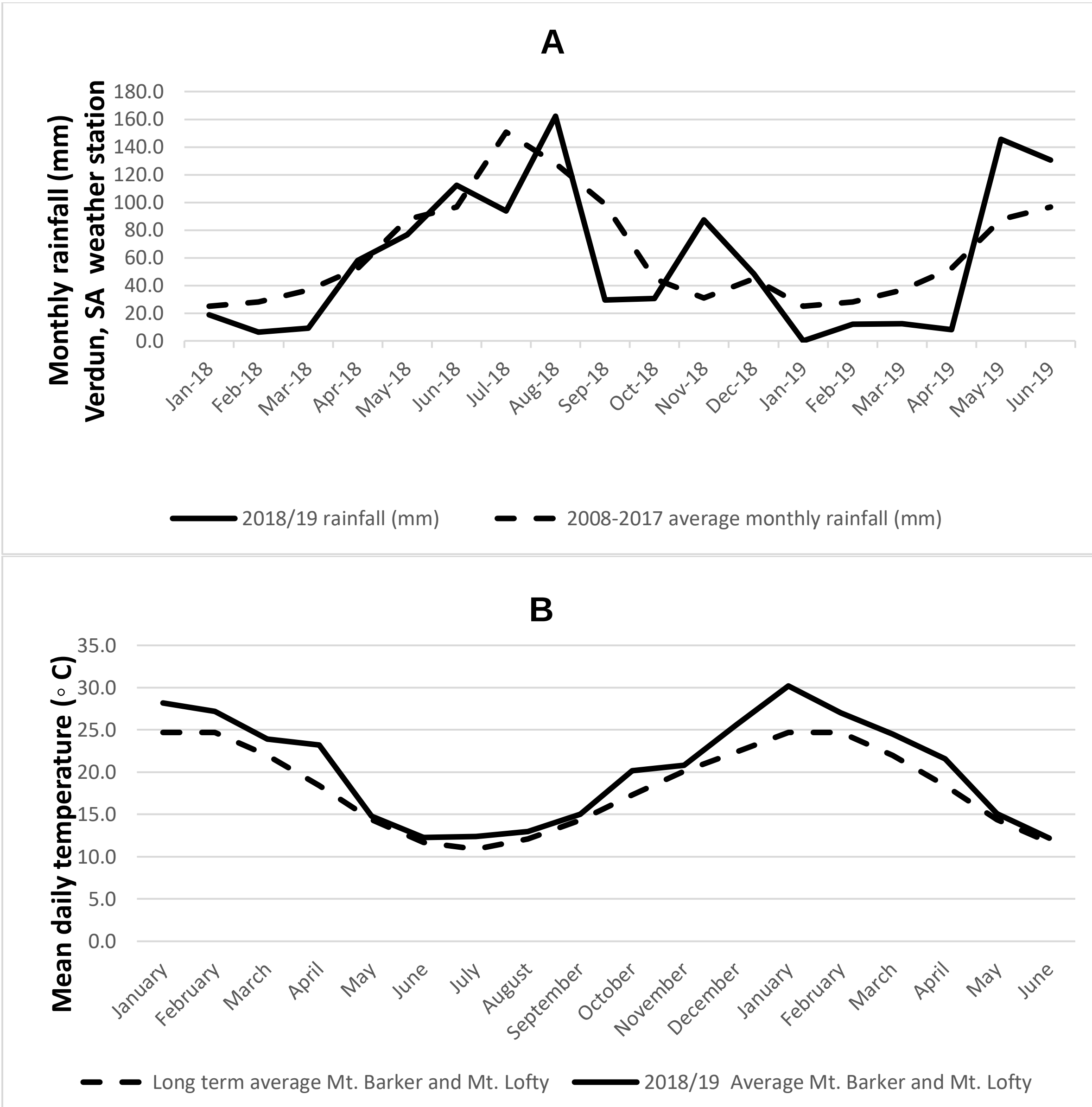

Figure 1. A. monthly rainfall (mm) at Verdun weather station 3 km from Winderlup compared with the monthly rainfall average for 2008-2017. B. Mean daily temperatures (°C) for the period of the trial compared with the mean daily long term average between Mt. Barker and Mt. Lofty weather stations.

The soil is a Kurosol with a shallow (between 100 and 170 mm) acidic sandy loam over clay on rock (Data_SA 2016). The sandy loam becomes non-wetting when it dries. Soil samples (0-100 mm) taken across the site in January 2018 showed that pH varied between 5.7 and 6.3 ($CaCl_2$), available P (Colwell) was between 12 and 33 mg kg^{-1} and available N (mainly nitrate) ranged from 5 to 28 mg kg^{-1}. The site was a sclerophyll stringy bark (*Eucalyptus obliqua* L. Hér. and *Eucalypus baxteri* Benth.) woodland until the late 1800s when it was cleared for orchards. The fruit trees were removed in about 1917. It has since been used at various times for grazing sheep, alpacas and beef cattle. In the past 15 years, grazing has been of low intensity with little fertiliser use and no cultivation.

An initial botanical survey of the site on 17 January 2018 found no native grasses and few perennial grasses. It was dominated by annual grasses especially bromes (*Bromus* spp.L.), wild oats (*Avena fatua* L.) and silver grass (*Vulpia bromoides* (L.) Gray) and subterranean clover (*Trifolium subterraneum* L.). The predominant broadleaf weed was Cape weed (*Arctotheca calendula* (L.) Levyns). These species have arrived on the property mostly through wind-blown seed, contaminated machinery and transport by animals (e.g. excrement, fleece contamination).

Experimental design

The trial was a randomised complete block design with six blocks. It was fenced to exclude livestock, kangaroos and rabbits. Each block had a 'nil treatment' control and seven soil seed bank management treatments, which were: 1) herbicide 2) harrow, 3) till/harrow, 4) soil solarisation 5) topsoil removal, 6) soil inversion, and 7) burning. Treatments started on 18th January 2018. Roundup Biactive ™ (glyphosate present as Isopropylamine salt @ 360g/L) was used at a rate of 15 ml/L of water to kill vegetation prior to harrowing, soil solarisation, and burning and to control weeds emerging in the winter and spring following treatment (Table 1).

Each plot was 1 x 1 m with a 0.5 m buffer between plots and 1.5 m between blocks. The verges were kept mown.

Treatments started at different times due to their differing methods for controlling surface weeds and the soil seed bank. For example, soil solarisation has to be undertaken in the hottest months of the summer, while burning benefits from dry biomass but must also be conducted safely, which is generally only possible in the late winter in Mylor. There was weed germination due to a wetter than average October/November leading to a mass germination from the soil seed bank. Therefore, all weed bank management treatments were sprayed with Roundup Biactive™ herbicide(15 ml per litre of water) on 2 December 2018.

For the period of soil solarisation with polyethylene (17th January to 12th March 2018), soil temperatures at 50 mm depth were measured using a T-Tec data logger probe in one solarisation plot and one till/harrow plot. On 20 May 2019, weed emergence was monitored using the quadrat point method (Crocker and Tiver 1948; Levy 1927) by placing a 1 m^2 grid over each plot. The grid had at 200, 300, 400, 500, 600, 700 and 800 cm in both directions to create a grid with 49 intersection points. Anything that touched the point was recorded as one of the categories broadleaf/clover, grass, bare ground. The sum of grass and broadleaf cover may be greater than 100% as some points contacted both grass and broadleaf plants.

Table 1. Details of Mylor 2018 trial treatments, dates and methods.

