Directions for best management of Kangaroo grass (*Themeda triandra* Forssk.) re-establishment in southeastern Australian native grassland remnants

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made.

Bram Mason

Dedicated to the loving memory of Les & Irene Mason and Brian & Doris Langdon.

Themeda triandra in flower

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Formatting and Conventions

The format style and conventions used in this thesis are consistent with those required by the Journal of *Ecological Management and Restoration*. The exceptions to this are Chapters one and seven and Chapter five, which, has been formatted for the Journal *Austral Ecology*.

The thesis is laid out in sections equivalent to a series of papers, for Chapters two to six to allow for ease of publication of the material.

Abstract

Western basalt Plains native grasslands, which extend from the west of Melbourne in Victoria to the South Australian border, are recognised as one of the most threatened ecosystems in Victoria (DC&E 1992). Ecosystem functions and their ability to support life are disappearing along with the remnant grasslands. The research project reported in this thesis aimed to develop methods to re-establish the dominant native grass, Kangaroo grass (*Themeda triandra*) in ways designed to out-compete invasive grassy weeds. In the past there has been some research done in re-establishing Kangaroo grass in Serrated Tussock (*Nassella trichotoma*) infested grasslands but results have been variable.

With the decreasing area of native grassland comes the problem of decreasing supply of native species seed available for re-establishment projects. The amount of Kangaroo grass seed harvested per gram of flowering material was found to be significantly greater ($p<0.01$) when a brush header was used in comparison with traditional bailing and harvesting with a sickle bar mower. Percentage germination of Kangaroo grass seed was not significantly different when two-month-old seed was compared with one year, two year, three year or four-year-old seed stored at room temperature. The percentage germination of Kangaroo grass seed was found to be significantly lower ($p<0.05$) than initial germination percentage after storage for five years at room temperature. Under ideal germination conditions in a growth cabinet (see Chapter two), Kangaroo grass seed (unaltered) was found to achieve maximum germination percentage after seven days.

Establishing Kangaroo grass during drought or a low precipitation year was investigated. The optimum conditions for using the "spray and hay" method of killing weeds and introducing Kangaroo grass seed to the ground were investigated. Kangaroo grass seedling density and percentage cover were significantly greater in soil tilled to a depth of five cm compared with no till

treatments. By comparison during a close to average precipitation year, soil cultivation did not significantly affect Kangaroo grass seedling density or percentage cover.

The soil seedbank and soil physical properties associated with Kangaroo grass re-establishment in cultivated areas was investigated. A trend was found of increasing density of Kangaroo grass seed available in the soil seedbank with increasing depth of cultivation to a maximum depth of five cm. This was considered likely to have been affecting the density of germinating Kangaroo grass seedlings and establishment. Treatments cultivated to five cm (similar to an agricultural fallow treatment) were found to produce a dense soil layer on the soil surface and a greater availability of soil moisture in the root zone below this layer, when compared with no-till treatments during a drought year.

Soil nitrogen mineralization and its influence in weed invasion and native grass re-establishment were investigated in plots where Chilean Needle grass (*N. neesiana*) was removed by a combination of herbicide application and tilling. In soil under Chilean Needle grass plants, net nitrogen mineralization was low compared with the net nitrogen mineralization in soil with no vegetative growth. This suggests that Chilean Needle grass has a potential large storage capacity for available forms of soil nitrogen. Mineralization of soil nitrogen was significantly greater in till re-establishment treatments when compared with no-till re-establishment treatments and in soil under alive Chilean Needle grass in *in situ* trials during spring. This suggests that vegetation in cultivated treatments have a greater potential to increase in biomass when compared to plants in un-cultivated treatments.

The effects of cultivation, fertilization and irrigation on germination and growth of Kangaroo grass and Chilean Needle grass seedlings following removal of Chilean Needle grass by herbicide and tilling were investigated. Cultivation of soil gave the most substantial increase in biomass for newly established Kangaroo grass seedlings assessed after six months. Subsequent fertilization

and irrigation had no significant influence on germination of Kangaroo grass seedlings at the rates trialed. Fertilization and irrigation did increase the average biomass of Kangaroo grass seedlings but this was not statistically significant at the rates investigated when compared with till no addition treatments.

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Chapter One

General Introduction

The traditional Aboriginal owners of Australia first utilized the benefits of Australia's southeastern native grasslands and grassy woodlands to provide them with a sustainable lifestyle. These ecosystems provided a staple diet of tubers and protein. This type of sustainable agricultural system survived for many thousands of years. With the invasion of European settlers to Australia came the introduction of European agricultural techniques. Initially these techniques produced bountiful yields for the colonisers. Two hundred years later we find the ecosystem of southeastern Australia undergoing a dramatic reduction in its capacity to sustainably support current agricultural techniques. Salinisation and water catchment degradation are two major problems in Victoria today. Past experience has told us that native grasslands may hold some of the answer to preventing further degradation of Victoria's environment.

The body of research into management of Australia's native grasslands is beginning to evolve. However some past ideas on management such as "fence and leave alone" have contributed to further degradation of native grasslands. It is now known that some type of vegetation management is required to maintain native biodiversity and reduce invasion of exotic species (Craigie & Hocking 1999). Vegetation management research has come in the way of burning, grazing and slashing to remove accumulating aboveground biomass (Craigie & Hocking 1999).

Repair and re-establishment of native grasslands has had various degrees of success. The groundbreaking research by McDougall (1989) has provided a platform for a number of Kangaroo grass (*Themeda triandra* Forssk.) research projects. McDougall (1989) suggested spreading ripe Kangaroo grass hay containing seed within flowers onto areas where Kangaroo grass establishment was desired. The thatch was then removed by burning in early spring. Other researchers trialed the McDougall technique but found that results for emerging density of Kangaroo grass seedlings could be unpredictable (Dare 1997; Mason 1998). Phillips (2000) highlighted some basic timing and seed quality aspects that need to be incorporated into Kangaroo grass establishment projects. Phillips (2000) suggested weed control in autumn, introduction of Kangaroo grass seeds in July and removal of thatch in spring as a sequence that produced a high conversion of seed into seedlings. Phillips (2000) termed this method as the 'spray and hay' method. Further trials of this method indicated poor reproducibility and problems during low precipitation years (Dare 1997; Mason 1998).

Aims

The key aim of the project reported in this thesis was the investigation of methods to reliably re-establish Kangaroo grass in native grasslands invaded by exotic grassy weeds during normal and low precipitation years. The ecological mechanisms operating within the re-establishment process were also investigated to provide information on how to best optimise the re-establishment method. Chilean Needle grass (*Nassella neesiana*) has been declared one of many Weeds of National Significance (WONS 2000) and was used in some instances as a model for removal of weeds and replacement by native Kangaroo grass.

Format of thesis

This study first investigated establishment methods for Kangaroo grass during low precipitation years and then went on to consider soil properties associated with establishment followed by methods to optimise germination and growth according to the soil property findings. The thesis structure reflects this progression. Review of literature, site characteristics and methodologies used are included in each chapter. Re-establishment trials were carried out in the field to reflect processes that would occur under large-scale establishment.

- Chapter two investigates methods of seed harvesting and germination characteristics of Kangaroo grass.
- Chapter three investigates the use of soil cultivation in Kangaroo grass re-establishment and effects on germination during low precipitation years.
- Chapter four investigates what happens to the soil seedbank and soil physical properties during cultivation and Kangaroo grass re-establishment.
- Chapter five reports on the availability of soil nutrients in grasslands invaded by Chilean Needle grass and during the re-establishment process and why available soil nutrients influence growth to a greater extent in cultivated treatments.
- Chapter six uses results from the previous Chapters to investigate methods of optimising germination and growth of Kangaroo grass using fertilization and irrigation.

Chapter Two

Kangaroo grass (*Themeda triandra*) seed characteristics of plants in the Western grassland plains of Melbourne: Implications for revegetation

Introduction

Grasslands dominated by Kangaroo grass are rapidly decreasing in size and number in the Western regions of Melbourne (DC&E 1992). With the destruction of remnants comes the problem of accessing enough Kangaroo grass seed for revegetation projects. This means that the price for harvested material tends to be expensive and may increase further in price with the continuing destruction of grassland habitat. The expense of Kangaroo grass seed can then adversely affect the viability of Kangaroo grass revegetation projects. A 15 kg bag of Kangaroo grass florets containing seed in 2002 varied in price from $700 for a low quality bag to $4000 for a high quality bag containing other native forb (native herbaceous plant) seed and no weed seed (Hocking, personal communication). With the potential ongoing increase in price for wild native seed, a coordinated approach to protecting remnant areas and harvesting from them needs to be implemented. This may help with reducing wastage of native seed and any conflicts between seed harvesting contractors.

Methods for harvesting Kangaroo grass seed in a concentrated form allows for revegetation projects to become more cost effective. Concentration used here refers to the amount of seed per gram of harvested material. In the past, Kangaroo grass revegetation projects have used labour intensive harvesting methods such as slashing with a sickle bar mower, followed by raking and bagging of slashed material (McDougall 1989; Phillips 2000; Waters *et al.* 2000). This type of method has obtained seed concentrations of between 0.1 seeds per g of harvested material to 0.5 seeds per g (Phillips 2000), two seeds per g (Windsor & Clements 2001) and on some occasions up to eight seeds per g (presented in Figure 2.1). Research and development into the most effective mechanisation for harvesting lowland grassland native grass seed in general is underway, for example Briggs (2001).

Problems can also arise with the quality of seed harvested. The quality of seed in terms of percentage germination will vary with the climatic conditions during the year of plant growth and flowering (Waters *et al.* 2000), and also between bioregions and within swards. The 2002 - 2003 Kangaroo grass seed-producing season was extremely poor in terms of seed quality in the West of Melbourne. The drought affecting most of South-Eastern Australia was thought to be a major contributor to poor seed production. Soil moisture at one location in the West of Melbourne was as low as two percent (Table 5.2) in November 2002. Adverse climatic conditions can be partially accounted for in Kangaroo grass revegetation projects by knowing the quality and quantity of seed in harvested material. If seed quantity and quality are known, the density of seed to be introduced to the ground can be calculated to give an estimate of how many seedlings will germinate in a given area. In a past Kangaroo grass revegetation project, results indicate that approximately 25 percent of seeds that reach the ground will germinate and survive into mature plants (Phillips 2000). Therefore, percentage survivorship of emerging seedlings also needs to be accounted for in any estimation of density of seeds to be added. Phillips (2000) suggested that the reduction in seedling density to approximately 25 percent of initial density is mainly due to intra and inter-species competition, along with less than ideal conditions for individual seed germination in the field, and predation.

Past research has identified dormancy of Kangaroo grass seed as being a problem in terms of limiting the percentage germination of seed (Hagon 1976; Phillips 2000). Dormancy of seed may also be in response to climatic or site conditions. There are methods to reduce dormancy of Kangaroo grass seed such as: addition of plant hormones like gibberellic acid and/or kinetin; dry storage; temperature stratification and; removal of glumes and/or lemma and palea (Hagon, 1976). Removal of the lemma and palea from Australian native grasses has also been reported by Lodge & Whalley (1981) to significantly increase germination. Groves *et al.* (1982) suggested 12 months of storage to break dormancy mechanisms in native grasses. Most of these dormancy release methods require a manual manipulation of individual seeds or florets. This can be very labour intensive in projects that require a large amount of seed. Waters *et al.* (2000) suggested that proper drying of seed and storage at approximately five to 20°C should be acceptable for most native grass species. Simple storage of seed at constant temperature and humidity is less labour intensive than other methods that have been suggested to achieve a high level germination. Seed storage was therefore a subject of investigation in the study reported here.

A series of trials were set up to investigate the quality and quantity of Kangaroo grass seed resulting from a range of harvesting and storage regimes. The first trial was designed to test the effect of harvester type on seed quantity and quality. The second trial tested the effect of storage at relatively constant temperature and humidity on Kangaroo grass seed percentage germination. The final trial was designed to test the time taken for one-year old Kangaroo grass seed to reach maximum germination under incubator conditions.

Methods

All references to mature Kangaroo grass seed represent seed classified as being hard and dark olive to black in colour as per recommendations by McDougall (1989) and Phillips (2000).

Harvester Effects

Samples of Kangaroo grass florets containing seed were obtained from locations in the West of Melbourne from a radius of approximately 10 km in January 1998 at the stage when seed was dark in colour with a hard centre. All samples were taken within one week. Sickle bar mower samples were slashed with the sickle bar, raked and then bagged and air-dried. Kangaroo grass hay bails were obtained from Parks Victoria (Organ Pipes National Park grassland Unit). The samples from the brush header harvester were obtained from Greybox and grasslands Indigenous Nursery, pre-dried and bagged. Approximately one ha of harvested Kangaroo grass using the brush header produced enough seed and florets to fill one wool pack. The quantity of harvested Kangaroo grass used in this trial was; sixteen wool packs using the sickle bar mower, eight wool packs from the brush header and eight traditional hay bails. All harvested samples were than sub-sampled (approximately 200 g per sub-sample). Three sub-samples were taken for each bale or 15 kg wool pack or hail bail and then sorted for ripe seed number and percentage germination.

Storage Effects

Kangaroo grass seed samples which had been collected and assessed for seed content and germinability and stored every year beginning in 1998 by Dr Colin Hocking (Victoria University) from native grasslands in the West of Melbourne for a range of revegetation projects were tested for percentage germination. Each sample of chaff weighed approximately one kg. Samples were stored at a temperature of between 15°C and 25°C. The percentage germination of seed was assessed approximately six months after harvest and at a time interval of one, two, three, four or five years after harvest.

Ripe seed number

The number of ripe seed per wool pack or hail bail was calculated using the following method: Each wool pack and hay bail was weighed to give a total sample weight. Each sub-sample was weighed and then every Kangaroo grass seed determined to have an awn and be hard and dark in colour was counted. Seeds were separated from chaff in the sub-sample from the wool packs or hail bails, via shaking the sample over a piece of butches' paper The ratio of number of seeds per weight of sub-sample was then used to estimate the number of seeds in each wool pack or hay bail.

Germination Rate

From each sub-sample from the wool packs or hail bails, seeds were separated via shaking the sample over a piece of butches' paper. Of the seeds separated, 100 seeds were randomly taken for use in incubation trials. Seeds were de-awned and placed into nutrient free agar in 250 mL flasks with lids. The flasks were then placed into an incubator set up for 12 hours day at 35°C and 12 hours night at 20°C for a period of 14 days. At the end of this period, germinated seeds (those seeds with cotyledons emerging from the seed) were counted and expressed as a percentage of the total amount of seed from each sub-sample used in the incubation trial (100).

Statistical analysis

All results were tested for significant differences using SPSS v11. Differences between harvester types were tested using a General Linear Model univariate ANOVA, testing for between subject effects and Tukey Posthoc multiple comparisons. Differences between initial and final germination results for seed storage experiments were conducted using a General Linear Model univariate ANOVA. A repeated measures General Linear Model was used to determine the day at which maximum percentage germination was significantly different from initial (at day two) percentage germination.

Limitations

The hay bails obtained from Parks Victoria and Greybox and grasslands were harvested from various locations in the West of Melbourne, Victoria within the Port Phillip and Western Port Catchment and Victorian Volcanic Plains Bioregion. This is a limitation to the study as various sites were used as harvesting locations not the one site. Another limitation was not having comparable areas harvested by each harvesting method. Awn length and intactness was also not characterised during this study, which meant that effects of harvesting method on awn could not be determined scientifically in this thesis.

Results

Harvester Type

The average quantities of Kangaroo grass seeds per g resulting from the different harvesting methods are presented in Figure 2.1. Seed quantity was significantly higher ($p<0.01$) at approximately 30 seeds per g in samples from the brush header in comparison to samples from the sickle bar mower and bailer, which were less than 10 seeds per g.

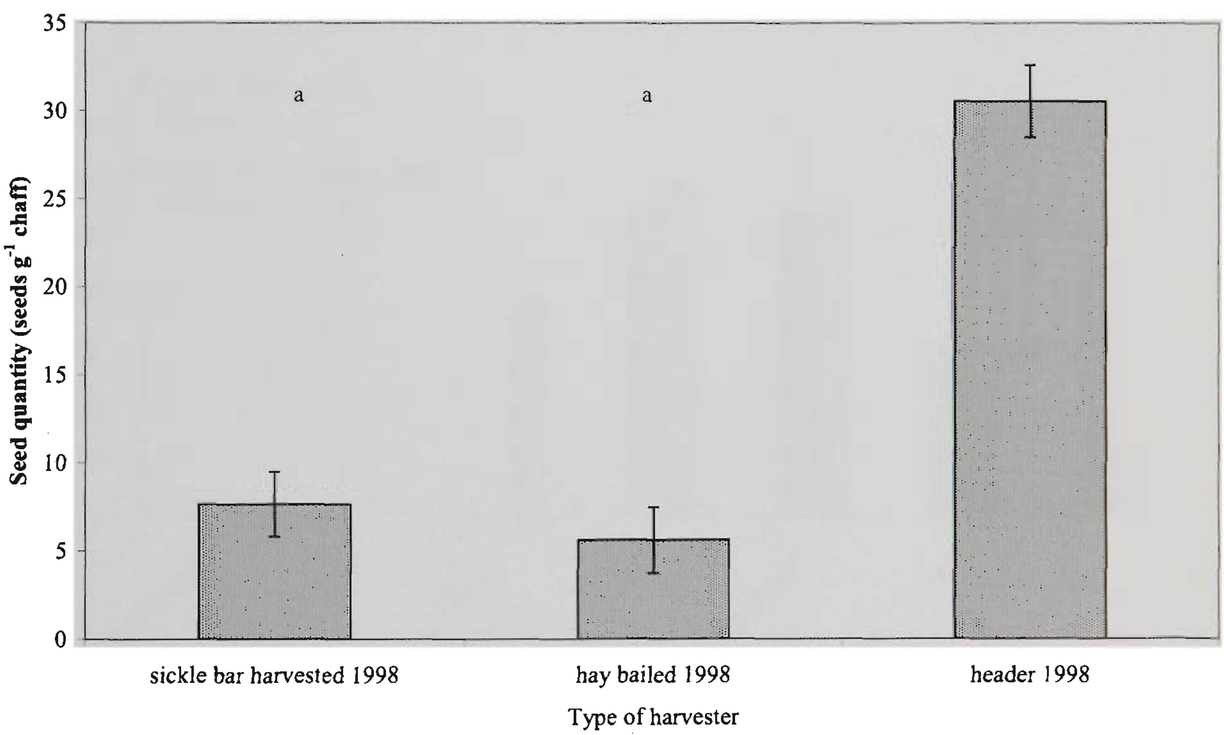

Figure 2.1: Kangaroo grass seed quantity per g of harvested material using various methods of harvesting; Bars indicate standard error (n range = 8 to 16). Key: a indicates a significant difference at $p< 0.01$ between harvesting method and header harvesting method.

Seed Storage

Seed viability did not differ significantly from initial viability until year five. Percentage germination of seeds then significantly ($p<0.05$) dropped from 45 percent to 12 percent (Figure 2.2). Figure 2.2 also shows percentage germination of Kangaroo grass seed over one year. There was no significant difference ($p>0.05$) in percentage germination of Kangaroo grass seed at two months since harvest; seven months since harvest or one year since harvest (Figure 2.2). Each age classification on Figure 2.2 presents the initial germination rate six months after harvest and the germination rate at the indicated storage time. Seed harvested in 2003 is from the same batch as one-year-old seed.

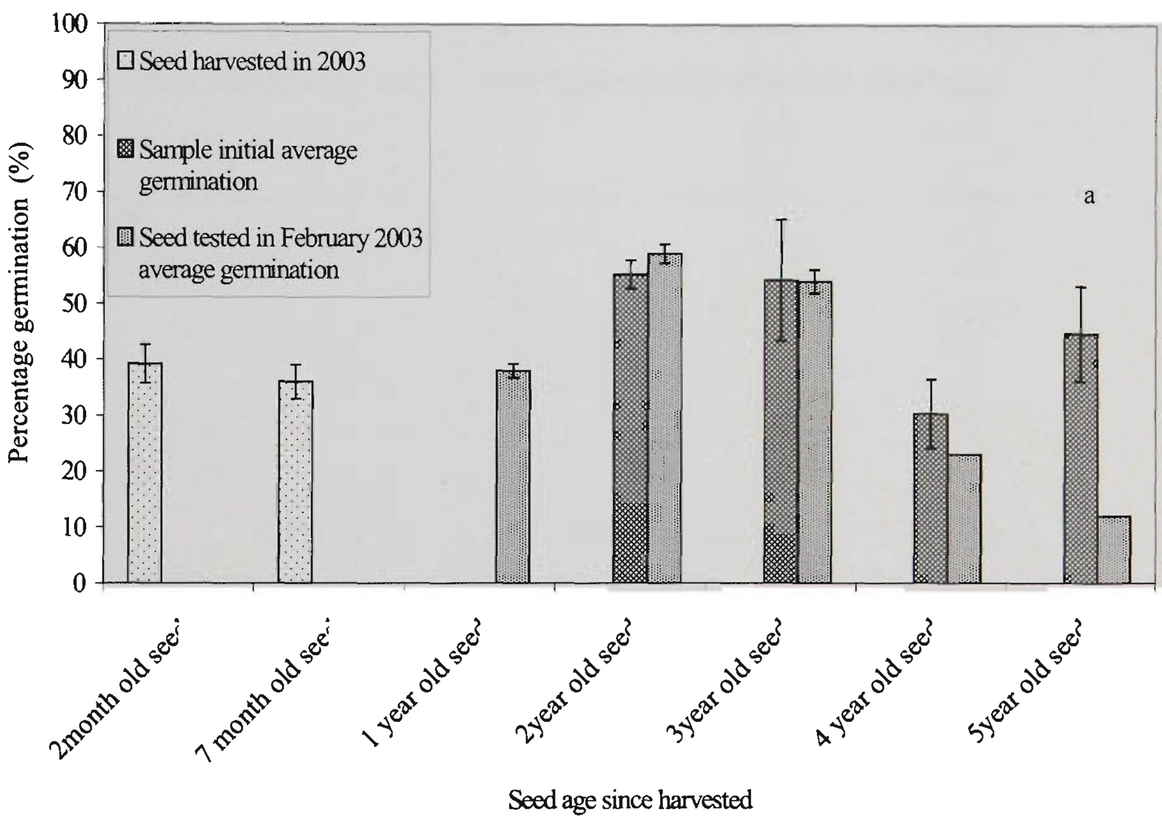

Figure 2.2: Kangaroo grass seed germination potential stored at room temperature for up to five years; Bars indicate standard error (4 replicates, 25 seeds per replicate). Key: 'a' indicates significant difference between initial and final germination rate at $p<0.05$.

Seed germination

Germination responses over time in days are presented for both ripe and non-ripe (Classified as having a soft centre, unfilled or being light in colour) Kangaroo grass seed trialed two months after germination in Figure 2.3. Seed classified as being non-ripe are presented as a control for the sorting of seeds. Kangaroo grass seeds classified as being non-ripe had a white to light olive appearance and were mostly soft to firm upon squeezing with forceps. Kangaroo grass seeds classified as being ripe had a dark olive to black appearance and were hard upon squeezing with forceps. Kangaroo grass seeds classified as being non-ripe had a maximum germination of 10 percent at the 13th day. Seed classified as being ripe had a maximum germination of 35 percent at the 13th day. At day seven neither ripe seed nor non-ripe seed were statistically different ($p>0.01$) from the maximum germination percentage at day 13. At day seven both ripe and non-ripe seed were statistically different ($p<0.01$) from results for each seed type at day one and day two.

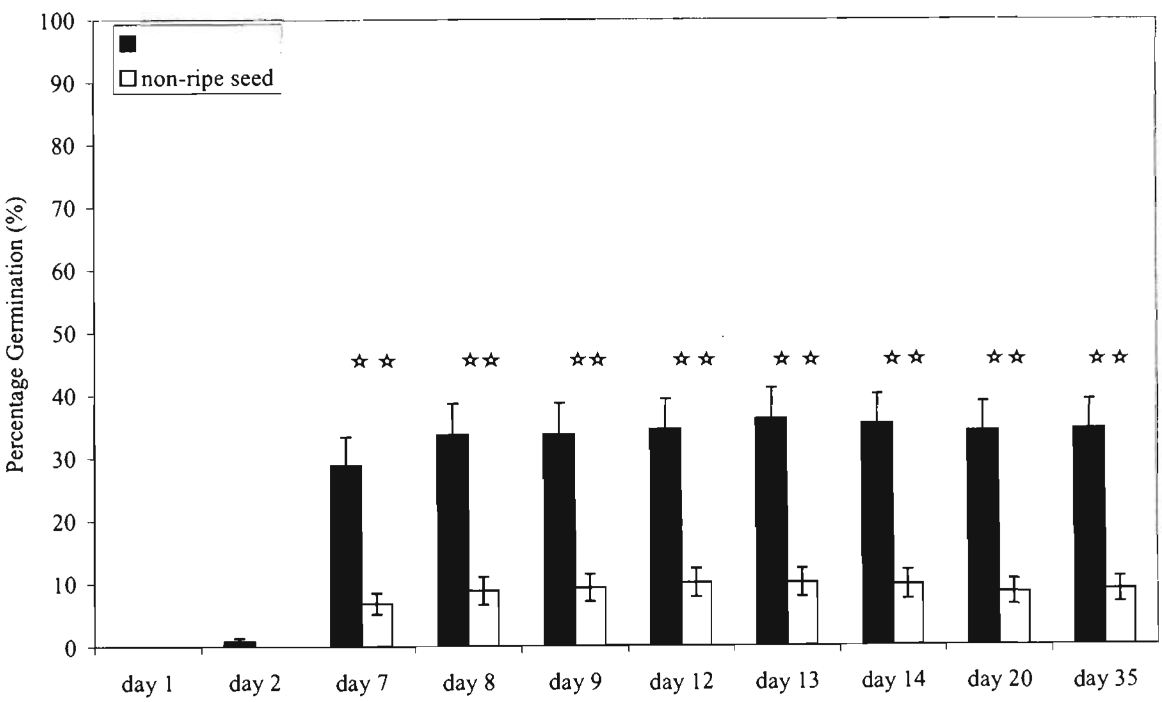

Figure 2.3: *In vivo* average Kangaroo grass seed germination of seed classified as ripe and non-ripe; Bars indicate standard error (n=10 equivalent to 250 seeds per trial). Key: Two stars indicate a significant change ($p< 0.01$) from day 2 results and other results.

Discussion

Harvester Type

With the ongoing dramatic reduction in native grasslands in the West of Melbourne high prices for good quality native grass seed are likely to continue. Increasing price may render many native grassland revegetation projects unviable. Small-scale native grass revegetation projects could become more viable if the amount of native seed that can be obtained per unit price could be increased. In the past seed collection mechanisms were inefficient in terms of the amount of seed collected per mass of collected material. Past seed collection techniques have included cutting seed laden culms with a sickle bar mower, raking the slashed material and then bagging the slashed material (Phillips 2000). Other methods employed include traditional hay bailing and the use of a modified brush cutter with a rotating brush and collection bag. In this trial, a brush harvester method allowed the amount of seed collected per mass of culm and head to be increased in comparison with the other harvesting methods tested here. A 15 kg wool pack of native grass seed harvested using non-brush header harvesting methods would only be expected to provide approximately 15,000 potentially viable seeds. The brush header used in the trials reported here can be expected to provide up to approximately 450,000 potentially viable seeds in a 15 kg wool pack. In terms of area that can be revegetated, a 15 kg wool pack of Kangaroo grass seed collected by a brush header could potentially revegetate almost 70 percent more than a 15 kg wool pack harvested with a sickle bar mower. As a brief comparison, a native grass seed harvester (similar to a brush header) currently being used by the Department of Land and Water Conservation in NSW was recorded as harvesting 200 kg of Kangaroo grass florets in 16 hours from 70 acres (28 ha) (Briggs 2001). The comparison of harvested area per unit time cannot be tested here due to results not being collected. The brush header harvester if set up is adapted, can be used to harvest a same unit area multiple times enabling seed ripening at different times to be collected. More research on increasing cost benefit s associated with the brush header is recommended.

Seed Storage

Grassland revegetation projects need to know the optimum time frame for how any collected native grass seed can be germinated in the field at maximum efficiency. Results reported here (Figure 2.2) suggest that Kangaroo grass seed can only be stored for up to four years under ideal conditions, after which germination potential significantly decreases. A more precautionary approach would be to store seed for no longer than three years, even under ideal conditions. Phillips (2000) investigated Kangaroo grass seed storage for a period of eleven months from harvest date and found an increase in germination rate with time. The trial by Phillips (2000) also suggests that a difference in the location of where seed is harvested can affect results for germination. The Phillips (2000) trial was not as extensive in terms of time of seed storage as results presented from this trial. McDougall (1989) recognised that further work on Kangaroo grass seed storage was needed to influence revegetation efficiency. However minimal literature is available on this subject and it requires further investigation.

The Kangaroo grass seed tested for immediate germination potential was harvested in 2002. The results from testing this seed indicate that the seed will reach maximum germination potential within two months. Phillips (2000) indicates that an increase in germination rate within a one year period may also be achieved, however this was not further examined here. Lodge & Whalley (1981) noted germination in native grasses two weeks after harvesting. This could have an impact on the time-line for many grassland revegetation projects. As long as sites to be revegetated are free from weeds, Kangaroo grass seed could be spread onto the site within two months after harvest. The type of weed over which re-establishment is occurring greatly influences the initial and on-going method of control (See Chapter 3). Allowance needs to be made for any contamination of harvested material with weed seed. Other factors that are known to play a part in grassland revegetation are: Timing of weed control; using a thatch over the seed; the reduction in on-ground seed density due to insectivory; and when to remove the thatch (Phillips 2000; Mason & Hocking 2002; Mason & Hocking 2003).

Percentage germination of seed stored for five years at room temperature was significantly lower ($p<0.05$) than initial percentage germination of this seed. Grassland management research indicates that native vegetation must have the above ground biomass removed either via burning, slashing and catching or grazing on a regular basis (Stuwe & Parsons 1977; McDougall 1989; Lunt & Morgan 1998). If aboveground biomass is not removed on a regular basis, dominant native grassland vegetation (typically Kangaroo grass) starts to senesce in many cases and eventually dies (Hocking & Mason 2001). During the senescence process the dominant grasses swamp native forbs and have an adverse effect on recruitment of these species (Lunt 1994; Morgan 1997). When Kangaroo grass plants are in the senescent stage they produce only a minimal amount of seed if any at all. The problems of this are that if native vegetation does not produce seed on a regular basis any soil-stored seed will be at a potentially greatly reduced viability over time. It has been reported that soil stored seed in the field can have dormancy mechanisms broken more quickly than what laboratory tests suggest (Tothill 1977). In this case, if the senescing vegetation were physically disturbed to the point where mature tussocks die; only minimal recruitment from the soil-stored seed would be expected. This, along with germination of weed seed incorporated into the soil seedbank, could lead to massive weed invasion into native grassland areas. At this stage major repair works would need to be implemented such as re-sowing of native species. More research is needed on the viability of soil-stored seeds of native grassland plants over time and grassland vegetation re-establishment methods.