TREATMENT	DATES in 2018	METHODS
Control		
Nil treatment		
Herbicide		
Spray with Roundup Biactive™ * Spray with Roundup Biactive™*	22 August 2 December	Spray all vegetation
Harrow/herbicide		
Spray with Roundup Biactive™*	17 May	Harrow to 50 mm depth twice in both directions using a landscaping rake. Remove dead vegetation and small stones.
Harrow to stimulate weed germination	23 August	
Spot spray*** with Roundup Biactive™*	25 September	
Spray with Roundup Biactive™*	2 December	
Till/harrow/herbicide		
Till top 100 mm of soil, harrow top 50 mm.	17 January	Till with a small rotary hoe set to cultivate the top ± 100 mm. Harrow to 50 mm depth twice in both directions using a landscaping rake. Remove dead vegetation and small stones.
Spray with Roundup Biactive™*	17 May	
Harrow top 30-50 mm to stimulate germination	23 August	
Spot spray*** with Roundup Biactive™*	25 September	
Spray with Roundup Biactive™*	2 December	
Till/soil solarisation/harrow/herbicide		
Till, harrow, irrigate and apply 50 μm polyethylene plastic for 53 days	18 January	Till with a small rotary hoe set to cultivate the top ± 100 mm. Remove dead vegetation and small stones. Water with soil wetting agent** and irrigate to 50 mm depth. Cover with 50 μm low density polyethylene and bury edges 50-100 mm.
Remove polyethylene	12 March	
Harrow top 30-50 mm with a landscaping rake to about 50 mm depth	14 May	Harrow to 50 mm depth twice in both directions using a landscaping rake to stimulate germination of hard-seeded species (e.g. clovers)
Spray with Roundup Biactive™*	22 August	
Spot spray*** with Roundup Biactive™*	25 September	plot 17 and 27 only– the other plots were bare
Spray with Roundup Biactive™*	2 December	

Table 1 (cont'd). Details of Mylor 2018 trial treatments, dates and methods.

Topsoil removal/herbicide		
Topsoil removal	24 August	Remove top 50 mm of soil with a trenching shovel.
Spot spray*** with Roundup Biactive™*	25 September	
Spot spray*** with Roundup Biactive™*	2 December	
Soil inversion/herbicide		
Invert soil to a depth of approximately 100 mm	14 May	A shovel was used to invert the soil.
Spray with Roundup Biactive™*	22 August	
Spot spray*** with Roundup Biactive™*	25 September	
Spray with Roundup Biactive™*	2 December	
Burning/herbicide		
Spray with Roundup Biactive™* to kill foliage for burning	22 August	The standing biomass was killed to provide dry fuel. Burned with an LPG fuelled Cambridge Weed Burner until all vegetation was removed.
Burn plots	10 September	
Spot spray*** weeds in plot 20 (no weeds in other plots)	25 September	
Spray with Roundup Biactive™*	2 December	

*Roundup Biactive™ contains glyphosate as Isopropylamine salt @ 360 g/L. This was applied at 15 ml/L of water

** Brunnings Easy Wetta (10 to < 30% surfactant) applied at 20 ml $m^{2\ -1}$.

***spot spraying means there were only a few weeds and only these were sprayed

Statistical analyses

Data of bare ground and broadleaf cover were analysed by univariate one-way ANOVA (SPSS version 26) with treatment type as a fixed factor. The grass cover data was strongly skewed because the control plots had 100% grass cover and the treatments had very little. It was assessed with a Kruskal-Wallis non-parametric test (SPSS version 26). Significant differences in bare ground, grass and broadleaf cover among means of treatments were compared by Tukey test ($P \geq 0.05$). Only significant differences ($P \leq 0.05$) are described.

Results

Soil temperatures were recorded over 40 days (the period the polyethylene remained intact) in a tilled plot and an adjacent tilled and solarised plot. The solarised plot had a higher daily maximum temperature for the first 23 days than the tilled plot (Fig 2.). After this period, there was little difference between the two treatments even though the polyethylene remained intact. The maximum temperature at 50 mm depth during the 40-day solarisation period was 43.5 °C for tilled soil and 48.5 °C for soil covered with polyethylene while the maximum ambient air temperature, averaged between the two closest weather stations (Kuitpo and Mt. Lofty) was 38.95 °C. The difference in maximum temperature at 50 mm depth was greatest in the first 10 days; on average 7.4 ºC higher in the solarised plot.

When vegetation coverage was recorded on 20 May 2019, there were noticeable differences in weed germination between treatments. This date was selected because there had been a mass germination of weed in the surrounding paddock in response to the autumn rains and it was also the ideal time for sowing C3 native grasses (although none were sown in this experiment).

There was 100% grass cover in the control plots. All management treatments had less than 10% grass cover with no differences among them (Fig. 3). There was least broadleaf weed cover in the nil treatment controls (8% ± 4 %), solarisation (21% ± 3%) and topsoil removal (24% ± 3%) treatments. Other treatments had higher broadleaf weed cover. Till/harrow (46 ± 4 %), inversion (49% ± 2%) and harrow (55% ± 7) had more broadleaf weeds but significantly less broadleaf weed cover than the herbicide only (67% ± 4 %) and burned plots 74% (± 5%).

All treatments had significantly more bare ground than the control, which had no bare ground. The percentage of bare ground was highest after solarisation (77% ± 3%) and topsoil removal (71% ± 3%). Harrow (38% ± 6%), till/harrow (50% ± 3%) and soil inversion (50% ± 2%) did not differ significantly. There was least bare ground after spraying (28% ± 4%) and burning (23% ± 4%).

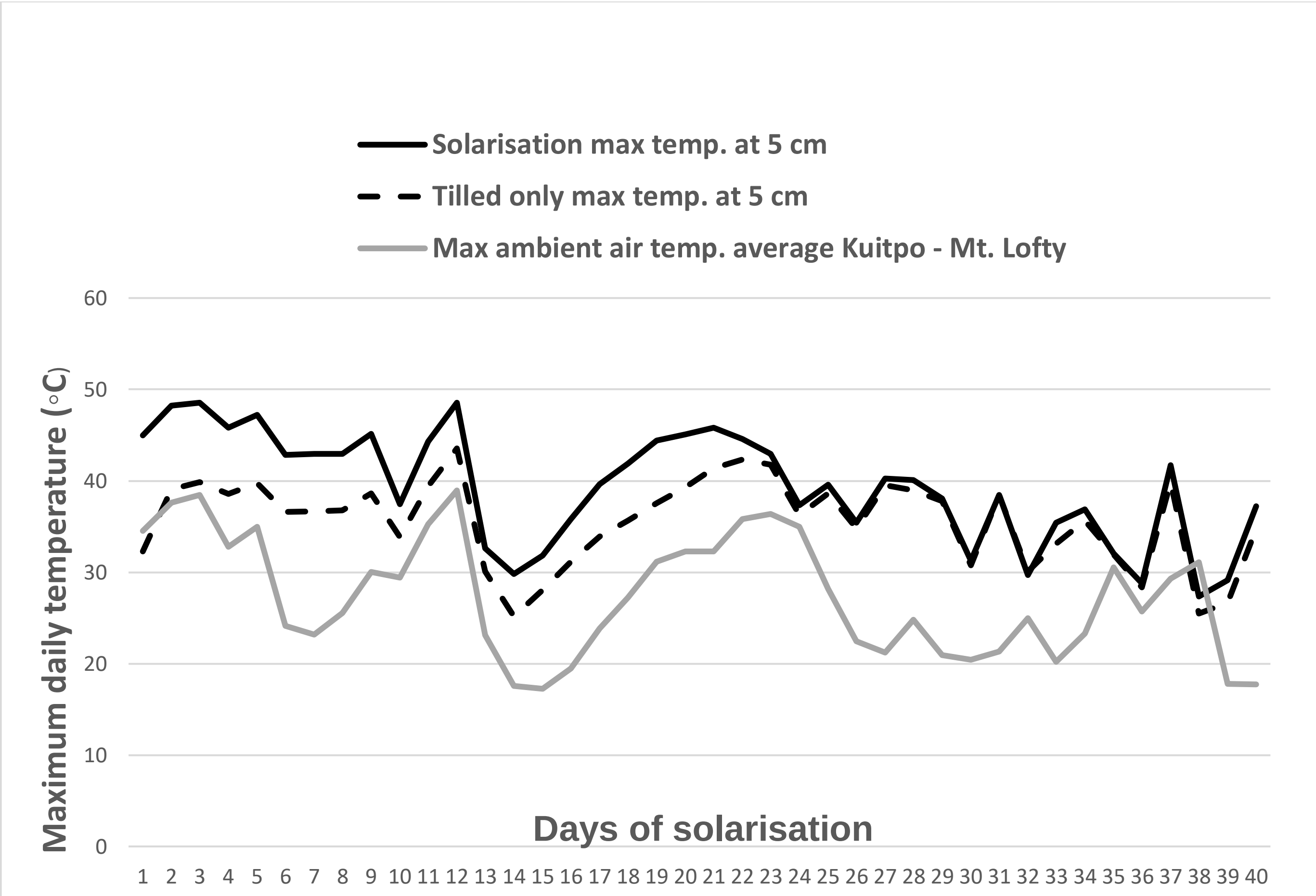

Figure 2. Maximum daily temperatures for a solarisation and an adjacent tilled plot at 50 mm depth and maximum ambient air temperature (average of Mt. Lofty and Kuitpo weather stations).

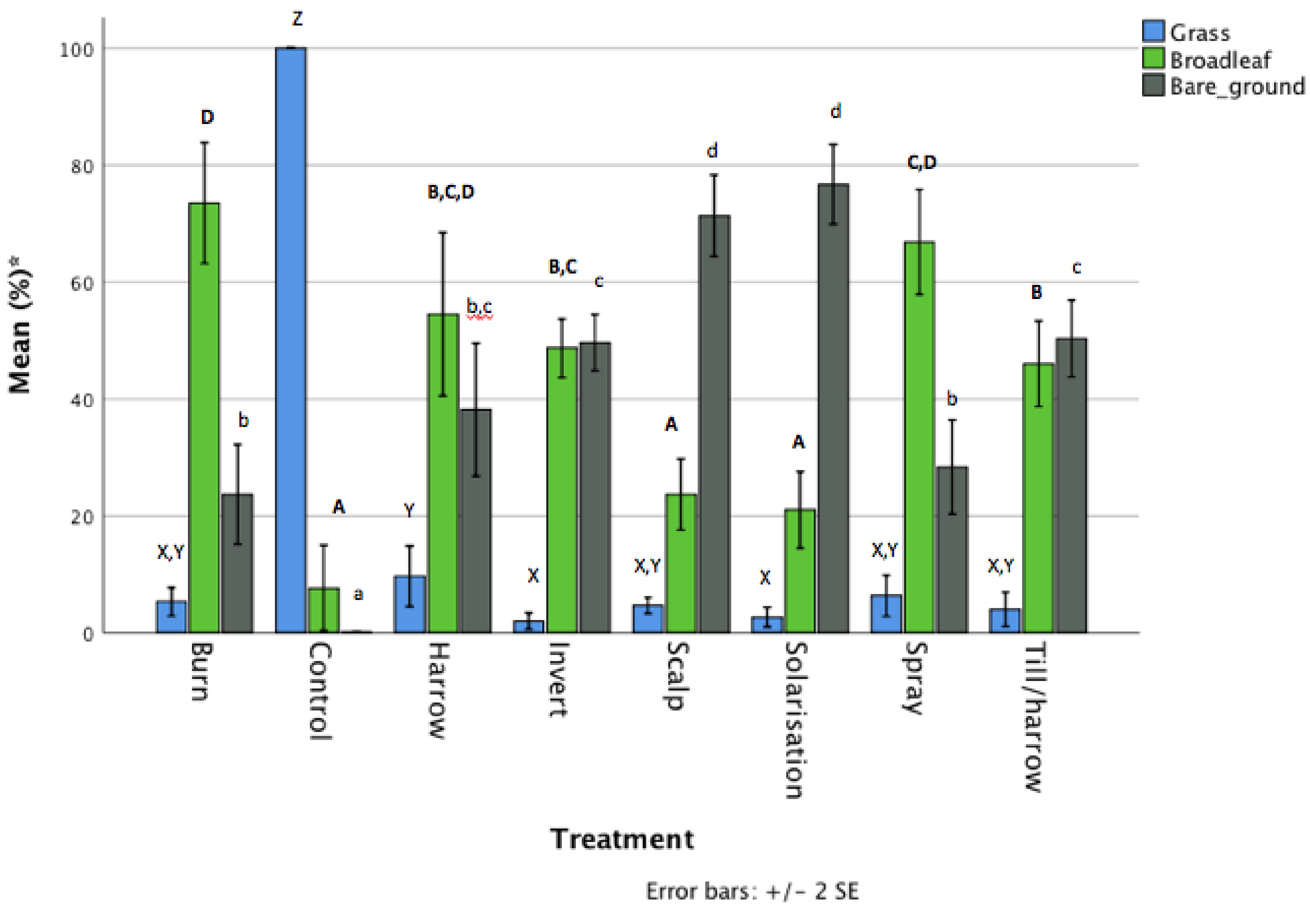

Figure 3. Percentage grass and broadleaf cover and bare ground on 20 May 2019. Significant differences (P ≤ 0.05) among treatments in grass cover are indicated by X,Y and Z, in broadleaf cover by upper case letters A,B,C and D and in bare-ground cover by lower case letters a, b, c and d.

Discussion

The hypothesis that topsoil removal would be the most effective weed control treatment is not supported because solarisation had similar weed cover. In practice, both methods could be made more effective by spraying any emerging weeds with knock-down herbicide again prior to sowing native grasses. In this experiment, emerging plants were predominantly clovers.

Consistent with the findings of others (Brown *et al.* 2017; Gibson-Roy *et al.* 2010), topsoil removal was an effective method to reduce the soil seed bank. A limitation of topsoil removal in this study was the depth of removed topsoil (50 mm) due to topsoil depth of only 100-170 mm at the trial site. In other studies topsoil removal was to a greater depth (Gibson-Roy and McDonald 2014), varying from 100 mm (Brown *et al.* 2017) to 400 mm (Klimkowska *et al.* 2010), which may remove more of the weed seed bank (Gibson-Roy and McDonald 2014).

Both topsoil removal and solarisation have advantages and disadvantages. Topsoil removal has the advantage of permanently removing propagules from the site (Gibson-Roy 2014). An additional benefit can be a reduction of soil nutrients as they tend to favour introduced species, especially nitrophilous annuals (Prober *et al.* 2002). The disadvantages include the cost of machinery (Jaunatre *et al.* 2014), what to do with the removed topsoil and removal of the soil depth that has high organic matter content and is the most biologically active (Maschmedt 2002).

Soil solarisation is known to work best with annual weeds with a shallow (i.e. top 5-100 mm) seed/propagule bank (Stapleton *et al.* 2008). In agreement with Linke (1994), we found that hard-seeded species like clover germinated after the polyethylene had been removed. The maximum temperature at 50 mm depth was 48.5º C. Other studies reporting successful weed seed bank control through soil solarisation with polyethylene reported temperatures ranging from 45 to 57º C at the same depth (Horowitz *et al.* 1983; Linke 1994; Sauerborn *et al.* 1989). The temperature required for seed death is species related and depends on a combination of soil temperature/heat duration, soil moisture, seed morphology and seed dormancy (Vidotto *et al.* 2013).

An advantage of soil solarisation is the relatively short treatment period. Other methods, for example chemical fallow, have to be maintained for months or years and therefore lead to longer periods of bare ground and higher erosion risks. The disadvantages of solarisation include firstly the need for wet soil at the hottest time of the year; requiring well-timed rain or irrigation and secondly, labour costs for laying and removing the polyethylene. Thirdly, polyethylene cannot be recycled in many locations and therefore it creates a landfill burden. To avoid landfill, biofilms that break down to water, CO_2 and microbial biomass could be tested.

Herbicide alone did not significantly reduce the soil seed bank with only 28% bare ground by seeding time. This is consistent with the findings of Gibson-Roy *et al.* (2010). Whilst we are unable to quantify the effect of using herbicide with the other treatments, we do know that, without it, more weeds would have been present for all treatments.