Seed Germination

To be able to confidently predict an area that a given number of Kangaroo grass seeds can be spread to give a viable stand of Kangaroo grass plants, the following should be accounted for: the desired density of resultant plants (a standard 60 plants per m^2 for Kangaroo grass has been suggested by Phillips 2000) and; how many seeds are present in a given mass of harvested material. Another very important factor that must be known for a successful grassland revegetation project is the viability and in particular the percentage germination rate of the seeds to be used (Lodge & Whalley 1981; Phillips 2000; Mason & Hocking 2002). Knowing the percentage germination rate, the amount of seed introduced onto a unit area to produce a wanted number of seedlings can be calculated. If 60 seedlings per m^2 are required and their percentage germination rate is 30 percent, 200 viable seeds would need to be introduced to one m^2. Unfortunately other factors operate in the environment that cannot be easily controlled. Phillips (2000) found that approximately 25 percent of Kangaroo grass seeds that are germinable would turn into surviving seedlings. So the calculation of density of seeds added would need to be modified to account for this survivorship factor. According to the calculations above, 800 viable seeds would need to be introduced per m^2 to result in 60 seedlings that would survive into mature plants. Past research has used a 14-day germination period for estimation of percentage germination of Kangaroo grass seeds (Hagon 1976; McDougall 1989; Mason 1998; Phillips 2000). The results of the study reported here indicate that a minimum of eight days could be used to obtain reliable information about Kangaroo grass seed percentage germination rate, although this is based on seed that was two months old.

Conclusion

For any grassland revegetation project to work effectively there are a number of key components that must be understood. The quality and quantity of seed to be used is one of these. Climatic conditions will affect the quantity and quality of any seed to be harvested. Climatic conditions cannot be modified or easily abated; however there are ways in which to maximize the harvest. The time of harvesting and harvester effectiveness will partially affect quality and quantity of the seed obtained from a sward. Storage of seed to reduce any potential dormancy factors has also been widely reported. The viability of seed along with the quantity of seed can be used as a predictor of the successfulness of a revegetation project. Trials presented for Kangaroo grass seed harvested with a modified brush header (the Bandicoot®) produced a significantly higher ($p < 0.01$) amount of seed per gram of harvested material when compared with material harvested by sickle bar mower or traditional bailing. Minimal germination inhibition factors appear to be operating in stored seed. Degradation of seed quality was most evident in the fifth year of storage when the percentage germination significantly reduced ($p<0.05$) in comparison to initial percentage germination. Maximum percentage germination in growth cabinets was reached after 13 days but was not significantly different ($p>0.05$) to day seven results. This knowledge of a method for obtaining less Kangaroo grass chaff with high levels of Kangaroo grass seed along with optimum and maximum seed storage times and time taken for seed to reach maximum germination under ideal conditions, can all be used to optimise Kangaroo grass revegetation projects.

Chapter Three

What works best in a normal and drought year?

Introduction

Kangaroo grass establishment in Australia has had mixed results (Hagon & Groves 1977; McDougall 1989; Todd 1991; Dare 1997; Phillips & Hocking 1996; Mason 1998; Cameron & Briggs 2000; Phillips 2000; Waters *et al.* 2000; Windsor & Clements 2001; Mason & Hocking 2002). Any favourable results have been difficult to consistently reproduce. Currently, the most common method for Kangaroo grass re-establishment is the 'spray and hay' method, first proposed by Mc Dougall (1989) and refined by Phillips & Hocking (1996) and Phillips (2000). The refined spray and hay method involves weed kill in autumn, introduction of Kangaroo grass thatch harvested in December or January depending upon seasonal conditions, containing ripe seeds in July and removal of thatch in October (Phillips, 2000). Some researchers have tried to identify the possible reasons for these inconsistent results. Phillips (2000) outlined specific timing as one key aspect to Kangaroo grass re-establishment. These include; when to kill exotics before revegetation begins; when to introduce Kangaroo grass seed to the site; and when to remove the thatch layer. However, the method was still not consistently reproducible. The 'spray and hay' method was least reproducible during less than average precipitation periods during the summer season (Hocking. Unpublished results).

Research into factors, which increase the success of Kangaroo grass establishment projects, has concentrated on irrigation, thatching, fertiliser addition and favourable microsites. Available soil moisture and the penetration of Kangaroo grass seed into the top few cm of soil has been shown to be a factor for seed soil penetration under laboratory conditions (Hagon & Chan 1977; Sindel *et al.* 1993: O'Connor 1996). These results are in slight contrast to those from field establishment trials by McDougall (1989) and Windsor &

Clements (2001), which indicated that irrigation is initially beneficial but not essential in Kangaroo grass germination. Using a thatch over any Kangaroo grass seed incorporated into the soil has had both favourable results (Hagon & Groves 1977; Phillips & Hocking 1996; Phillips 2000; Mason & Hocking 2002) and non-significant results (McDougall 1989). Fertiliser addition has been trialed for the purpose of increasing Kangaroo grass seedling germination. Hagon & Groves (1977) found fertilizer addition only had a minor effect on germination. Waters *et al.* (2000) indicated there might be a potential in using fertilizer for ongoing management of native grasses but there is little benefit during the establishment phase. Sindel *et al.* (1993) and Winkel *et al.* (1991) highlighted the importance of soil microsites and their importance for Kangaroo grass seed incorporation into the soil layer. Fluctuations in humidity cause the awn to twist and move the seed along the top of the soil (Sindel *et al.* 1993). Any rough part of the soil surface such as a rock or crack increases seed burial (Sindel *et al.* 1993). Two main conclusions from the results of Sindel *et al.* (1993) were that: Firstly, soil compaction reduces Kangaroo grass seedling recruitment and inversely rough soil with a maximum number of microsites increases seedling recruitment; and secondly Kangaroo grass seed may be provided with and absorb more water if the soil layer is high in organic matter.

One major side purpose for the establishment of Kangaroo grass can be for use in weed control through competitive replacement. The most common weed where competitive replacement with Kangaroo grass has been researched is Serrated Tussock (Phillips & Hocking 1996; Phillips 2000). For purposes of weed control before establishment of Kangaroo grass the herbicides glyphosate and atrazine have both been used (McDougall 1989; Dare 1997; Mason 1998; Phillips 2000; Mason & Hocking, 2002). Phillips (2000) reported re-establishment of Serrated Tussock in plots where glyphosate had been used as the primary herbicide. There are potential problems associated with the unselective nature of glyphosate being used in native grasslands (Phillips & Hocking 1996)

Atrazine is a selective herbicide that kills plants that have a C3 photosynthetic system (winter/spring growth) without appearing to harm plants that have a C4 photosynthetic system (summer growth) (McDougall 1989). Atrazine has a hydrophobic nature and requires soil application around target plants (Hance & Holly 1990). Being hydrophobic, atrazine requires high soil moisture to effectively work. This makes timing of application crucial. Atrazine is often not effective during the dry summer months when applied on sites around Melbourne, Australia (Hocking. Unpublished results). Atrazine has also been noted to persist in the soil profile for up to nine months (Hance & Holly 1990). A note must be made here about the use of atrazine. Use of atrazine in many States requires a permit. Triazines (which include atrazine) have been statistically linked to increasing the risk of breast cancer in medium and high levels of exposure (Kettles *et al.* 1997). This raises questions as to how safe atrazine and other triazine-based herbicides are to use in sensitive situations such as remnant grassland reserves. The use of atrazine is currently under review by the National Registration Authority for Agricultural and Veterinary Chemicals, Australia (National Registration Authority 2002).

A search of literature for alternative safe herbicides has resulted in minimal outcomes. However one solution kept reappearing. This was an acetic acid (as approximately four percent acetic acid vinegar) and surfactant mixture (USDA 2002). No trials appeared to have been conducted using the acetic acid herbicide on *Nassella* species. The use of atrazine and acetic acid in the trials reported here was for the purpose of environmental weed control research. The National Registration Authority for Agricultural and Veterinary Chemicals must be referred to before using either chemical.

The study reported in Chapter three investigated the influence that different levels of soil disturbance have on the establishment of Kangaroo grass and re-establishment of Chilean Needle grass and Serrated Tussock. A side pilot trial investigated the effectiveness of acetic acid and surfactant herbicide on above ground vegetation death of Chilean Needle grass and Serrated Tussock, and subsequent effects on Kangaroo grass re-establishment.

The re-establishment method used in the soil disturbance and alternative herbicide trials are based on the outcomes of an earlier study by Phillips (2000) and recommendations by Mason (see Chapter two) to use

Kangaroo grass chaff containing ripe seed instead of using Kangaroo grass hay (florets and culms) containing seed. This required that a large amount of Kangaroo grass seedless hay be available to spread over the chaff material as a thatch. Sufficient Kangaroo grass hay was obtained for one trial in this study but a cheaper and more available alternative was needed for future trials. A pilot investigation was set up to identify if the readily available and cheap wheat straw (*Triticum aestivum*) could be used as a suitable alternative to Kangaroo grass hay as the thatch layer used in the spray and hay method.

Methods

Soil Disturbance Experiments

Areas once dominated by Kangaroo grass but subsequently dominated by a mixture of Chilean Needle grass and Serrated Tussock were selected for the re-establishment trials. All sites were in the West or North West of Melbourne, Victoria, Australia (Table 3.1 and Appendix One: Figure A1 Map of field research sites). A replicated random factorial block design was used for experimentation. Plots were two x two metres incorporating a half-metre buffer zone around each 1.5 x 1.5 m assessment plot. All treatments were replicated eight times. Three levels of soil disturbance were incorporated into this trial: No soil disturbance, one centimetre deep soil disturbance and five centimetre deep soil disturbance. The further investigation of a three centimetre deep disturbance is presented for some treatments. The no soil disturbance treatment was a direct repeat of the basic spray and hay re-establishment method investigated by Phillips & Hocking (1996). The soil disturbance plots followed the basic spray and hay method with the inclusion of soil disturbance one day before Kangaroo grass seed and thatch were laid.

Site Name	Co-ordinates		Reserve Name	Soil Description	Year trial initiated
	NW corner	**SE corner**			
AR1	5819450 N, 55 305475 E	5819410 N, 55 305502 E	Iramoo - VU, Vic	Basaltic clay	2000
AR4	5819394 N, 55 305482 E	5819381 N, 55 305508 E	Iramoo - VU, Vic	Basaltic clay	2001
AR5	5819367 N, 55 305580 E	5819352 N, 55 305604 E	Iramoo - VU, Vic	Basaltic clay	2001
AR6	5819376 N, 55 305485E	5819344 N, 55 305520 E	Iramoo - VU, Vic	Basaltic clay	2002
Woodlands	1445008.45 E, 373813.98 S	1445010.22 E, 373814.05 S	Woodlands Historic Park, Vic	Dark sandy loam	2001

Table 3.1: Location and brief description of trial sites used in this investigation into Kangaroo grass re-establishment. VU = Victoria University – St Albans campus.

The basic spray and hay guidelines used were as follows: Above ground biomass of exotics was reduced in early autumn. Fire was used for this purpose throughout all trials presented through the use of a gas flamethrower. Once exotic regrowth had started in late autumn, plants were sprayed with atrazine (as Nufarm Nutrazine®) at 8.7 kg active ingredient. per ha. In most cases plant death did not occur for a further month after herbicide application. In August soil disturbance at the appropriate depths were applied to the treatment plots. A rake was used to scarify the surface for one cm deep disturbance; manual digging was used for three cm deep soil disturbance; and a rotary hoe with depth gauge was used for the five cm deep soil disturbance. Within two days following soil disturbance Kangaroo grass seed in a chaffy form was spread over the plots at a density calculated to reach a final plant density of 60 plants per m^2. This density was dependent upon the germination rate of harvested seed and the quantity of harvested seed per gram of harvested material. A 20 % survivorship of seedlings was used along with the germination rate to determine the amount of viable seeds that could be expressed as surviving seedlings. Locally derived Kangaroo grass seed was obtained from Greybox and grasslands Indigenous Nursery packed loosely in 15 kg wool packs (Chapter 2) and harvested over December to January of the year prior to trial. Calculations to estimate the rate of addition of the seed required working out the number of seed per g of chaffy material and also the percentage germination of ripe seed. All wool packs containing Kangaroo grass seed in the chaffy form were tested for the above parameters according to the methods described in Chapter 2 and an average was obtained. It was calculated that approximately 60 grams of chaffy material would produce 60 Kangaroo grass seedlings that would survive on a plot (data not shown). This weight of Kangaroo grass chaff was then spread out over each m^2 (Plate 1). After the chaff had been spread on to the field site, a layer of Kangaroo grass hay was used as a thatch over the chaffy material. The thatch was laid at one and a half kg per m^2. The thatch was then left on until late November for AR1 and early October for the other field sites. It was planned to remove the thatch in October for AR1, however this did not happen due to resource commitments. Vegetation density (seedlings / plants were counted within a one m^2 assessment area) and percentage cover (Cover was estimated by eye within a one m^2 assessment area) were then assessed at six and 18 months after establishment.

Plate 1: Addition of Kangaroo grass chaff containing seed to a no-till re-establishment treatment.

The soil disturbance experiment outlined above was repeated in 2001 on three sites and again in 2002 on one site. This replication over time was used to test the method over several years with differing precipitation patterns. Sites tested in 2001 were AR4, AR5 and Woodlands (Table 3.1). Sites AR4 and AR5 had an additional application of atrazine in February 2002 to combat reinvasion of both Chilean Needle grass and Serrated Tussock. These data are presented along with equivalent plots that were not sprayed for comparison.

Controls were used for this experiment in terms of a till with not seed applied; and a no till with any seed applied. Each of these controls had herbicide applied as with the establishment treatments.

A differentiation is made in the results between seedlings and mature plants. Seedlings were considered to have a basal diameter up to two centimetres. Where mature plants were considered to have a basal diameter greater than to centimetres. If the assessed plant was close to the two centimetre diameter, a decision was made based upon length and thickness of leaf material.

Alternative Herbicide

The alternative herbicide experiment was initiated in Autumn 2002 on randomised replicated plots within site AR6. Eight replicated 2 x 2 m plots were used for treatment. These plots incorporated a half-meter buffer zone. As this was a pilot trial only one concentration of alternative herbicide solution was used. Rates of acetic acid ranged between four and six percent acetic acid by titration. One-litre vinegar solutions had 10 ml of regular strength Morning Fresh ® dishwashing detergent added to the spray unit. The solution was then shaken for a few seconds and then applied to the 4 m^2 treatment plots at 500 ml per m^2. Soil pH was measured daily following application until it reached background levels via a hand held pH meter at approximately 10 am each morning. Vegetation death was assessed by estimating above ground dead material (discoloured or brown) by eye two months after application. Vegetation was re-assessed after six months to investigate effects on Kangaroo grass and exotic plant establishment. Controls in terms of distilled water applied at the same rate were used for this experiment.

Alternative thatch type

The basic spray and hay Kangaroo grass establishment method was used except for one modification trialed during a less than average precipitation year (2000). Two different thatch types were trialed: a Kangaroo grass hay thatch and a Wheat straw thatch (Plate 2) applied at the same rate. The trial was investigated on 2 x 2 m plots with six replicates of each treatment. Resulting vegetation germination or regrowth was then assessed after six months.