The smoke from burning leads to germination of the soil seed bank (Dixon *et al.* 2009) but, even combined with herbicide, this did not create more bare ground by sowing time than the no treatment control. This finding is also consistent with Gibson-Roy *et al.* (2010).

In agreement with other studies, three other methods: till/harrow, soil inversion and harrowing were only partially successful (Czerwiński *et al.* 2014; Gibson-Roy *et al.* 2010; Merfield 2013) even with the addition of herbicide. They resulted in about 50% broadleaf weed cover. These were mostly broadleaf weeds which would grow rapidly and smother any germinating native grasses in the coming months. For these methods to be more effective, they may require a longer pre-sowing weed treatment period (i.e. more than 18 months), with repeated soil treatment and/or post-sowing management, for example with broadleaf herbicides. However, longer soil preparation periods with bare ground have the potential to increase erosion risks.

It was hypothesised that till/harrow would be more effective than harrowing alone because tillage would bury a greater proportion of weed seed to a depth where it would not germinate. However, this hypothesis was not supported because the two treatments had similar percentage bare ground. A possible explanation is that these soils had not previously been tilled and the vegetation was dominated by annual weeds. If most seeds were close to the soil surface and readily germinated after regular harrowing, tillage would not provide an additional benefit. If harrowing alone is just as effective, this is a benefit to the soil and any subsequent crops because tillage can damage soil structure, diminish soil organic matter and reduce water holding capacity (Carter 2002).

For larger areas, soil solarisation requires machinery to lay and retrieve the polyethylene. It requires a smooth, vegetation-free soil surface and would not be suited to rocky sites. This method may become more attractive as new biodegradable plastics and sprayable polymers are currently being developed which may have applications for rangeland settings in the near future (Adhikari *et al.* 2016).

In conclusion, at this site with a Mediterranean climate, soil solarisation could be an effective alternative to topsoil removal for weed control prior to sowing plants with slow seedling development. Harrowing and topsoil inversion may be useful tools but would require longer treatment time to be effective. Since grasses were not sown in this experiment, further work is needed to investigate the effect of topsoil removal and solarisation on the establishment of native grasses.

Conflicts of Interest

The authors declare no conflicts of interest.

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CHAPTER 5. Waite Biofilm Trial

Statement of Authorship

Title of Paper	Waite Biofilm Trial
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Marne Durnin		
Contribution to the Paper	Research into biodegradable plastics and manufacturers. Identification of products used in the trial. Liaison with Biobag Australia. In collaboration with other authors: trial design, project timetable, development of data collection methods. In collaboration with Nick Timbs: trial set up, monitoring, sowing. Data collection and data analysis (in collaboration with the Biometry Hub and Petra Marschner). Drafting of the manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	8 March 2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Petra Marschner		
Contribution to the Paper	In collaboration with Kate and Marne: trial design, project timetable and development of data collection methods. With Marne: assistance with data analysis and manuscript editing.		
Signature		Date	09/03/2021

Name of Co-Author	Dr. Kate Delaporte		
Contribution to the Paper	With Marne and Petra: trial design, project timetable and development of data collection methods. Oversight of site selection, species selection, site management.		
Signature		Date	12/3/21

Please cut and paste additional co-author panels here as required.

Statement of Authorship (continued)

Name of Co-Author	Nick Timbs		
Contribution to the Paper	Trial site maintenance, site preparation, application of treatments, assistance with sowing and data collection.		
Signature		Date	10/3/2021

Assessing the potential of solarisation with polyethylene and biodegradable plastic for reducing the annual seed bank

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Keywords: native grass, soil seed bank, solarisation, polyethylene, biodegradable plastic

Abstract

Native grasses have potential to improve pastures dominated by annual weeds but their establishment requires effective management of the soil seed bank. Soil solarisation with low density polyethylene (LDPE) is a widely used method of seed bank management in Mediterranean climates. However, its use creates a long-term landfill burden in most locations because it is neither recyclable nor biodegradable. Biodegradable plastic film is becoming available in Australia and elsewhere. We compared tillage and solarisation with LDPE or with biodegradable plastic (biofilm) in a randomised block experiment. Herbicides were also used to control emerging weeds. Maximum daily soil temperatures were highest under the LDPE (41.7 ± 0.4 °C) compared to the biofilm (39.8 ± 0.3 °C). Native grasses (*Rytidosperma caespitosum* (Gaudich.) Connor & Edgar and *Microlaena stipoides* var. Burra) were sown after solarisation. They established well on all sown treatments and percentage groundcover was: LDPE (51% ± 7%), tillage (34% ± 7%) and biofilm (33% ± 7%). The remaining plot area was mainly bare ground due to herbicide treatments. The lack of treatment differences is likely due to the small plot size, high seedbank variability within treatments and herbicide application to all plots except the control. It is concluded that native grasses

may establish well with herbicide treatments alone and that solarisation with either LDPE or biofilm may not provide an additional benefit but a larger-scale experiment using a modified design is recommended.

Introduction

In a Mediterranean climate, many introduced perennial grass species do not survive the dry summers and eventually die, leaving pastures dominated by annual grasses and broadleaf weeds (Whalley *et al.* 2005; Wilson and Simpson 1994). Several studies have suggested that native pasture grasses with traits for drought tolerance, year-long flowering and persistence in dry conditions could improve pastures when introduced perennials do not survive (Firn 2007; Sanford *et al.* 2005).

The potential benefits of native grasses include adaptation to climate (especially variable rainfall), the ability to grow and flower whenever conditions are suitable (Mitchell *et al.* 2015), the ability to survive and grow at low nutrient availability (Lodge 1994; Lodge 1996) and adaptation to a range of soil conditions including acidity and salinity (Lodge 1996). Additionally, deep-rooted perennial grasses have potential to increase soil organic matter (Carter 2002a; Carter and Gregorich 2010). Soil organic matter improves aggregate stability, water holding capacity and aeration (Carter 2002b; Carter and Gregorich 2010; Degens 1997).

Despite their potential benefits, the slow seedling growth of many native pasture grasses means they are easily out-competed for light and moisture by annual grasses and broadleaf weeds. This makes them difficult to establish from seed in a pasture setting (Semple *et al.* 1999).

The competing annual grasses and broadleaf weeds mainly come from the soil seed bank, which consists of seeds that can germinate when conditions are suitable (Park and Allaby 2017). Effective weed management therefore requires management of both surface weeds and the soil seed bank. In Mediterranean climates, soil solarisation with plastic sheets can be an effective seed bank management method in agriculture and horticulture (Adhikari *et al.* 2016; Stapleton 2000). For solarisation, moist soil is covered with a plastic sheet for 30-50 days at the height of summer. Heating of the soil through UV transmission leads to seed death and/or degradation (Marshall *et al.* 2013).

Low density polyethylene (LDPE) is most commonly used for solarisation because its mechanical properties make it easy to apply and retrieve and because it transmits UV radiation well (Ammala *et al.* 2011). In 2001, agricultural LDPE film use in Australia was about 4000 tonnes annually and between 700,000 t (Espi *et al.* 2006) and 1 million t per annum worldwide (Halley *et al.* 2001). However, LDPE is not degradable and there are few recycling facilities thus, after use, it becomes a considerable source of long-term pollution as well as a landfill burden (Brodhagen *et al.* 2015). With increasing awareness of the environmental problems LDPE creates and changes in regulatory frameworks, there has been increasing worldwide research into biopolymers with potential for biodegradability and use in agriculture (Adhikari *et al.* 2016).

One such commercially compostable film is manufactured from Mater-Bi resin by Novamont, Italy. Made from corn starch, vegetable oil products and biodegradable synthetic polyesters (Adhikari *et al.* 2016), it is certified as commercially compostable under the European Standards UNI EN 13432 and EN 17033 (Novamont 2020). Under these standards, biodegradable plastic must break down to only water, CO_2 methane, biomass and mineral salts within a specified time (European Standards 2020). Little is known about the potential of biodegradable films for soil solarisation and native grass establishment. Biodegradable plastic made from Mater-Bi resin has greater porosity than LDPE (Morton 2021, personal communication). The aim of this experiment was to compare the effectiveness of soil solarisation with biodegradable plastic and LDPE plastic for giving native grasses a germination and establishment window. The first hypothesis was that solarisation controls weeds better than tillage alone. The second hypothesis was that the soil beneath the biofilm will not get as hot because of its higher porosity, therefore weed control will be less effective that with LDPE.