The affect of thatching during a well below average precipitation year (drought) on seedling emergence and establishment were also investigated.

Plate 2: Wheat thatch re-establishment treatment surrounded by Kangaroo grass thatch treatments. The distance between plastic mesh measured two m. Note: only wheat thatch had been applied at the time of this photo.

Statistical analysis

Statistical analysis was conducted using SPSS v 11.0. All data were tested for normality via box plots, and measures of skewness and kurtosis were obtained. Transformations were conducted according to Zar (1984) if required to achieve normality. General Linear Model univariate analyses with or without posthoc tests (as appropriate) were used for simple comparisons. General Linear Model multivariate analyses with or without posthoc tests (as appropriate) were used to look at competition effects. With lambda in tables not mentioned here

Results

Soil Disturbance Experiments

Kangaroo grass establishment in below or well below average precipitation years

In a below average precipitation year (Figure 3.1) Kangaroo grass germination was higher by approximately 10 percent in treatments with soil disturbance than no soil disturbance at site AR1 (Figure 3.2). This difference was statistically significant for Kangaroo grass seedling percentage cover at $p<0.05$ (Figure 3.2 and Table 3.2). Plate 3 represents a no-till Kangaroo grass re-establishment trial six months after emergence. Plate 4 represents a till Kangaroo grass re-establishment treatment after six months.

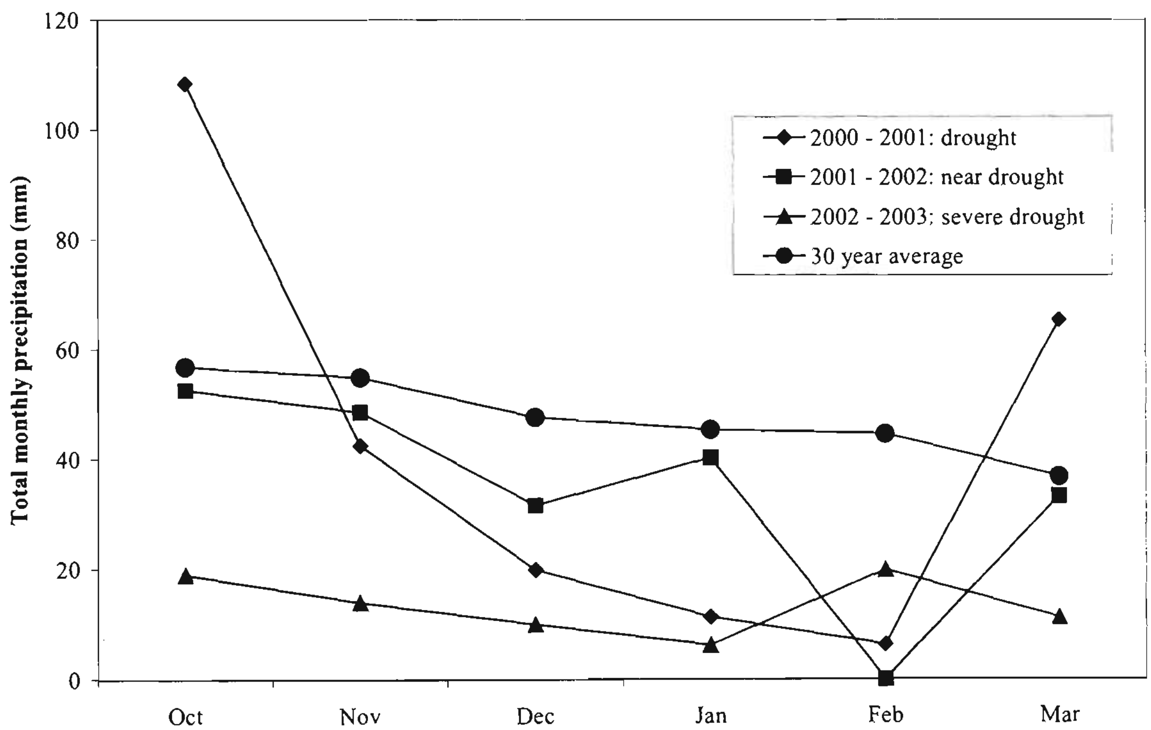

Figure 3.1: Total monthly and the 30-year average, precipitation during Kangaroo grass growing season. Data collected by Rockbank meteorological station (associated with Australian Bureau of Meteorology) Victoria, approximately five kilometres from Iramoo Reserve study sites (AR1, AR4, AR5 & AR6).

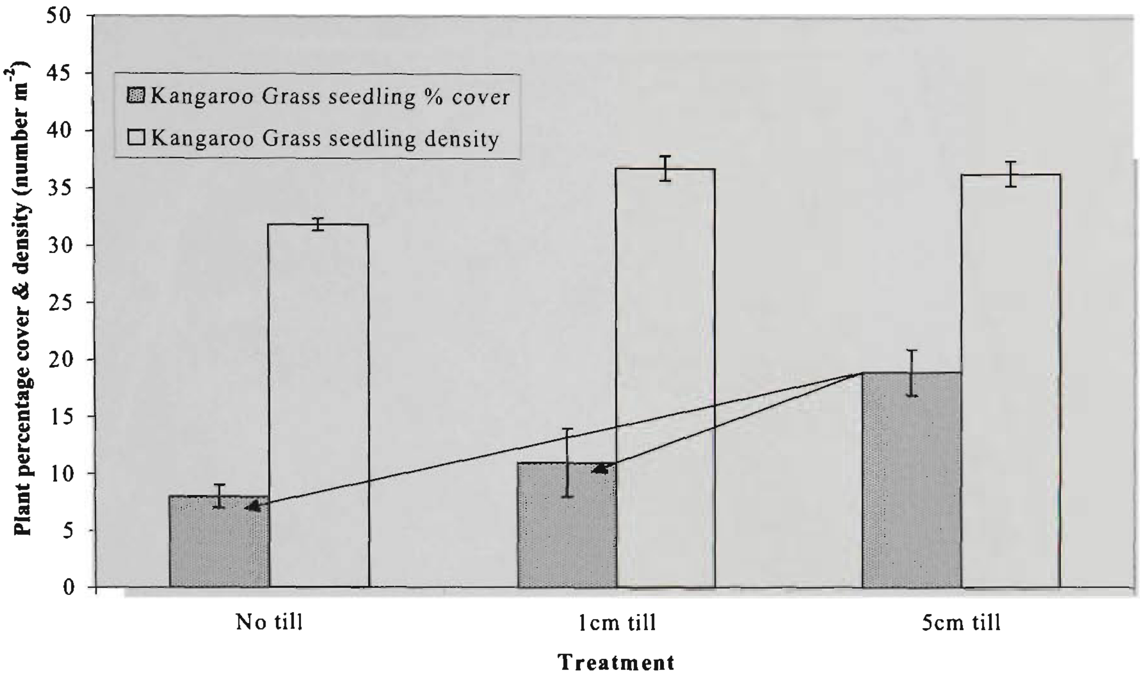

Figure 3.2: Average Kangaroo grass seedling percentage cover and density in three revegetation treatments in plot AR1 during a below average precipitation growing season; Bars indicate standard error (n=8). Arrows indicate a significant difference at ($p<0.05$) between depths of soil disturbance.

Plate 3: No till Kangaroo grass re-establishment treatment in plot AR1 after a dry summer, photo taken in March 2001. Quadrant dimensions equalled one m^2.

Plate 4: Five cm till Kangaroo grass re-establishment treatment in plot AR1, photo taken in March 2001. Quadrant dimensions equalled one m^2.

When this trial was repeated during a well below average precipitation year the results showed an even clearer trend. Both Kangaroo grass seedling percentage cover (by approximately 20 percent) and plant density (by approximately 25 seedlings per m^2) in five cm tilled plots was significantly greater ($p<0.01$) when compared with no till plots (Figure 3.3, Plate 5 and Table 3.2).

Kangaroo grass establishment in a year with close to average precipitation

Trials on plots AR4, AR5 and Woodlands received a total monthly precipitation close to the average for the preceding 30 years during the Kangaroo grass germination period (Figure 3.1). A multivariate test was performed on all data from sites AR4, AR5 and Woodlands for significance of site, treatment and any interactions on Kangaroo grass seedling establishment and Chilean Needle grass. For data collected six months after the start of the establishment period, treatment and site factors were both significant for Kangaroo grass seedling establishment (Wilks' Lambda $p<0.01$;

from SPSS results). To determine differences between soil disturbance treatments, data from each site were analysed separately. A multivariate test was then performed on treatment and site for all data except the 18-month assessment data. Only the factor 'treatment' was established as being significant (Wilks' Lambda $p<0.00$). Site data and interactions between site and treatment for all data at the 18-month period were not significant. Data in Figure 3.4 and Tables 3.3, 3.4 and 3.5 were statistically tested and indicate that there was no significant difference ($p>0.05$) between any soil disturbance treatments for establishment of Kangaroo grass seedling density 18 months after seedling emergence. There was a major difference between field sites in Kangaroo grass seedling density with Woodlands showing a highest value of 293 ± 59.2 seedlings per m^2 compared with sites AR4 (108 ± 20) and AR5 (268 ± 41.1).

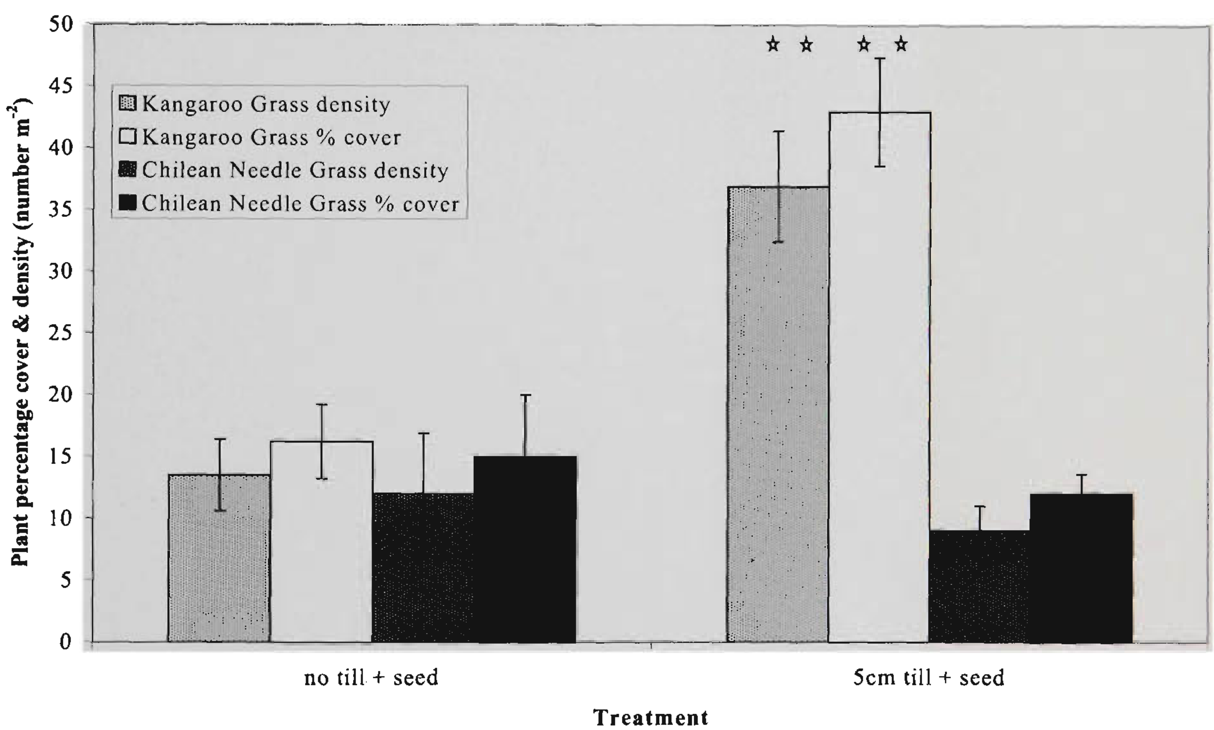

Figure 3.3: Average plant percentage cover and density in two Kangaroo grass revegetation treatments in plot AR6 after a well below average precipitation (drought) growing season. Assessments were undertaken in April 2003, approximately six months after first seedling emergence; Bars indicate standard error (n=8). Key: * * Indicates a significant difference at ($p<0.01$) for plant type between no till and five cm till treatments.

Plate 5: Five cm till Kangaroo grass re-establishment treatment plot AR6 March 2003. Quadrant dimensions equalled one m^2.

Site	Precipitation	Assessment time	Soil disturbance	Kangaroo grass Plants	Kangaroo grass Seedlings	Chilean Needle grass plants	Chilean Needle grass Seedlings	Serrated Tussock Plants	Serrated Tussock Seedlings
AR1 2001	**Below Average**	**6 month**	None	1.21 ± 0.09	31.86 ± 0.53	4.36 ± 0.13	16.79 ± 0.35	0	0
			1 cm till	0.38 ± 0.11	36.88 ± 1.08	2.63 ± 0.23	17.25 ± 0.44	0	0
			5 cm till	0.75 ± 0.14	36.38 ± 1.09	0.25 ± 0.11*	12.50 ± 0.38	0	0
AR1 2002	**Close to Average**	**18 month**	None	12.50 ± 1.94	0.40 ± 0.26	4.90 ± 1.29	5.80 ± 1.33	4.50 ± 1.05	0
			5 cm till	17.00 ± 3.83	0.90 ± 0.35	1.80 ± 0.70	7.60 ± 5.00	2.40 ± 1.20	0
AR6 2003	**Well Below Average (Drought)**	**6 month**	None		14.00 ± 2.90$\psi\Phi$	12.00 ± 4.90	5.00 ± 1.6Δ	1.00 ± 0.4	1.00 ± 0.90
			5 cm till		37.00 ± 4.50$\psi\psi$	9.00 ± 2.00	1.00 ± 0.7Δ	1.00 ± 0.6Δ	0.00 ± 0.30
			No till control		0	1.00 ± 0.30	13.00 ± 1.9	1.00 ± 0.4	5.00 ± 1.80
			Till control		0	5.00 ± 1.90	5.00 ± 2.1	1.00 ± 0.3	2.00 ± 0.60

Table 3.2: Average plant density of Kangaroo grass, Chilean Needle grass and Serrated Tussock in revegetation sites AR1 and AR6 during a below average precipitation and a well below average precipitation (drought) year. Standard error is shown (n=8). Sites AR1 and AR6 are both situated on basaltic clay soils and were dominated by Chilean Needle grass. Key: * indicates a significant difference ($p<0.05$), ** indicates a significant difference ($p<0.01$) between stated depth of soil disturbance and no soil disturbance plus seed addition result for particular species; ψ indicates a significant difference ($p<0.05$), $\psi\psi$ indicates a significant difference ($p<0.01$) between stated depth of soil disturbance and 5 cm till no seed addition result for particular species; Φ indicates a significant difference ($p<0.05$), $\Phi\Phi$ indicates a significant difference ($p<0.01$) between no till plus seed and 5 cm till plus seed addition result for particular species.; Δ indicates a significant difference ($p<0.05$), $\Delta\Delta$ indicates a significant difference ($p<0.01$) between indicated depth of soil disturbance and no till control for particular species.

Site	Precipitation	Assessment time	Soil disturbance	Kangaroo grass Plants	Kangaroo grass Seedlings	Chilean Needle grass plants	Chilean Needle grass Seedlings	Serrated Tussock Plants	Serrated Tussock Seedlings
AR4 2002	**Close to Average**	**6 month**	None		108 ± 20ψψ	1 ± 0.5ψψ		1 ± 0.8	
			1 cm till		106 ± 13ψψ	0ψψ			
			3 cm till		90 ± 23ψψ	1 ± 0.4ψψ		1 ± 0.8	
			5 cm till		102 ± 17ψψ	1 ± 0.3ψψ			
			Till control		0	19 ± 2.6	1 ± 0.8	1 ± 1.0	
AR4 2003	**Well Below Average (Drought)**	**18 month**	None	56 ± 16.1ψψ		10 ± 3.7		5 ± 3.9	1 ± 0.8
			1 cm till	77 ± 10.6ψψ		4 ± 2.6ψ		1 ± 0.8	
			3 cm till	59 ± 10.8ψψ		7 ± 2.5		6 ± 3.9	
			5 cm till	66 ± 11.0ψψ		5 ± 2.0		2 ± 1.6	
			Till control	0		17 ± 1.6	1 ± 0.8	6 ± 1.4	

Table 3.3: Average plant density of Kangaroo grass, Chilean Needle grass and Serrated Tussock in revegetation site AR4 during a close to average precipitation year and immediately after a well below average precipitation (drought) year. Standard error is shown (n=8). Site AR4 has a basaltic clay soil and was dominated with Chilean Needle grass. Key: ψ indicates a significant difference ($p<0.05$), ψψ indicates a significant difference ($p<0.01$) between stated depth of soil disturbance and 5 cm till no seed addition result for particular species; Φ indicates a significant difference ($p<0.05$), ΦΦ indicates a significant difference ($p<0.01$) between no till plus seed and 5 cm till plus seed addition result for indicated species.

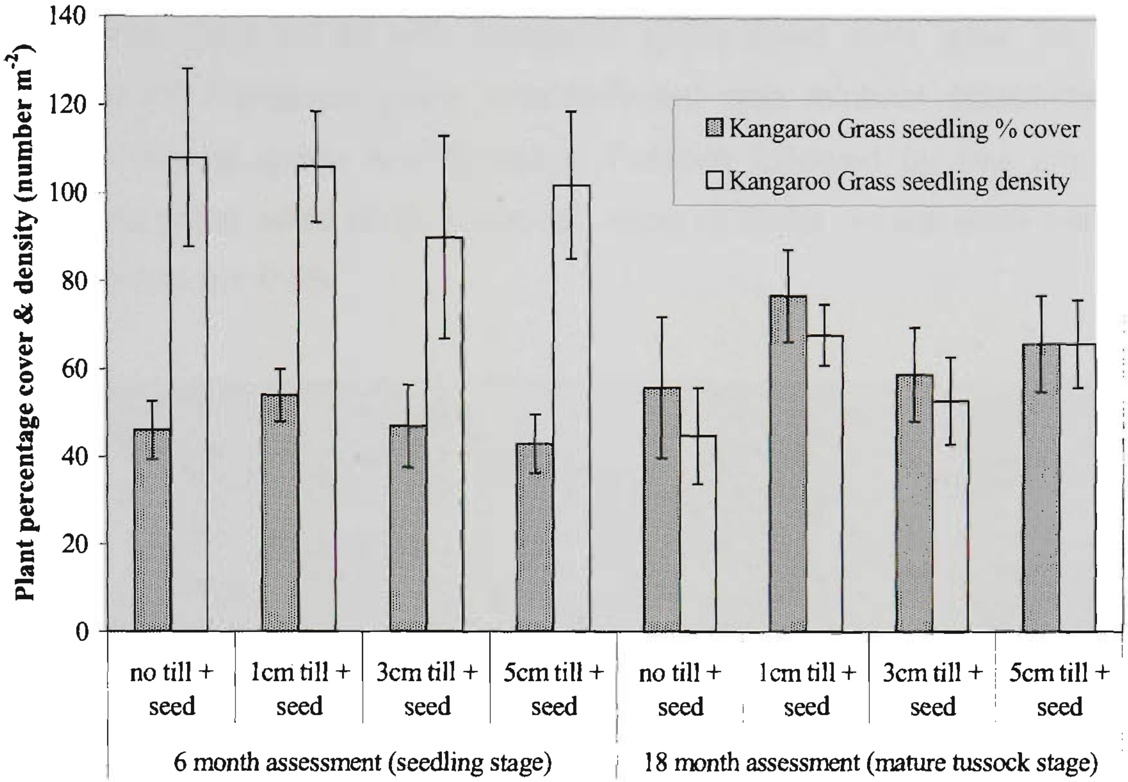

Figure 3.4: Average plant percentage cover and density in four Kangaroo grass revegetation treatments in plot AR4 established during a close to average precipitation growing season and surviving through a well below average precipitation (drought) period; Bars indicate standard error (n=8).

Site AR4 – Based at Iramoo grasslands

On site AR4, Kangaroo grass seedling density reached 90 to 108 seedlings (individual replicates) per m^2 (Figure 3.4 indicates averages and Plate 6) by the 18-month assessment date. Only minimal re-establishment of Chilean Needle grass and Serrated Tussock occurred six months after thatch was removed from the field site in plots that had atrazine applied in February 2002 (Table 3.3). Plots that were not treated in February 2002 with atrazine ranged between 10 percent to 28 percent lower percentage cover of mature dead Chilean Needle grass when compared to no till, one cm till, and 3 cm till plus seed Kangaroo grass seed treatments ($p<0.05$). No difference ($p>0.05$) was found between February applied atrazine plots when compared to all other plots for alive and dead percentage cover and density of Serrated Tussock and Kangaroo grass. However after 18 months both Chilean Needle grass and Serrated Tussock had re-established at highest numbers in till with no Kangaroo grass seed and in, no soil disturbance plus Kangaroo grass seed treatments. One cm till with Kangaroo grass seed plots gave the highest densities of Kangaroo grass establishment and minimal establishment of Chilean Needle grass and Serrated Tussock followed by five cm till with Kangaroo grass seed plots. However, none of these results were statistically significant at $p > 0.05$.

Plate 6: Kangaroo grass emergence in till re-establishment treatments in plots AR4, November 2001.

Site AR5 – Based at Iramoo grasslands – average precipitation year

At site AR5 Kangaroo grass seedling density was between 118 and 268 seedlings per m^2 at the six-month assessment time (Table 3.4 and Plate 7). Soil treatment was initially significant for Kangaroo grass seedling density at $p<0.05$. However when the data were further analysed using a Tukey posthoc test, no significant difference ($p>0.05$) was found between levels of soil disturbance for Kangaroo grass emergence. As with site AR4, all treatments except controls and replicates of five cm till plus Kangaroo grass seed treatments were sprayed with atrazine in February 2002. Plots that were not sprayed in February 2002 had a significantly greater percentage cover of Serrated Tussock compared with no till plus Kangaroo grass seed ($p<0.01$), one cm till plus seed ($p<0.01$) and three cm till plus seed ($p<0.01$) treatments. Re-establishment of Chilean Needle grass was only minor after six months on all treatments to which herbicide had been applied (Table 3.4). However, after 18 months Chilean Needle grass had reached highest densities in till plus no Kangaroo grass seed treatments (16 $\pm$5.0 per m^2) and no till plus Kangaroo grass seed treatments (9 $\pm$ 2.9 per m^2) (Table 3.4). Serrated Tussock re-established in all treatments after 18 months (Table 3.4). Treatments of no till plus Kangaroo grass seed addition had low densities of Serrated Tussock establishment compared with soil disturbance treatments.

Plate 7: Kangaroo grass in 5cm till treatment 18 months after emergence, March 2003. Quadrant dimensions equalled one m^2.

Site	Precipitation	Assessment time	Soil disturbance	Kangaroo grass Plants	Kangaroo grass Seedlings	Chilean Needle grass plants	Chilean Needle grass seedlings	Serrated Tussock Plants	Serrated Tussock Seedlings
AR5 2002	**Close to Average**	**6 month**	None		$268 \pm 41.1\psi$			$1 \pm 0.4\psi\psi$	
			1 cm till		$258 \pm 41.0\psi$			$1 \pm 0.7\psi\psi$	
			3 cm till		$265 \pm 60.4\psi$			$2 \pm 1.0\psi\psi$	
			5 cm till		$118 \pm 17.7\psi$			$0\psi\psi$	
			Till control		0			13 ± 3.5	2 ± 1.5
AR5 2003	**Well Below Average (Drought)**	**18 month**	None	$72 \pm 16.1\psi$		9 ± 2.9		2 ± 1.6	
			1 cm till	$58 \pm 18.7\psi$		$2 \pm 1.1\psi$		7 ± 2.5	
			3 cm till	$47 \pm 11.1\psi$		$4 \pm 2.0\psi$		8 ± 3.0	
			5 cm till	$69 \pm 11.1\psi$		$0\psi\psi$		7 ± 2.5	
			Till control	0		16 ± 5.0		5 ± 3.1	

Table 3.4: Average plant density of Kangaroo grass, Chilean Needle grass and Serrated Tussock in revegetation site AR5 during a close to average precipitation year and immediately after a well below average precipitation (drought) year. Standard error is shown (n=8). Site AR5 has a basaltic clay soil and was dominated with Serrated Tussock. Key: ψ indicates a significant difference ($p<0.05$), $\psi\psi$ indicates a significant difference ($p<0.01$) between stated depth of soil disturbance and 5 cm till no seed addition result for particular species. *Site Woodlands – Based at Woodlands Historic Park – average precipitation year*

At Woodlands trial site Kangaroo grass seedling density averaged between 269 and 325 seedlings per m^2 (Table 3.5 and Plate 8) six months after initial germination. Soil treatment was not statistically significant ($p>0.05$) for Kangaroo grass seedling emergence. Early re-establishment of Chilean Needle grass occurred in all treatments. The lowest average density was found in five cm till plus Kangaroo grass seed treatments (3 ± 1.8 per m^2) and the highest average density was found in one cm till plus Kangaroo grass seed treatments (13 ± 5.3 per m^2). At 18 months Chilean Needle grass re-established in seedling densities up to 83 ± 27.1 per m^2 and mature plant densities of 9 ± 2.5 per m^2 in plots tilled that had no Kangaroo grass seed added (Table 3.5). Other treatments only had minimal re-establishment of Chilean Needle grass after 18 months.

Plate 8: Kangaroo grass in five cm till re-establishment treatment at Woodlands 18 months after emergence, March 2003. Quadrant dimensions equaled one m^2.

Competition

An indication of inter-plant competition was obtained by comparing seedling densities at six months and 18 months after thatch was removed. Major reductions in Kangaroo grass densities were found in the transient stage from seedling to mature plants. Figure 3.5 indicates the percentage drop in plant density for each soil treatment between six and eighteen months. Data are average percentage reductions in Kangaroo grass seedling density from each field site (AR1, AR4, AR5 and Woodlands) for each soil disturbance treatment. The percentage reduction in seedling density ranges from 66 percent in no till treatments to 52 percent in five cm till treatments. A simple univariate ANOVA was performed on this data and no statistical difference (p>0.05) was found between soil treatments.

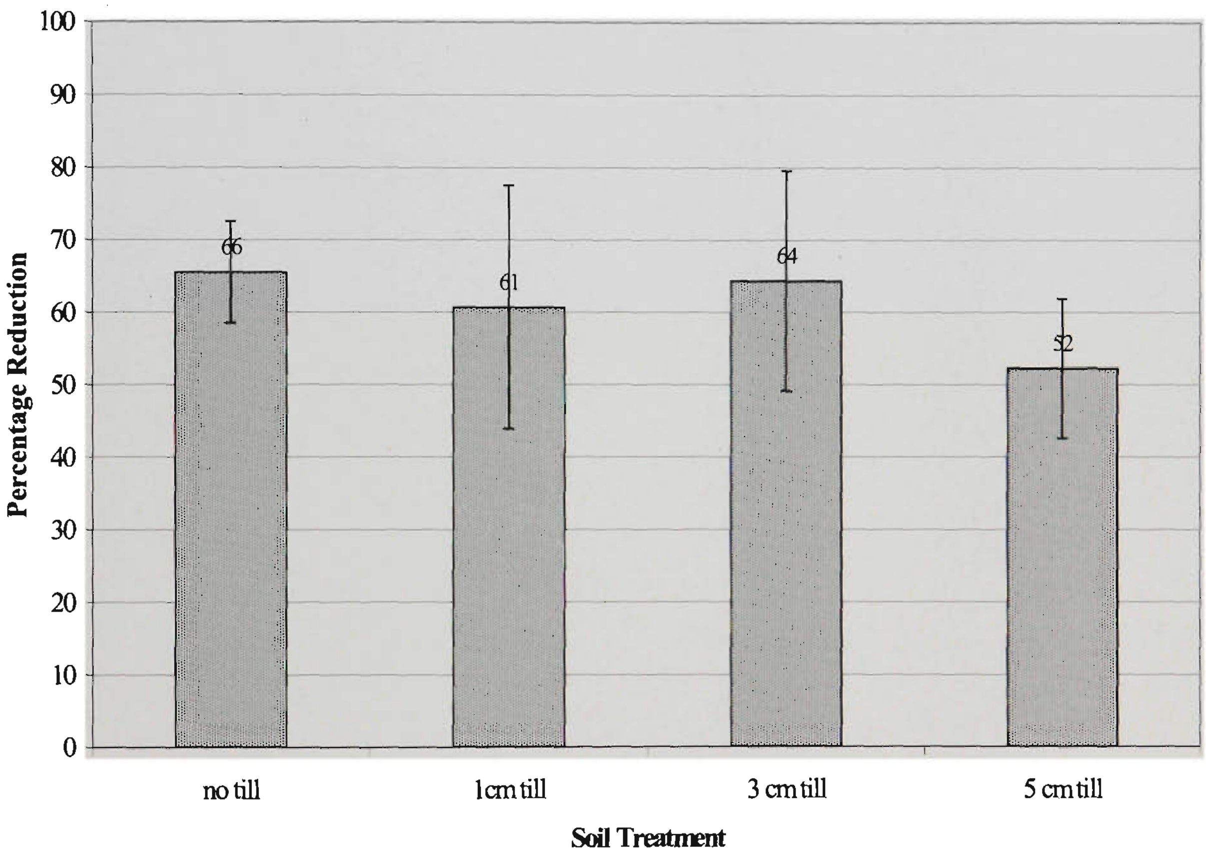

Figure 3.5: Percentage Reduction in density of Kangaroo grass from six-month-old stage (seedling) compared with 18-month-old stage (mature) in soil disturbance re-establishment plots; Bars indicate standard error (n=4). Data displayed are derived from average Kangaroo grass densities in plots AR1, AR4, AR5 and Woodlands. No significant difference between soil disturbance treatments was found (p>0.05).

At sites AR4 and AR5 Chilean Needle grass and Serrated Tussock densities increased in all treatments when comparing the six-month assessment with the 18-month assessment data (Table 3.3 & Table 3.4). At site AR1 Chilean Needle grass density decreased slightly when comparing the six month and 18 month assessment data (Table 3.2). Serrated Tussock density increased in site AR1 when comparing the six-month and 18-month assessment data (Table 3.2).