Materials and Methods

A randomised block trial was conducted in the Waite Arboretum (-33.0325, 138.629444), in Adelaide, South Australia from January to May 2020. The region has a Mediterranean climate with high evaporation during hot dry summers. Most rain falls in the cool wet winters (May-July). Average annual rainfall is 547 mm (based on all years on record at the nearest weather station) but only 374 mm of rain was recorded in 2019 (BOM 2020a). This was 68% of average and the driest year on record (BOM 2020a).

The pre-European vegetation of the site was open grassy woodland, which was cleared for agricultural and grazing land during the 1800s (Gardner 2015). Between 1928 and 2020, over 2,500 plant specimens from all over the world were planted and successfully established in the Arboretum, surviving on annual rainfall after establishment. Up until 1990, sheep grazed on the site to manage the ground vegetation (Gardener 2015). More recently, the ground vegetation has been managed by mowing and spraying to remove weeds beneath the tree canopies.

The soil in the Waite Arboretum is a Chromosol based on Australian soil classification or Rhodoxeralf according to US Soil Taxonomy, with clay loam to 400 mm which overlays a heavy clay up to 900 mm depth. In February 2020, the soil from the top 100 mm of the trial site had the following properties: pH 6.1 -

6.6 (H_2O), P (Colwell) was 37 – 60 mg kg^{-1} and nitrate N was between 12 and 20 mg kg^{-1}. Ammonium was not detectable.

A vegetation survey of the trial site on 14 January 2020 showed it was dominated by annual grasses (including *Hordeum* sp. L., *Ehrharta* sp. Thunb., and *Bromus* spp. L.) and broad leaf weeds (particularly *Plantago* sp., *Arctotheca calendula* (L.) Levyns, *Polygonum aviculare* L., and *Lepidium africanum* (Burm. F.) DC). No native grasses were found.

Experimental design

The experiment was a randomised block trial with four blocks. Each block had four 1 m^2 plots which were: 'nil treatment' controls; tilled plots; tilled plots covered with 11 μm thick LDPE and tilled plots covered with 14 μm thick biofilm made from Mater-Bi resin. A 0.5 m buffer between plots and a 1 m buffer along the outside of the plots was sprayed to minimise seed drifting onto the plots (Figure 1).

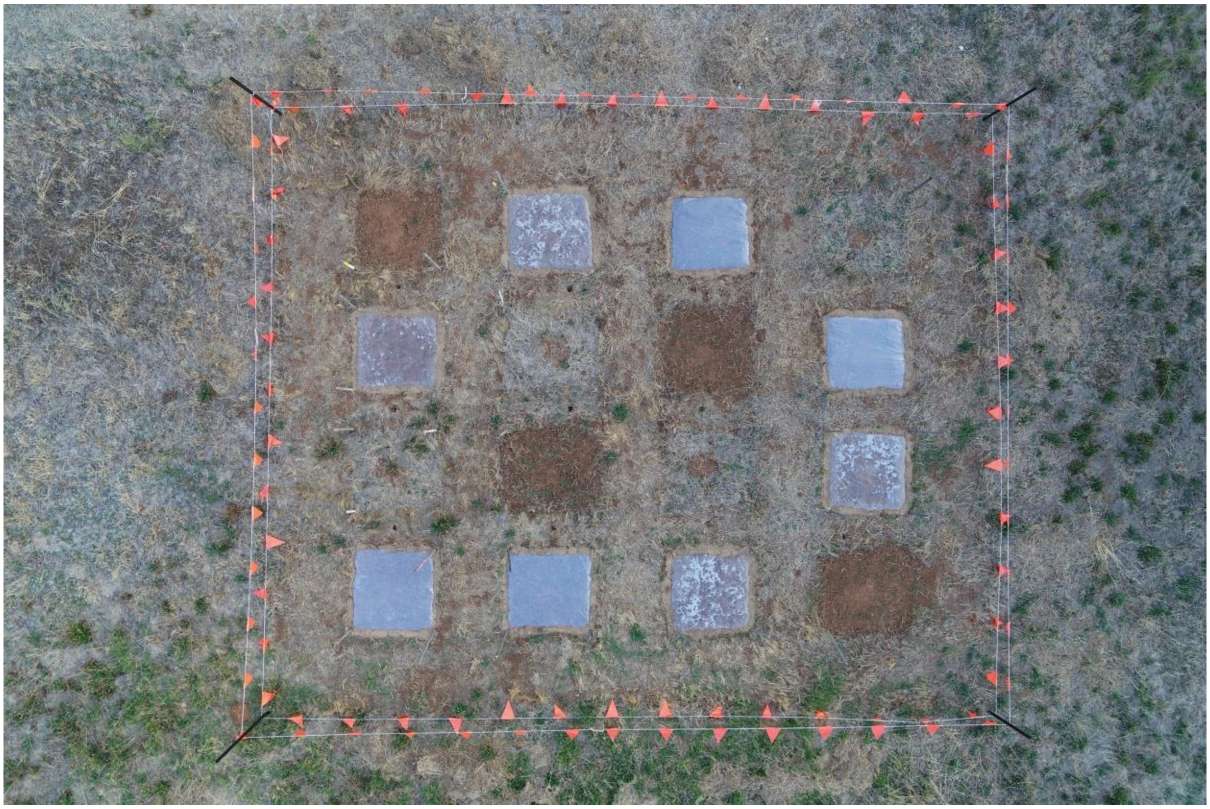

Figure 1. Drone photograph of the Waite Biofilm Trial taken 16 February 2020. Image courtesy of Dr. Ramesh Raja Segaran, URAF, University of Adelaide.

On 4 February 2020, the plots were irrigated to field capacity. To record soil temperature, Hastings 'Tinytag' data loggers were buried in the centre of each plot at 50 mm depth and remained in place until 13 March 2020. The plastic sheets (either LDPE or biofilm) were placed on the plots with the edges of the plastic sheets buried to about 50 mm depth.

Before sowing native grasses, any weeds present on the plots were sprayed on 7 May 2020 with 7 ml L^{-1} Roundup 570™ plus 0.5 ml L^{-1} oxyflourfen. 0n 19th May 2020, each plot (except the nil treatment control) was sown with two rows each of Weeping rice grass (*Microlaena stipoides* var. Burra) and Wallaby grass (*Rytidosperma caespitosum* (Gaudich.) Connor & Edgar) and later sprayed with 7 ml L^{-1} Roundup 570™ to kill emerging weeds. On 10th August 2020, except the 'nil treatment' controls, broadleaf weeds were sprayed with 1.3 ml L^{-1} of Apparent™ MCPA 750 to give the native grasses maximum opportunity to establish. This application rate was too low and did not kill the broadleaf weeds, therefore a second spray with 2.7 ml L^{-1} was applied on 16 Sept 2020.

Data collection

Weed emergence and percentage bare ground on the plots were recorded on 6 May 2020 using a quadrat point intercept method (Crocker and Tiver 1948; Levy 1927). This time was chosen because the soil was moist, triggering a mass germination of autumn weeds and it was the typical sowing time for C3 native grasses. A 1 m^2 grid with fixed wires at 200, 300, 400, 500, 600, 700 and 800 mm was placed over the plot. The wires intersected at 49 points. At each intersection point, a wire was lowered to the ground and anything that touched the wire was recorded as either 'grass' or 'broadleaf' or both. If there was no vegetation, it was recorded as bare ground. The species present on the plots were also recorded.

On 2 December 2020, percentage ground cover was surveyed using a 1 m^2 (inner dimension) square with wires every 200 mm in both directions to create a grid with 25 x 200 mm square quadrats. For each quadrat, the percentage vegetation cover for native grass, broadleaf weeds (including clover), grassy weeds and bare ground was estimated and then averaged to give an overall composition for each plot.