Site	Precipitation	Assessment time	Soil disturbance	Kangaroo grass Plants	Kangaroo grass Seedlings	Chilean Needle grass plants	Chilean Needle grass seedlings	Serrated Tussock Plants	Serrated Tussock Seedlings
Woodlands 2002	**Close to Average**	**6 month**	None		293 ± 59.2ΔΔ		6 ± 2.0		
			1 cm till		285 ± 48.1ΔΔ		13 ± 5.3		
			3 cm till		269 ± 38.4ΔΔ		9 ± 2.7		
			5 cm till		325 ± 64.9ΔΔ		3 ± 1.8		
			No till control		1 ± 0.5		12 ± 8.8		
Woodlands 2003	**Well Below Average**	**18 month**	None	58 ± 13.4		0 ± 0.1			
			1 cm till	65 ± 9.8		0 ± 0.1	3 ± 3.0		
	(Drought & Edge Fire)		3 cm till	62 ± 9.9			1 ± 0.4		
			5 cm till	70 ± 7.9		1 ± 0.3			
			No till control	NA		9 ± 2.5	83 ± 27.1		

Table 3.5: Average plant density of Kangaroo grass and Chilean Needle grass at the Woodlands National Park revegetation site during a close to average precipitation year and immediately after a well below average (drought) precipitation year with a fire burning up to the edge of the site. Standard error is shown (n=8). The Woodlands site has a sandy loam soil and was dominated with Chilean Needle grass. Key: Δ indicates a significant difference ($p<0.05$), ΔΔ indicates a significant difference ($p<0.01$) between indicated depth of soil disturbance and no till control for particular species

Alternative Herbicide:

Soil pH was monitored prior to acetic acid addition and at daily intervals thereafter until soil pH returned to original application levels (Table 3.6). Soil pH initially dropped from an average of 7.6 to an average of 7.3 when the herbicide was applied. Soil pH when measured at 20 and 48 hours after acetic acid application was significantly ($p<0.001$) lower when compared with control treatments. Soil pH measured 72 hours after application of acetic acid was also significantly ($p<0.01$) different compared with control treatments. Soil pH measured 96 hours after application of acetic acid was still significantly ($p<0.05$) different when compared with control treatments. Soil pH in acetic acid treatments was not statistically different to control soil pH when measured 168 hours (7 days) after application.

	Soil pH in Acetic acid treatments	**Soil pH in Control treatments**	**Significance (p value)**
Before application	7.6 ± 0.1	7.6 ± 0.1	1.000
20 hours after application	7.3 ± 0.1	7.8 ± 0.1	0.001***
48 hours after application	7.3 ± 0.1	7.8 ± 0.1	0.001***
72 hours after application	7.5 ± 0.1	7.9 ± 0.0	0.002**
96 hours after application	7.8 ± 0.1	8.0 ± 0.0	0.020*
168 hours after application	8.0 ± 0.0	8.0 ± 0.0	1.000

Table 3.6: Average soil pH associated with acetic acid herbicide treatments and control water application treatments, standard error (n=6) and p value indicated. Key: * indicates a significant difference at $p<0.05$, ** indicates a significant difference $p<0.01$ and *** indicates a significant difference at $0<0.001$ between soil pH in acetic acid and control treatments.

Vegetation assessment two months after herbicide application

Chilean Needle grass density was significantly greater ($p<0.05$) by 11 plants per m^2 in control plots when compared to acetic acid herbicide treatments and 13 plants per m^2 when compared with atrazine treatments two months after herbicide application (Table 3.7). Chilean Needle grass percentage cover was significantly greater ($p<0.05$) by 39 percent in control plots when compared with acetic acid herbicide treatments and 34 percent when compared with atrazine treatments two months after herbicide application (Table 3.7). Serrated Tussock density was significantly greater ($p<0.05$) by six plants per m^2 in control plots when compared with acetic acid herbicide treatments but not significantly greater ($p>0.05$) at 1.5 plants per m^2 when compared with atrazine treatments two months after herbicide application (Table 3.7). Serrated Tussock percentage cover was significantly greater ($p<0.05$) by 17 percent in control plots when compared with acetic acid herbicide treatments two months after herbicide application (Table 3.7). Serrated Tussock percentage cover was higher in atrazine treatments by four percent when compared with control plots two months after herbicide application (Table 3.7).

Assessment ten months after herbicide application

Assessments were undertaken in April 2003 after the standard spray and hay method had been applied to the herbicide treated plots. Both acetic acid treatments ($p<0.05$) and atrazine treatments ($p<0.01$) had a significantly greater Kangaroo grass seedling density and percentage cover when compared with control plots (Table 3.7). However, atrazine treated plots had a significantly ($p<0.01$) greater density of Kangaroo grass seedlings (29.75 seedlings per m^2) compared with acetic acid treatments. No other results of the 10-month assessment were statistically significant ($p>0.05$) when the acetic acid and atrazine treatments were compared for Kangaroo grass seedling density or percentage cover.

Chilean Needle grass seedling density was significantly greater ($p<0.01$) by 6.6 seedlings per m^2 in acetic acid treatments and 8.0 seedlings per m^2 in atrazine treatments when compared to the control plots. Chilean Needle grass seedling percentage cover was significantly greater ($p<0.01$) by 11.6 percent in acetic acid treatments and ($p<0.05$) 10.9 percent in atrazine treatments when compared to the control plots.

Chilean Needle grass mature plant density was significantly less ($p<0.01$) by 10.75 plants per m^2 in acetic acid treatments and 11.75 plants per m^2 in atrazine treatments when compared with the control plots. Chilean

Needle grass mature plant percentage cover was significantly less ($p<0.01$) by 34 percent in acetic acid treatments (an average of 9.38 ± 3.5 percent cover) and 41 percent in atrazine treatments (an average of 2.5 ± 1.34 percent cover) when compared with the control plots (an average of 43.8 ± 7.5 percent cover).

Serrated Tussock seedling and mature plant density and percentage cover were not statistically different ($p>0.05$) when comparing acetic acid treatments with the control ten months after herbicide application. Serrated Tussock mature plant density was significantly ($p<0.01$) reduced by 4.8 plants per m^2 in atrazine treatments when compared with the control ten months after herbicide application. Serrated Tussock mature plant percentage cover was significantly ($p<0.01$) reduced by 18.4 percent in atrazine treatments when compared with the control ten months after herbicide application.

	Kangaroo grass seedlings	Chilean Needle grass seedlings	Chilean Needle grass plants	Serrated Tussock seedlings	Serrated Tussock plants
Two months after herbicide application					
Plant density (number m^{-2})					
Control			19.88 ± 1.69		6.25 ± 0.29
Acetic Acid			8.57 ± 3.30*		0.29 ± 2.02*
Atrazine			6.88 ± 1.60*		4.75 ± 1.56
Plant percentage cover					
Control			55.00 ± 2.85		17.63 ± 0.71
Acetic Acid			15.86 ± 10.05*		0.71 ± 6.90*
Atrazine			21.25 ± 4.80*		21.25 ± 5.73$^{\Psi\Psi}$
Ten months after herbicide application					
Plant density (number m^{-2})					
Control	0.00	0.75 ± 0.31	12.88 ± 1.86	0.63 ± 0.38	5.00 ± 1.82
Acetic Acid	7.13 ± 3.92 *$^{\Psi\Psi}$	7.38 ± 2.13**	2.13 ± 0.79**		1.75 ± 0.92
Atrazine	36.88 ± 4.53**$^{\Psi\Psi}$	8.75 ± 2.02**	1.13 ± 0.67**	0.63 ± 0.63	0.25 ± 0.25**
Plant percentage cover					
Control		1.00 ± 0.78	43.75 ± 7.54	0.38 ± 0.18	20.00 ± 6.75
Acetic Acid	8.88 ± 4.86*	12.63 ± 3.12*	9.38 ± 3.46**		9.38 ± 4.67
Atrazine	43.13 ± 4.43**	11.88 ± 1.62**	2.50 ± 1.34**	0.63 ± 0.63	0.63 ± 0.63**

Table 3.7: Average plant percentage cover and density in un-treated control plots, acetic acid treated plots and atrazine treated plots two months after herbicide application and 10 months after herbicide application which incorporated re-establishment with Kangaroo grass, standard error included (n=8). * Indicates a significant difference at ($p<0.05$) and ** indicates a significant difference at ($p<0.01$) between control and stated herbicide. $^{\Psi}$ indicates a significant difference at ($p<0.05$) and $^{\Psi\Psi}$ Indicates a significant difference at ($p<0.01$) between herbicides.

Alternative Thatch type

Using the spray and hay Kangaroo grass re-establishment method, alternate thatch types were compared over untilled soil: a Kangaroo grass hay thatch and a Wheat straw thatch. Kangaroo grass seedling density and Chilean Needle grass seedling and mature plant density from the different thatch type treatments (Figure 3.6) were compared. No statistical difference ($p>0.05$) was found for any plant type assessed between the different thatch types.

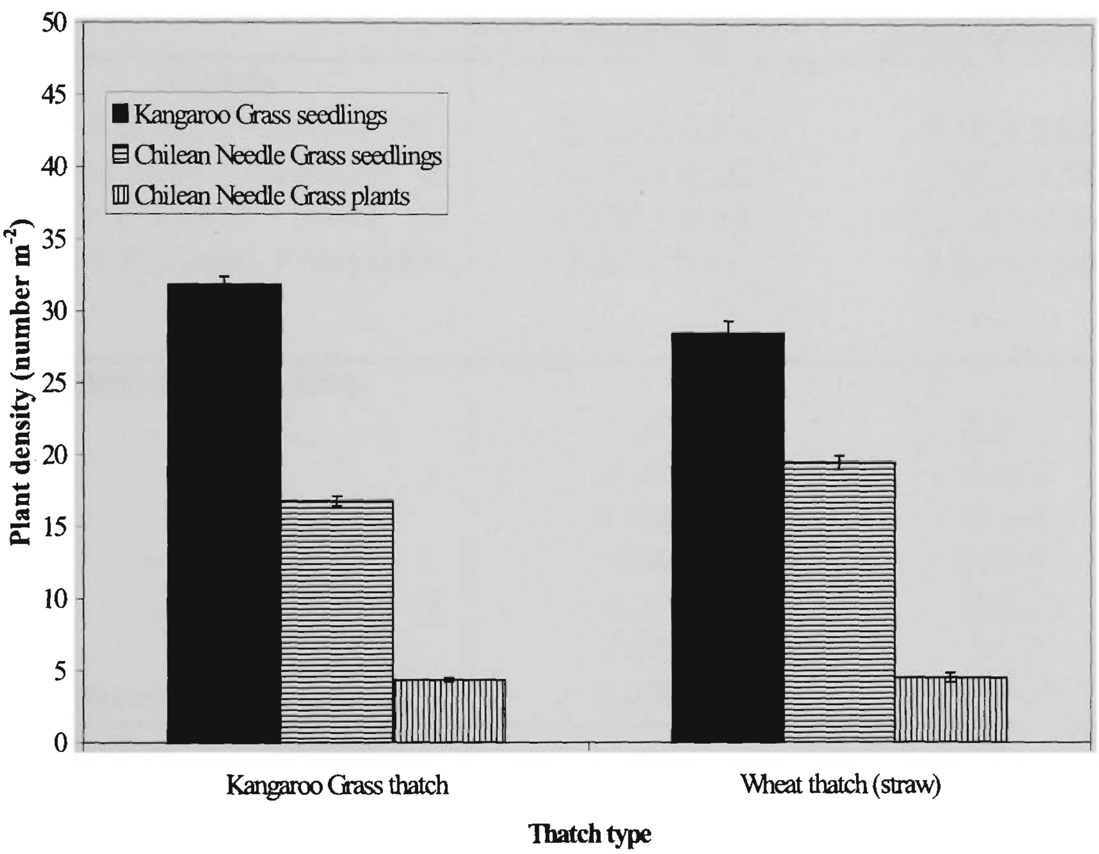

Figure 3.6: Average plant density in a Kangaroo grass re-establishment trial using two different thatch types at the same thatch density; Bars indicate Standard error (n=6).

The effect of thatch addition during a drought year was investigated on site AR6. Establishment density of Kangaroo grass and Chilean Needle grass are indicated in Table 3.8. Note that results for no-till plus thatch and till plus thatch are the same as reported in Table 3.2. Treatments with no thatch resulted in the lowest density of Kangaroo grass seedlings established. No-till, no thatch treatments average density of Kangaroo grass was 7 ± 2.53 seedlings per m^2, till no thatch treatment average density of Kangaroo grass was 15 ± 6.20 seedlings per m^2. Both no-till no thatch ($p<0.001$) and till no thatch ($p<0.05$) treatments had a significantly lower density of Kangaroo grass seedlings when compared with till and thatch treatments. Till no thatch treatments had the lowest density of Chilean Needle grass at 1.63 ± 0.56 seedlings per m^2. No-till and thatch treatments had the highest density of Chilean Needle grass seedlings at 12.38 ± 4.86 seedlings per m^2.

	Kangaroo grass seedlings	**Chilean Needle grass seedlings**
Density		
Till + KG seed + thatch (1)	36.88 ± 4.53	8.75 ± 2.02
Till + KG seed + no thatch (2)	15.38 ± 6.20	1.63 ± 0.56
No till + KG seed + thatch (3)	13.50 ± 2.90	12.38 ± 4.86
No till + KG seed + no thatch (4)	7.00 ± 2.53	3.63 ± 0.94
Statistics (p value)		
1 x 2	0.016*	0.014*
1 x 3	0.022*	0.952
1 x 4	0.000**	0.208
2 x 3	0.999	0.004**
2 x 4	0.366	0.586
3 x 4	0.301	0.076
Between subject effects	0.000**	0.002**
Multivariate Effects		
Wilks' Lambda	0.000**	

Table 3.8: Average density of Kangaroo grass and Chilean Needle grass seedlings in treatments with and without a thatch during a well below average precipitation year; Bars indicate standard error (n=8). Key: * indicates a significant difference at $p<0.05$, ** indicates a significant difference at $p<0.01$.

Discussion

Summary of results to be discussed below

Climatic conditions	Result in plant density	Result in plant percentage cover
2000 - 2001 below average precipitation	No significant differences	Significant differences
2001 - 2002 close to average precipitation	No significant differences	No significant differences
2002 – 2003 well below average precipitation	Significant differences	Significant differences

Table 3.9: Comparison of till re-establishment treatments versus no till re-establishment treatments for Kangaroo grass seedlings under differing climatic conditions.

Soil Disturbance Experiments

Below average precipitation year (2000 –2001 plot AR1)

Kangaroo grass seedling emergence was recorded in competitive replacement treatments that used the spray and hay method (Phillips & Hocking 1996; Phillips 2000) with and without soil disturbance. During this below average precipitation year, soil disturbance was found to significantly ($p<0.05$) increase the percentage cover of Kangaroo grass seedlings compared with no soil disturbance re-establishment trials. However, soil disturbance did not significantly ($p>0.05$) increase the density of Kangaroo grass establishing. Laboratory trials by Sindel *et al.* (1993) indicated that a rough soil surface could increase the amount of seed soil penetration. It was anticipated that soil disturbance in field trials presented in this report would also increase the amount of seed soil penetration as soil disturbance provided a rough soil surface. A greater degree of seed soil penetration should theoretically increase seedling emergence through having more seed available to germinate. This was not the case during the below average precipitation year. Other factors that could have affected seedling emergence are discussed below.

Abiotic factors may have played a greater role in seedling germination and survivorship than seed availability. Water availability is needed for imbibition of seed, germination, plant survivorship and quantity of biomass produced. Research by McWilliam *et al.* (1969) emphasises the importance of available light, nutrients and water in the early stages of seedling emergence. Imbibition of seed in the soil can be affected by a combination of factors such as the potential energy of soil water and, the rate of movement of soil water across the seed-soil interface and into the seed (Hadas & Russo 1974a). Optimal seed-soil water contact occurs when the average aggregate size is one-fifth to one-tenth of the seed diameter (Hadas & Russo 1974b). Cultivation initially increases soil porosity (volume of pores). Porosity of soil affects the movement of water through soil. In particular it is the size, number, orientation, distribution and continuity of pores that affect hydraulic conductivity (Rowell 1994).

It is suggested that water availability was high enough in both soil disturbance and no soil disturbance re-establishment treatments during the below average precipitation year so as not to produce a significant effect between these two treatments. Further analysis of porosity and soil moisture in relation to depth of tilling and establishment of Kangaroo grass seedlings is presented in Chapter four.

Cultivation (soil disturbance) can also be used for control of weeds (Parsons 1995) but when used in native grassland situations where active native species reintroduction has not occurred, cultivation may increase the potential of exotics to establish (Hobbs & Huenneke 1992; Wijesuriya & Hocking 1998; Wijesuriya 1999; Prieur-Richard & Lavorel 2000). Linked to cultivation, high levels of available nutrients are also reported to positively influence plant survivorship and biomass production. These relationships are further investigated in the context of Kangaroo grass establishment in Chapter five.

Outcomes in a well below average precipitation year (2002 – 2003 plot AR6)

Over a drought period during the summer of 2002 – 2003 in Melbourne, Victoria, soil disturbance at five cm depth followed by implementing the spray and hay method resulted in a significantly greater density and percentage cover of Kangaroo grass seedlings than for no tilling treatments. The density of Kangaroo grass seedlings present in sites six months after emergence (37 $\pm$ 4.5 per m^2) is not as high as estimated (60 per m^2). The resulting density represents 12 percent of the amount of germinal seed added to the site, which is about half that predicted using seed addition calculations reported by Phillips (2000). Kangaroo grass seed density on conversion to seedlings in plots not tilled were on average 14 $\pm$ 2.9 per m^2. This is only five percent of the amount of germinal seed added to the treatment. Evidence reported in Chapter four suggests that under greater water stress situations as in a drought, the influence of fallowing on soil porosity and evaporation are increased. Greater availability of water and reduction of evaporation in tilled plots compared to non-tilled plots are likely to have contributed significantly to the higher density of Kangaroo grass seedlings in tilled plots, compared with no till treatments.

Average Chilean Needle grass and Serrated Tussock re-establishment on tilled plots was lower in comparison to no-tilled plots but this difference was not statistically significant ($p>0.05$).

Outcomes in close to average precipitation year (2001 – 2002 plots AR4, AR5 & Woodlands)

Results six months after emergence

Results from Kangaroo grass competitive replacement trials during a close to average precipitation year (2002) showed a difference between sites but not between soil disturbance treatments. Excellent rates of Kangaroo grass seedling establishment occurred for all treatments and on all sites during this establishment season. The difference between sites for Kangaroo grass seedling emergence needs further investigation.

The conversion of germinable Kangaroo grass seed into seedlings surviving to the six month since emergence stage was excellent for all sites and treatments. Site AR4 had the lowest Kangaroo grass seedling emergence density of all the sites tested. However, this emergence was at the lowest at 90 $\pm$ 23 seedlings per m^2 and highest at 108 $\pm$ 20 seedlings per m^2. This results in a 30 percent and 36 percent conversion of germinal seed into seedlings added to the plot. This is higher than the average reported by Phillips (2000) and a similar conversion to that reported by O'Connor (1996) of approximately 35 percent in the best treatments.

Sites AR5 and Woodlands had emerging Kangaroo grass seedling densities ranging from 258 $\pm$ 41per m^2 (AR5 3 cm till plus Kangaroo grass seed) to 325 $\pm$ 65 per m^2 (Woodlands, 5 cm till plus Kangaroo grass seed). This is equivalent to an 86 percent to 108 percent conversion of germinal seed added to the plot germinating and surviving to the six month old stage. An error of at least 10 percent is associated with laboratory based, seed percentage germination tests and may account for some of the apparent 108 percent survivorship along with any recruitment from the naturally occurring seedbank. A Tukey post hoc test was performed to determine where the difference occurred between tilling depths but no significant difference ($p>0.05$) was found.

Only minimal differences were found between treatments at all sites for Chilean Needle grass and Serrated Tussock seedling and mature plant densities six months after Kangaroo grass seedling emergence. This may suggest that the re-establishment rate of these species is determined by plant biological factors such as dormancy more than abiotic factors. The application of atrazine three months after Kangaroo grass emergence did not statistically affect Kangaroo grass densities at the six month since emergence stage. Dead Chilean Needle grass and Serrated Tussock densities and cover were significantly higher ($p<0.05$) in plots that had atrazine applied three months after Kangaroo grass establishment compared with no atrazine application treatments. This result was anticipated as the herbicide atrazine has been shown to have a more positive effect on plant death than abiotic factors (Table 3.7).

During this period of Kangaroo grass re-establishment treatments the total monthly precipitation was close to the 30-year average. As a result the soil being treated was closer to field capacity than in trials performed during drier years. At field capacity most soil pores will contain soil water in equilibrium between gravimetric draining, evapotranspiration and evaporation.

At this stage the permanent wilting point (where plants die due to moisture stress) will take longer to be reached than in soils with a lower field capacity (Rowell 1994). The apparent lack of difference in Kangaroo grass seedling densities between treatments with different depths of soil disturbance is thought to be because all treatments had sufficient water available for imbibition and germination of seeds.

Results 18 months after emergence

Results in this section were assessed during March 2003 during a well below average precipitation year. After 18 months Kangaroo grass plant densities had declined at all sites and all treatments. Kangaroo grass plant densities ranged from 47 $\pm$ 11.1 per m^2 (site AR5, 3 cm till plus Kangaroo grass seed) to 77 $\pm$ 10.6 per m^2 (site AR4, 1 cm till plus Kangaroo grass seed). There were no statistical differences ($p>0.05$) in Kangaroo grass density between sites and treatments when data collected 18 months after removal of thatch were compared. The reductions in Kangaroo grass density between the six-month and 18 month assessments are shown in Figure 3.5. No till plus the standard spray and hay method resulted in the greatest percentage decline of Kangaroo grass density at 66 percent. The till treatments at five cm depth plus standard spray and hay method resulted in the lowest percentage decline of Kangaroo grass density at 52 percent.

Chilean Needle grass mature tussock densities were in the 18-month assessment data average higher in no till plus Kangaroo grass seed re-establishment plots when compared to the five cm till plus Kangaroo grass seed treatment plots. However this difference was not statistically significant ($p>0.05$). At the Woodlands site the re-establishment of Chilean Needle grass seedlings in till with no seed addition plots was high compared with the seed addition plots. Problems with loss of replicates due to kangaroo and rabbit disturbance mean that no statistical analysis could be conducted on the any added seed treatments. No definite trends were noted in Serrated Tussock seedling and mature plant re-establishment in the 18-month assessment data.

Alternative Herbicide

Two months after application

An acetic acid and surfactant based herbicide was trialed for its capacity to rapidly kill Chilean Needle grass and Serrated Tussock. The effectiveness of the alternative herbicide was compared with that of atrazine as Nufarm Nutrazine®. Soil pH returned to background levels three days after acetic acid and surfactant application. Both acetic acid and atrazine significantly ($p<0.05$) reduced the density and percentage cover of Chilean Needle grass by more than 30 percent in comparison to untreated plots. The effect of the herbicide atrazine is considered to have been limited due to low soil water availability and the hydrophobic nature of atrazine (Hance & Holly 1990). The acetic acid herbicide was significantly ($p<0.05$) more effective at killing Serrated Tussock above ground vegetation than atrazine two months after herbicide application. Acetic acid plus surfactant herbicide is considered to give an effective rapid kill of aboveground vegetative matter to both Chilean Needle grass and Serrated Tussock under trial conditions. This result is promising but needs further development to achieve below ground vegetative matter death given both these species are perennial. More investigation is also required under a broad range of field conditions before the acetic acid and surfactant herbicide could be considered a viable alternative herbicide.

Effects on Kangaroo grass re-establishment during a drought

The acetic acid and atrazine treated plots were subjected to the 'spray and hay' plus five cm deep till Kangaroo grass competitive replacement method after the above ground vegetation was dead. Kangaroo grass emerging seedling densities from acetic acid treatments were significantly ($p<0.01$) lower than atrazine treatments. The density of Kangaroo grass seedlings emerging in acetic acid treatments is thought not to be at a density that will survive further weed invasion. McDougall (1989) suggested that a population of 40 per m^2 surviving seedlings is required for the population to be sustainable. Results from Phillips (2000) in which Serrated Tussock was competitively replaced with Kangaroo grass suggested that 60 per m^2 surviving seedlings are required for the stand to resist re-invasion. Chilean Needle grass seedling density was significantly higher in both herbicide treatments in comparison with the control. This is most likely due to higher levels of available above and belowground resources in treatments where a large percentage of the vegetation has been killed and tilled (Wijesuriya 1999). Chilean Needle grass mature plant density was significantly lower in both herbicide treatment plots in comparison to untreated plots after Kangaroo grass establishment. Both Chilean Needle grass seedling and mature plants re-established at non-statistically different rates when the herbicides acetic acid and atrazine were compared.

Serrated Tussock had begun to re-establish in the acetic acid treated plots 10 months after application. The re-establishment of Serrated Tussock is thought to have been in direct competition for resources with the emerging Kangaroo grass seedlings. During a year with higher precipitation additional problems resulting from re-establishment of both Serrated Tussock and Chilean Needle grass into acetic acid treated areas would be likely to occur. There may be potential for use of this alternative herbicide in integrated weed management approaches, however further research is needed.

Alternative Thatch Type

The spray and hay Kangaroo grass establishment method requires a thatch be used to cover any Kangaroo grass seed that has been laid on the ground (Phillips & Hocking 1996; Phillips 2000) presumably to aid in retention of soil moisture. The thatch is removed preferably by fire in early October. Due to the greatly limited area of native grassland remaining (DCE 1992; Kirkpatrick *et al.* 1995), Kangaroo grass hay can be limited. Cheaper and more abundant wheat hay was investigated as a thatch. The wheat thatch (mostly straw without seed heads) was found to be an acceptable replacement for Kangaroo grass thatch. No statistical difference ($p>0.05$) was noted for Kangaroo grass seedling emergence, or between densities and percentage cover of Chilean Needle grass or Serrated Tussock, between treatments using each of the thatch types. Only two wheat plants emerged in all of the field sites tested. This is thought to be an acceptably low risk and unlikely to act as an aggressive weed.

Results suggest that the incorporation of a thatch into Kangaroo grass re-establishment treatments during a below average precipitation year will significantly increase the density of establishing Kangaroo grass seedlings. However, thatched treatments also had a significantly greater density of Chilean Needle grass seedlings when compared with no thatch treatments. Not using a thatch in Kangaroo grass establishment treatments may give acceptable results during an average or above average precipitation year but further research is needed.

Implications for Management

The following are strategies that could be used to competitively establish Kangaroo grass over Chilean Needle grass and Serrated Tussock dominated sites in the west of Melbourne, Victoria. It must be noted that any form of soil disturbance has the potential to be a site for invading weed species. It is recommended that a desired plant species, under competitive conditions must be placed back into the space provided by the small-scale soil disturbance as reported above. The following are only guides and it must be noted that an assumption of upcoming seasons would be required.

In below average precipitation years

Reduce the biomass of unwanted weed species to approximately five cm in height, preferably by burning in early autumn. Once plant regrowth has started apply a suitable herbicide at label or government recommended rates. Be careful at this stage not to kill desired species. In late July or early August till the dead exotics. Be careful at this stage not to till areas dominated by desired species. Immediately after the till apply Kangaroo grass seed and a thatch layer. Apply Kangaroo grass chaff with viable seed to the ground in sufficient density to give 60 seedlings per m^2. This requires that Kangaroo grass seed and chaff be assessed for germinable seed content prior to addition to the re-establishment site. During drought conditions allow for only 13 percent of the germinal seed added to the site to survive to be mature tussocks. Lay a thatch (preferably Kangaroo grass although wheat straw will suffice) over the establishment area at approximately one and a half kg per m^2. In early October burn the thatch layer on the site so that minimal cover is left on the site. Monitor the site for weed invasion and Kangaroo grass establishment, which should occur after the thatch burn. The potential benefits of adding water and nutrients at this stage is currently under investigation.

In close to average precipitation years

During years with average precipitation wider selection of treatments may be available to achieve desired results of a high density of Kangaroo grass and low density of Chilean Needle grass and Serrated Tussock around the Melbourne region. The same method as for below average precipitation years can be used with the exception that a conversion rate of up to 20 percent of viable seeds to seedlings can be allowed for. Tilling will not appear to produce a significantly higher density of Kangaroo grass under these conditions. Tilling will increase the amount of exotic mature plant death but may at the same time increase re-establishment of exotic seedlings. Lower rates of seed addition may also be possible. Further research is needed to clarify the benefits of tilling and lower seed addition. Additional research is also needed to determine whether or not a thatch is necessary during average precipitation years, and if thatch were needed, what type and rate would be effective.

Conclusion

Kangaroo grass germination during a low precipitation year was significantly higher in plots that were tilled to a depth of one cm and five cm when compared with plots with no soil disturbance. In comparison during a year with higher total monthly precipitation, no significant difference was found in Kangaroo grass germination between plots with soil disturbance and plots without soil disturbance. In a subsequent disturbance trial during a severe drought year, Kangaroo grass germination was significantly higher in plots that were tilled when compared with plots without tilling. Caution needs to be used in the interpretation of these results. Soil disturbance has been shown to increase weed invasion in some native grasslands. If soil disturbance is to be used it should only be applied in areas with near full cover of exotic species (the dominant species) and in which native species need to be re-introduced competitively to minimise further exotic invasion. During drought-affected years, soil disturbance was used to give acceptable and reliable results of Kangaroo grass germination in conjunction with the modified spray and hay revegetation method presented here. During non-drought years soil disturbance does not appear to have any benefits. Two minor parallel trials investigated alternative herbicides and thatch types. An acetic acid surfactant solution gave close to 100 percent death of above ground vegetative matter two months after application (significantly different to plots sprayed with distilled water at $p<0.05$) for both Chilean Needle grass and Serrated Tussock. More testing is required before this solution could be considered an effective and reliable herbicide. Plants sprayed with the herbicide atrazine gave effective (significantly different to plots sprayed with distilled water at $p<0.05$) vegetation kill two months after application for Chilean Needle grass but not Serrated Tussock. In a comparison of Kangaroo grass hay and Wheat hay used as the thatch cover during the revegetation process, no significant difference was found ($p>0.05$) in Kangaroo grass or Chilean Needle grass seedling establishment density per m^2.

Chapter Four

Effects of soil disturbance on soil seedbank and soil moisture

Introduction

The techniques for Kangaroo grass (*Themeda triandra*) re-establishment are still in their infancy. Successful establishment of Kangaroo grass has been undertaken on small-scale areas and in relatively normal precipitation years (Phillips 2000). Under drought conditions results have been poor (Hocking unpublished data and Chapter three). Investigations reported in Chapter three found that low-level soil disturbance in areas dominated by weeds such as Serrated Tussock (*Nassella trichotoma*) and Chilean Needle grass (*N. neesiana*) can have a significant effect on increasing Kangaroo grass establishment during low precipitation years (drought years). Suggested reasons for this could include (1) the effects of soil disturbance on increasing the amount of Kangaroo grass seed entering the soil seedbank, or (2) changes in moisture dynamics in the soil which favour Kangaroo grass seedling establishment (Hagon & Chan, 1977; Sindel *et al.* 1993; O'Connor 1996).