Analyses

Soil temperatures at 50 mm depth were recorded every 30 minutes with Hastings 'Tinytag' data loggers. These data were analysed by univariate one-way ANOVA (IBM SPSS Statistics, version 26) with treatments as a fixed factor. The 6 May 2020 and 2 December 2020 data were analysed by univariate one-way ANOVA (IBM SPSS Statistics, version 26) with treatment type as a fixed factor and ground cover type (i.e. native grass, broadleaf weed, grassy weed and bare ground) as dependent variable. Significant differences among means of treatments were analysed by Tukey test ($P \leq 0.05$). The nil treatment controls had no native grasses and were not sown with native grasses, therefore they were not included in the analysis of native grass ground cover.

For all data, only significant differences are reported.

Results

Soil temperature

The biofilm made from Mater-Bi resin began to deteriorate from day 27 while the LDPE sheet was still intact when both sheets were removed after 41 days (on 13 March 2020).

Figure 1 shows that the maximum daily temperatures recorded at 50 mm depth were related to the maximum daily air temperature at Kent Town, Adelaide (BOM 2020b), which is about 6 km from the experimental site. The biofilm and LDPE solarisation treatments always had a higher maximum soil temperature than the control and the tilled treatment. However, the mean daily maximum temperatures under the biofilm (39.8 ± 0.3 °C) were consistently lower than the mean maximum temperatures under LDPE (41.7 ± 0.4 °C). The difference between the maximum temperature under the two films was greatest on the hottest days. Over the 27-day period, there was no difference in mean daily maximum soil temperature (at 50 mm depth) between the controls (33.9 ± 0.3 °C) and the tilled plots (34.1 ± 0.3 °C). After the biofilm started to deteriorate on 2 March and until the plastic covers were removed on 13 March 2020, the mean daily maximum soil temperature did not differ between the biofilm (34.3 ± 1.2 °C), the control (34.4 ± 1.2 °C) or the tilled treatment (31.7 ± 1.1 °C). The temperature with LDPE was higher at (36 ± 1.4 °C).

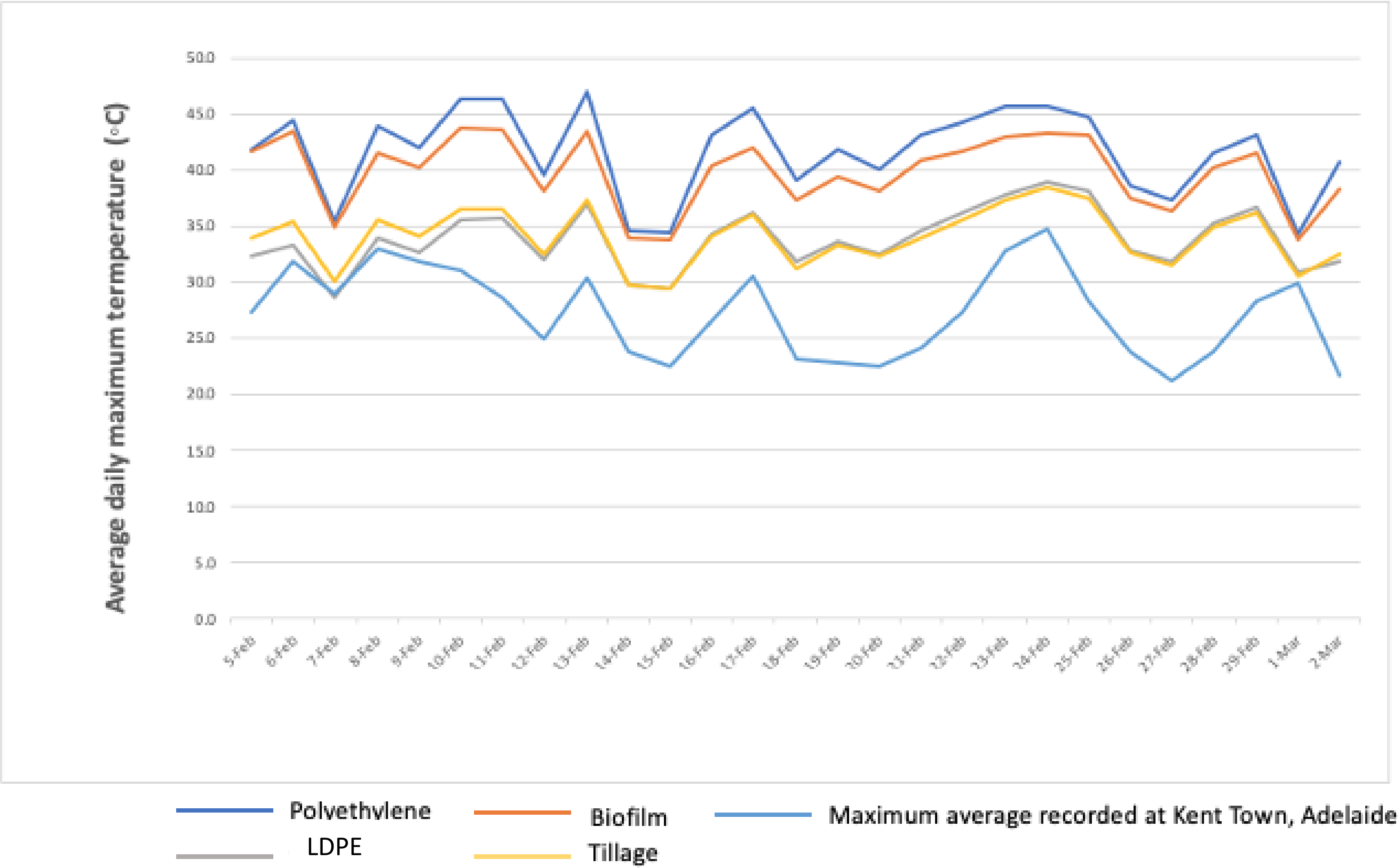

Figure 1. Daily maximum average temperature from 5 February to 2 March 2020, at 50 mm depth for the control, tillage, biofilm and LDPE treatments and daily maximum air temperature (measured at Kent Town, Adelaide)

Pre-sowing vegetation survey 6 May 2020

No native grasses were found. In addition to annual grass seedlings, which were not identified to species level, a range of broadleaf seedlings were also present. These were: *Trifolium* spp., *Arctotheca calendula* (L.) K., *Plantago lanceolata* L., *Heliotropium europaeum* L., *Dysphania pumilio* (R. Br.) Mosyakin & Clemants, *Oxalis pes-caprae* L., *Polygonum aviculare* L., *Trifolium* spp. and *Malva* spp.

The control plots had a high percentage of grass ground cover (80% ± 1%). There was less grass with tillage (35% ± 1%) and biofilm (39% ± 0.1%) plots. The LDPE treatment had very little grass cover (0.1% ± 1%).

There was no difference in broadleaf cover between the controls (78% ± 14%), tillage (61% ± 14%), or biofilm solarisation (37% ± 14%). The treatment with LDPE had less broadleaf cover than the control (20% ± 14%).

Solarisation with LDPE and biofilm led to more bare ground than the tillage treatment (4% ± 6%). However, bare ground percentage was significantly greater with LDPE solarisation (76% ± 6%) than with biofilm (35% ± 6%). It was noted that soil solarisation did not kill soursob (*Oxalis pes-caprae* L.). As a bulb weed with storage organs that may be deeper than 50 mm (the area of treatment effect), this was expected and consistent with the findings of others (Egley 1983; Linke 1994).

Vegetation survey 2 December 2020

All treatments had similar native grass establishment. Percentage cover by native grasses was 51% ± 7% with LDPE, 34% ± 7% with tillage and 33% ± 7% with biofilm. The lack of statistical difference between the treatments was possibly due to high variability among the LDPE plots (ranging from 35-70%) and low variability among the biofilm plots (28-35%).

Due to the MCPA applications on 10 August and 16 September 2020, all treatments had very little broadleaf weed cover. Broadleaf weed cover was 58% ± 4% in the unsprayed controls, 1% ± 4% with LDPE and biofilm and 2% ± 4% in the tillage treatment.

The treatments also had very little grassy weed cover compared to the unsprayed controls (42% ± 6%). Again, there were no significant differences between LDPE (5% ± 6%), biofilm (9% ± 6%) and tillage (<1% ± 6%). The controls had no bare ground. There were no differences in bare ground among the other treatments: LDPE (43% ± 10%), biofilm (58% ± 10%) and tillage (63 % ± 10%).

Discussion

There was no significant difference between sown treatments, therefore both the first (solarisation controls weeds better than tillage alone) and second (solarisation using LDPE would be more effective than solarisation with biofilm) hypotheses are not supported.

An unexpected finding is that neither of the solarisation treatments was more effective than tillage plus herbicides. There was good native grass establishment in all treatments except the control (no native grasses sown) which suggests that, at this location and in this season, solarisation was of little benefit and seed bank management with herbicides was sufficient for native grasses to establish.

The results also expose two key problems in the experimental design:

1) emerging weeds needed to be controlled with herbicide but there was no control plot to measure what effect the herbicides alone had, and;

2) among plots, there was variability both in weed species and in their distribution.

An explanation of these weaknesses and their possible solutions for future research follows.

Pre and post-sowing herbicides

Roundup 570™ (a non-specific herbicide) was used before sowing native grasses. Prior to using this herbicide, soil solarisation with LDPE produced most bare ground; which should have created the best window for native grass establishment. However, the use of herbicide on all treatments, except the control, created 100% bare ground at sowing time altering the impact of the solarisation treatments. Other researchers have found that herbicide alone is not an effective treatment (Brown *et al.* 2017) but there should have been a treatment with herbicide only so its effect could be measured.

A broadleaf herbicide was used after sowing to control weeds that were known to be poorly controlled by solarisation. The MCPA was initially applied at the lowest recommended rate of 1.3 ml L^{-1} as a cautious approach. This was insufficient to kill the weeds and a second spray was used at the higher rate of 2.7 ml L^{-1}. The native grasses were emerging during the interval between these applications. It is possible that, with the weed patchiness and the delay in weed kill, some native grasses were set back or died, leading to greater within treatment variability.

Seed bank variability and plot size

Soil solarisation does not effectively control weeds with hard seeds or with a seed bank deeper than about 50 mm (Linke 1994; Stapleton *et al.* 2000). After sowing, there was higher than expected variability of weed emergence among the plot, particularly broadleafs. Seed bank patchiness led to germination of weeds on some plots but not others leading to high within treatment variability. To reduce this variability, the experimental design would be stronger if the plots were larger (at least 4 m^2) and if there were more replicates. Figure 2 is an experimental design with larger plots and more treatments and replicates so that the effect of the herbicide treatments can be measured.

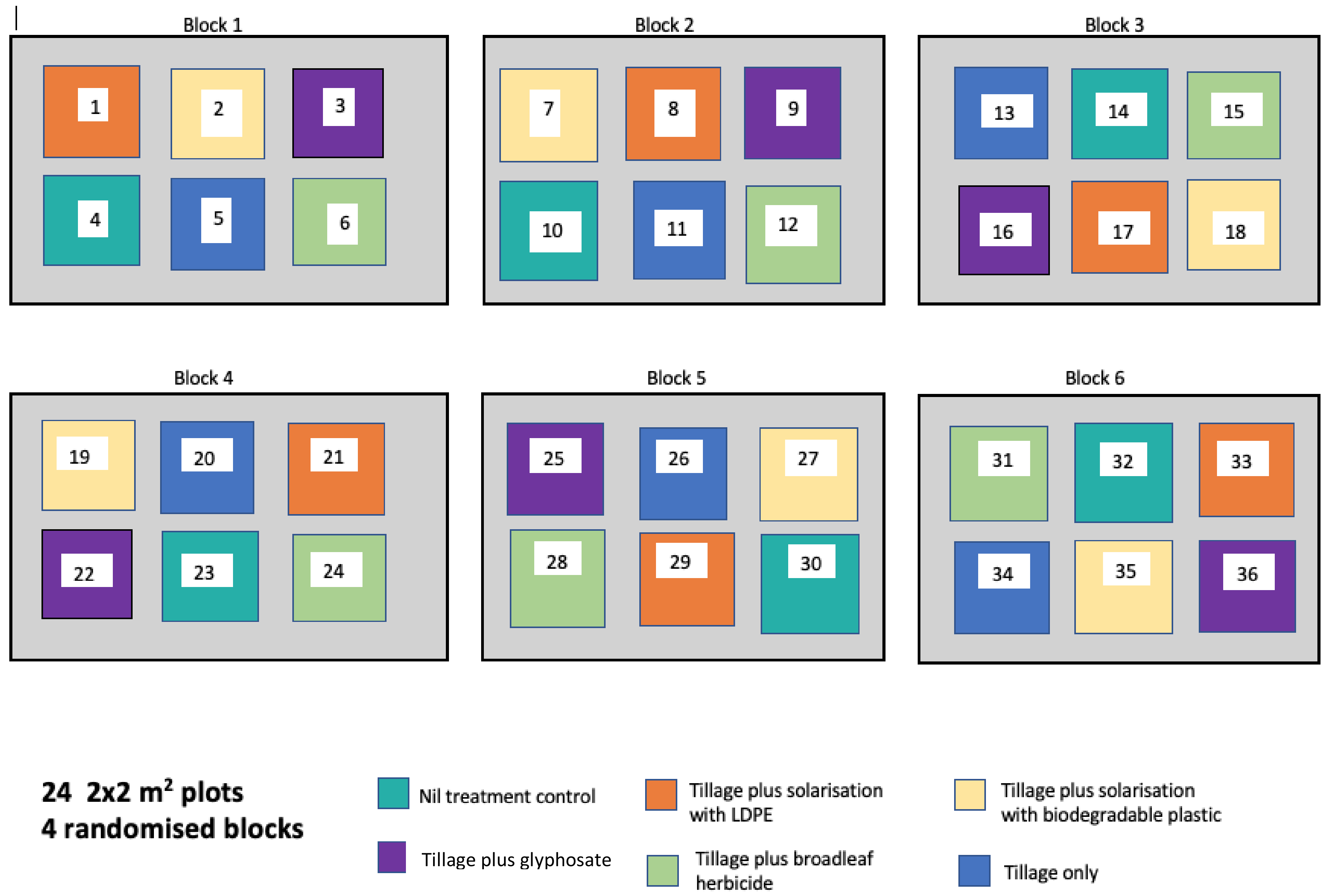

Figure 2. An experimental design that would allow the effect of the herbicides to be quantified and better account for the high variability in emerging weeds.

In conclusion, we found that at our site native grasses established well with shallow tillage and the use of herbicides as needed. Solarisation with either LDPE or biofilm made from Mater-Bi resin did not provide additional benefit when herbicides were also used. Future experiments would benefit from additional controls to separate the effect of the herbicides used.

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CHAPTER 6. Conclusions and future research

This thesis has explored ways to manage weeds and the soil seed bank so that slow growing native grasses can establish from seed, especially in an Australian Mediterranean climate. Weeds, particularly annuals, can compete strongly with young native grasses for critical resources like light and soil moisture (Semple *et al.* 1999). Reasons for sowing native grasses include better sward resilience and the potential for year-round feed. There are also potential applications for restoration of native grassy ecosystems.

The central research question of this thesis has been: “Which methods are suitable for establishing native grasses in annual-dominated pastures?” It was investigated through interviews with experienced native grass practitioners, a Test of Concept Area and two field trials, one at Mylor in the Adelaide Hills and the other in the Waite Arboretum in Adelaide. This chapter provides a summary of the work undertaken and the conclusions. Suggestions for further research are also given.

Native Grass Practitioners

Native grass practitioners were generous with their knowledge and their regional methods for establishing native grasses were documented. There was no generalised method that suited every site, so a range of potential establishment methods were used. Factors that were considered prior to establishing native grasses included soil type, slope, aspect, rainfall, budget and the composition of the soil seed bank. Although the literature suggests that C3 native grass establishment from seed in pastures was largely unsuccessful due to weed competition (Firn 2007; Semple *et al.* 1999), regional practitioners have had success using topsoil removal or 2-3 years of pre-sowing preparation using burning, till/harrow and herbicides.

Test of Concept Area

The need for weed control prior to and during establishment of native grasses was well known (Chivers and Raulings 2009). In the Test of Concept Area (TCA) at Mylor and in the Biofilm trial, I identified five additional requirements for establishment, which were:

1. control of grazing by stock, native or feral animals;
2. irrigation during long, hot, dry spells in the first summer;
3. sowing in rows to increase weeding efficiency;
4. determining which species and/or accessions were tolerant to the soil type and environmental conditions, and;
5. sowing seed thickly to provide sufficient competition to weeds (e.g. 16 kg/ha for *Microlaena stipoides*).

Prior to this study, it was known that summer active (C4) grasses, especially Kangaroo grass (*Themda triandra* Forssk.) established well under some conditions, particularly with burning of seed hay and/or a layer of mulch (Cole and Lunt 2005; Semple *et al.* 1999; Stafford 1991). However, methods for sowing and mulching larger areas were needed (Cole and Lunt 2005). A potential method for this in the TCA was to sow French white millet (*Panicum milleaceum*) in the midrow of Kangaroo grass (*Themeda triandra*). The millet grows quickly and can be slashed several times during the summer to provide a protective mulch for the slower-growing Kangaroo grass. This method has potential application for larger areas, especially those that are prone to erosion.

Mylor 2018 Trial (average annual rainfall ~900 mm)

In the field of grassland restoration, topsoil removal is an effective way to establish a range of native plants from seed. Topsoil removal has been widely used in Victoria, New South Wales and, more recently, on suitable sites in South Australia (Gibson-Roy 2008; Gibson-Roy 2014). Nevertheless, some local practitioner rejected the method because it removes the most biologically active layer of soil, is expensive and creates spoil.

The Mylor 2018 trial compared topsoil removal, soil solarisation, till/harrow, harrow only, topsoil inversion, burning and herbicide as pre-sowing weed control treatments. No native grasses were sown. It showed that topsoil removal was an effective method for creating bare ground so that native grasses might have an establishment window. At Mylor the soil varied from only 100 to 170 mm in depth. It was decided not to test this method further because the sandy, sloping site had high erosion risk once the topsoil was removed. It could also have created areas of exposed bedrock where no plants would grow.

Soil solarisation created a similar amount of bare ground as topsoil removal and also worked well for establishing native grasses in the TCA. However, this method also has a range of disadvantages including the time and cost to lay and retrieve the low-density polyethylene (LDPE), the need for soil to be at field capacity prior to laying the LDPE sheets at hottest/driest time of year and the plastic waste created. Despite these disadvantages, the method has potential application for small-scale habitat recreation in the Australian Mediterranean zone.

Till/harrow, harrow only and topsoil removal were comparable in weed reduction but less effective than topsoil removal or soil solarisation. To be effective enough for native grasses to establish well, they would need to be repeated over more than 18 months. Burning and herbicide were least effective and might only be useful if combined with other methods.

Waite Biofilm Trial (annual average rainfall ~550 mm)

The Waite Biofilm trial compared soil solarisation with low density polyethylene (LDPE), and a biodegradable plastic (biofilm) and till/harrow as a pre-treatment for sowing native grasses. Native grasses established well with all treatments and soil solarisation with either LDPE or biofilm did not provide an additional benefit over till/harrow. However, the results of this trial were confounded by the use of herbicides in all treatments except the control. The highly variable seed bank also led to high within-treatment variability. More robust experimental methods are needed for future experiments of this type and a suggested improvement was outlined at the end of Chapter 5. In summary, it was recommended that the experimental plots should be at least 2 x 2 m^2 and the experimental design should include replicated controls for each herbicide application.

Although till/harrow and herbicides were effective for native grass establishment in the Waite Biofilm trial, they did not control weeds effectively in the Mylor 2018 trial or the TCA. This higher rainfall environment would likely require about 3 years of repeated application of pre-seeding weed control prior to sowing native grasses. Other researchers have recommended more than 18 months of pre-sowing preparation for methods other than topsoil removal and solarisation (Semple *et al.* 1999). On the sloping, shallow, sandy soils at Mylor, cover crops would be needed to prevent erosion during this lengthy pre-sowing period.

In summary, there are thousands of hectares of degraded pasture in temperate Australia. As pastures degrade (i.e. through over-grazing, falling fertility, climate change etc.) they tend to become dominated by annual weeds that grow quickly in spring and die in summer, potentially leaving the ground bare and prone to erosion. The management of annual weeds is an ongoing challenge whether for pasture improvement, fire management, soil improvement or grassy ecosystem restoration.

There are considerable challenges to establishing native grasses. Native seed cost and supply chain reliability will be barriers to native grass adoption by graziers. For example, in the TCA and Waite Biofilm trial, a successful sowing rate for Weeping rice-grass (*M. stipoides*) was ~16 kg ha^{-1}. Seed alone would cost about $4,000 per hectare (depending on seed quality) from a commercial supplier (NativeSeeds 2020). Sowing the seed in rows allowed for more efficient weed control, easier seedling identification and better seed placement than by seed broadcasting. However, most native grass seed would have to be pelletised or suspended in a liquid to pass through a conventional seeder. The cost of this would have to be weighed against the weeding efficiencies. Temporary irrigation is a further expense, alternatively, sowing only in wetter years might be an option.

Given the high cost of native grass establishment in old pastures (especially in a high rainfall environment), it will be important for graziers to have a clear understanding of the aims, challenges and costs. For most graziers, sowing native grasses from seed will only be practical and affordable on a small scale (i.e. several hectares) and only when introduced grasses are not surviving. Nevertheless, there is potential for graziers to develop their own seed orchards or to form co-operatives for seed production, machinery purchases and to share knowledge. With increased adoption of various applications, demand for seed will increase and the regional seed market will grow.

Future research

Kangaroo grass (*Themeda triandra*) is a keystone species with potential to sequester the nitrate that facilitates annual weeds (Prober and Lunt 2009). Some think it should be established first to increase ecological resistance to weeds (Prober and Lunt 2009). It would be possible to combine Kangaroo grass establishment with herbicides, burning and seed removal (for example by using a forage harvester) to increase its establishment and reduce annual weed adundance. A randomised trial for reducing annual weeds, especially wild oats, using Kangaroo grass combined with other methods is needed in our region. Plot treatments could include an untreated control, a herbicide control, Kangaroo grass seeding only and three other treatments: Kangaroo grass seeding plus annual burning, herbicide and seed removal. Relative abundance of Kangaroo grass, annual grasses and broadleaf weeds could be measured (using a point-intercept method) to determine the establishment of Kangaroo grass and the abundance of exotic weeds.

A further area for research is whether a cover crop of millet facilitates Kangaroo grass establishment. In this study, it provided a protective environment and, after slashing, produced mulch to retain soil moisture and suppress summer weeds. A randomised trial is needed to test row spacings and planting densities. Treatments could include a control without millet, plus three row spacings of 400 mm, 500 mm and 600 mm with two sowing densities for the millet. Kangaroo grass biomass could be measured at the end of the first and second summers.

Another area for research is the potential benefit of native perennial grasses for the soil when introduced grasses are not surviving. By using both warm season and cool season species, it is possible to have native grasses that are growing actively year-round. In theory, this should lead to higher soil organic carbon levels than would be present from annual plant cover. Soil organic carbon might have substantial benefits in terms of water holding, soil structural improvement and carbon sequestration. This would have to be a long-term study (e.g. 5-10 years) to minimise interannual differences between the annual and the perennial ground cover. Near and medium infrared spectroscopy of soil samples is one established and cost-effective method for measuring soil organic carbon.

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