Increasing the availability of Kangaroo grass seed by manipulative addition has been reported to increase recruitment 37-fold in comparison to naturally occurring levels of seed in the soil seedbank (O'Connor 1996). Background seedbank densities of Kangaroo grass seed vary considerably between sites ranging from 0.3 per m^2 (in grassland near the eastern cape of South Africa, O'Connor 1996) to 1375 per m^2 at the Derrimut grassland reserve in Victoria, Australia (Lunt 1996). These figures are for total seeds not germinable seeds. Investigation into the soil seedbank in northern Australia found high densities of annual grasses but few perennial grasses (McIvor & Gardener 1991). Tasmanian woodland soils were found to contain a high proportion of native compared with exotic seeds in the seedbank but most of the native seeds were from herbaceous vegetation types (Pyrke 1994). These results are not surprising, as varying approaches to vegetation management and a host of climatic variables will affect the amount and type of seed produced and therefore present in the soil seedbank. Windsor & Clements (2001) found that three different rates of Kangaroo grass seed addition (225g, 450g and 900g) did not produce significant differences in Kangaroo grass seedling establishment density (measured as number of seedlings per m^2). This suggests that competition between seeds for resources necessary for germination and establishment may be of greater importance than gross seed densities when sufficient seed is available. McDougall (1989) and Phillips (2000) both mention that there can be large variation in the amount of seed harvested per plant. It is likely that large variation will be found in seed density across a Kangaroo grass site to be harvested and in the resulting harvested hay added to re-establishment sites. The removal of the thatch layer used in most re-establishment methods by fire also has the potential to reduce seed availability, especially of seed not buried in the soil. Assessment of soil seedbank during the establishment phase was conducted in the study reported here to give an indication of the amount of seed that is available for germination and establishment on revegetation sites.

A decline in soil moisture has been shown to reduce the establishment of Kangaroo grass and other grasses native to Australia (Hagon & Chan 1977; O'Connor 1996; Windsor & Clements 2001). The availability of soil water can depend upon the total amount of water delivered to the soil, the location of soil water within the soil profile and also on soil properties such as bulk density and porosity (Rowell 1994). Rowell (1994) described the interactions of soil water and porosity, which are summarised as follows: Soil water is associated with the size and number of pores within the soil profile; macropores (larger than 50μm) function to allow rapid drainage of soil water; once the macropores are emptied, drainage is slow and the soil is said to be at field capacity; micropores sizes (50-0.2μm) are important influences on evaporation and transpiration processes when the soil is between field capacity and the wilting point; an increase in soil porosity corresponds with a decrease in soil density. In addition, a zone in the soil profile that is less dense should have more available water associated with the increased number and size of micropores within its structure compared to a more dense profile. Rowell (1994) also reported that cultivation of soil can decrease soil bulk density in comparison to no cultivation. Therefore cultivation should be one way to increase the availability of soil moisture as the bulk density decreases. There is a potential however for cultivation to increase the rate of evaporation of soil water which has the potential to have adverse effects on seedling establishment.

The effects of soil seedbank availability associated with Kangaroo grass re-establishment trials were investigated in response to differing levels of soil disturbance over two years and four field sites in the West of Melbourne, Australia. The effects of different depths of soil disturbance during Kangaroo grass establishment on soil properties and the location and availability of soil water in the soil profile were also investigated.

Methods

Site Location

All except one field site were located at the Iramoo grassland Reserve associated with Victoria University in the suburb of St Albans, outer Western Melbourne, Victoria, Australia. Native Kangaroo grass and *Austrostipa* spp. dominate the Iramoo grassland reserve. Chilean Needle grass and Serrated Tussock are the dominant grassy weeds at this location and were the target species to replace with Kangaroo grass. The other field site used is located at Woodlands Historic Park in Greenvale, Victoria, Australia. The field sites are described in more detail in Chapter 3.

Calculation of Kangaroo grass seed addition

Calculations of the amount of Kangaroo grass seed to apply to revegetation plots followed protocols developed by Phillips (2000) and are reported in Chapters two and three. Chapter three treatments and therefore soil disturbance methodology was used in the experiments within this Chapter. Each bag of harvested Kangaroo grass was sub sampled three times to obtain an average seed density for each bag. Germination potential of seeds was tested in a 6.5 percent agar solution. The agar was set in 200 ml jars with lids. Kangaroo grass seeds were implanted without awns into the agar and incubated on a diurnal cycle of 35°C daylight for 12 hours, and 20°C darkness for 12 hours. Total incubation period was 14 days. The desired on-ground Kangaroo grass seedling density in establishment trials was 60 seedlings per m^2. The average seed density per harvested bag, percentage germination and estimated survivorship percentage are presented in Table 4.1. As seeds were broadcast on to the site these is potential that some seeds were subjected to predation.

Calculation of soil seedbank

Soil seedbank was determined using a modification of the seed extraction processes used by Buhler & Maxwell (1993); Tsuyuzaki (1994); Ter Heerdt *et al.* (1996); and Luschei *et al.* (1998). The process was briefly as follows: a soil auger with a diameter of 16 cm was used to take one soil sample randomly within each eight replicate for each treatment to a depth of five cm; a 0.5 M potassium chloride solution was added to the soil to help with breakdown of the soil structure and; soil samples were washed through a 1.0 mm sieve and 500μm sieve. These two sieves in combination allowed for the separate collection of Kangaroo grass, Chilean Needle grass and Serrated Tussock seeds. Seeds of each type were then sorted into hard or soft. Densities of hard seed found in the soil seedbank were compared with densities of viable seed added to treatment plots. Soil seedbank samples were taken at the beginning of the Kangaroo grass establishment period in October after the thatch layer had been removed by burning the re-establishment sites.

Soil Physical Properties

Soil dry bulk density, particle density, porosity and available soil moisture follow standard procedures outlined by Rowell (1994). Physical properties as mentioned above were only recorded from site AR6 located at the Iramoo grassland Reserve in March 2003 within one week of vegetation assessments.

Statistics

Data was statistical analysed using SPSS v 11.0. All data were tested for normality via box plots, skewness and kurtosis. Transformations were conducted according to Zar (1984) if required. General Linear Model univariate analyses with or without posthoc tests were used for simple comparisons. General Linear Model multivariate analyses with or without posthoc tests were used to look at between subject effects.

Results

Kangaroo grass seed addition

The density of Kangaroo grass seed applied to research plots is presented in Table 4.1. Errors shown in this table represent one standard deviation from the mean. The variation associated with seed samples applied to each site was relatively high. Values for both the average total amount of seed and average estimated amount of ripe seed are presented. The average total seed values can be compared with the minimum and maximum amount of seed found in soil seedbank analysis. The predicted theoretical amount of seed applied to each treatment was within the orders of magnitude obtained from the soil seedbank if errors are also considered. The large errors associated with analysis of seedbank for Kangaroo grass do not allow for meaningful statistical comparisons. It would appear that a large percentage of Kangaroo grass seed applied to the site was incorporated in to the soil seedbank. A control is also indicated on Figure 4.1 where no additional seed was incorporated in to the treatment. This control represents the background level of seeds in the soil seedbank.

Sample type	Iramoo 2001 (AR4 & AR5)	Woodlands 2001	Iramoo 2002 (AR6)
Calculated addition of seed to field site			
Average total seed added to one m^2	1152 ± 492	1367 ± 658	1667 ± 667
Average total ripe seeds with awns added to one m^2	857 ± 344	856 ± 340	833 ± 333
Average percentage of germinable seed (*in vitro*)	35% ± 10	35% ± 12	36% ± 11
Theoretical conversion of seed into seedlings in the field	20%	20%	20%
Theoretical density (per m^2) of surviving Kangaroo grass seedlings	60	60	60
Soil seedbank analysis			
Minimum number of hard seed found in seed bank per m^2	884 ± 525	3518 ± 3642	1534 ± 594
Maximum number of hard seed found in seed bank per m^2	3328 ± 2096	5469 ± 4298	2000 ± 980

Table 4.1: Calculation of average density (seed per m^2) of Kangaroo grass seed added to re-establishment field sites and resulting average minimum and maximum density of Kangaroo grass seed found in the soil seedbank to a depth of five cm, one standard deviation indicated (n varied between 15-24 for seed addition calculations, and equalled eight for soil seedbank calculations). Germinable seed was calculated from laboratory based germination trials (see Chapter two).

Soil seedbank analysis

Kangaroo grass

Every treatment that had Kangaroo grass seed applied resulted in a significant increase in the soil seedbank when compared with the control (no seed addition treatment, Figure 4.1). A statistically non-significant trend of increasing seedbank density with depth of soil disturbance is indicated. Only site AR5 showed a significant increase in seedbank density when the five cm till plus Kangaroo grass seed treatment was compared with the no till plus Kangaroo grass seed treatment. The control (no treatment) at the Woodlands site had the lowest background level of Kangaroo grass seed equalling a density of 105 ± 105 seeds per m^2 as expected.

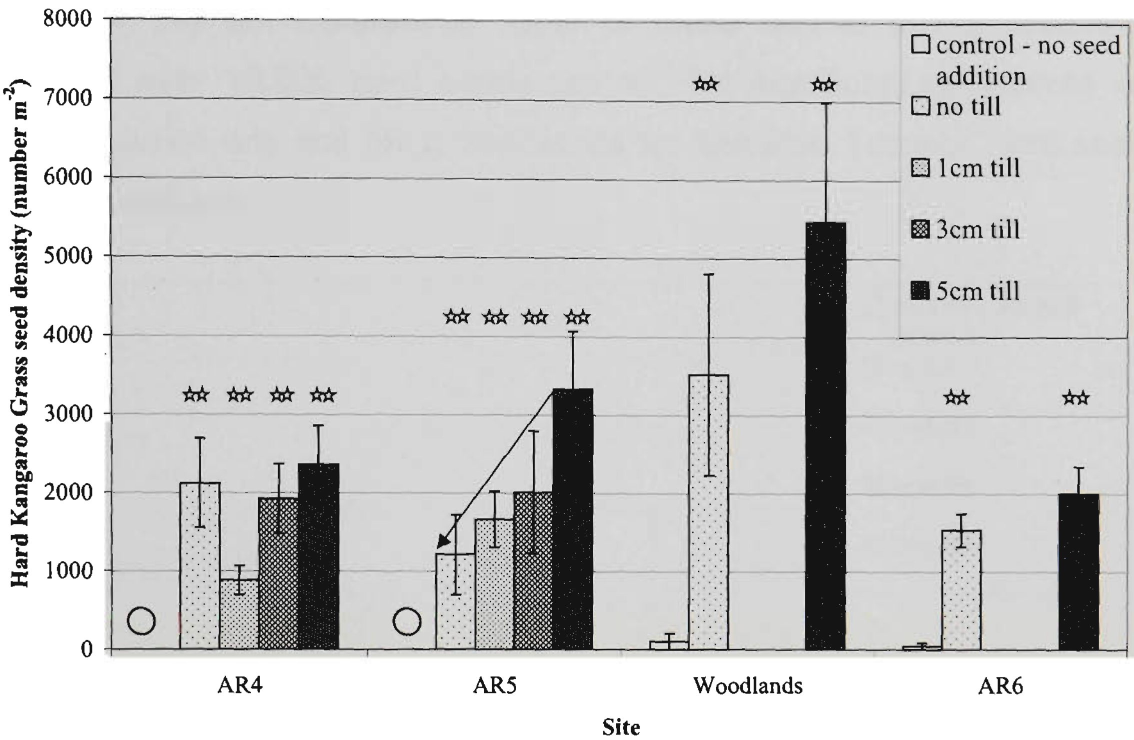

Figure 4.1: Average Kangaroo grass hard seed in soil seedbank associated with soil disturbance on four sites. Bars indicate standard error (n=8). Key: ** Indicates a significant difference ($p<0.01$) between seed density in soil disturbance treatment and control for each specific site. Arrow indicates a significant difference ($p<0.05$) between five cm till and no till for site AR5. No data were obtained from 3 cm till plots from sites Woodlands and AR6. Control results were all below 110 seeds per m^2. The legend indicates levels of increasing soil disturbance treatments. O Indicates zero result in control column.

Chilean Needle grass

There was a general trend of decreasing soil seedbank average density of Chilean Needle grass seed with increasing depth of soil disturbance (Figure 4.2). This trend was not statistically significant ($p>0.05$). The Chilean Needle grass seedbank ranged from approximately 500 hard seeds m^{-2} to 3500 hard seeds m^{-2}.

Serrated Tussock

No trends were obvious in any of the sites for Serrated Tussock hard seed density within the soil seedbank (Figure 4.3). Site AR5 showed the highest density of Serrated Tussock soil stored seed in treatments that had one cm and three cm soil disturbance. Both of these depths of soil disturbance averaged over 10,000 hard seeds per m^2. No significant differences were found between any soil tilling treatments for Serrated Tussock hard seed in the soil seedbank.

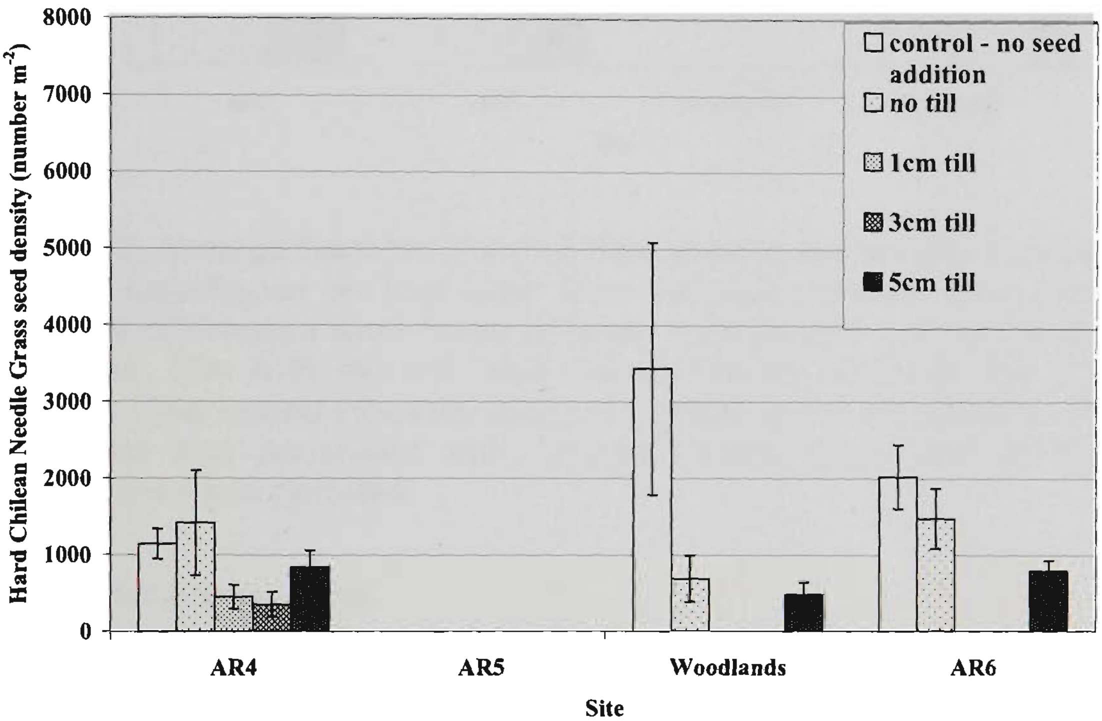

Figure 4.2: Average Chilean Needle grass hard seed in soil seedbank associated with soil disturbance on four sites. Bars indicate standard error (n=8). No significant differences were found between controls and treatments or within treatments. No data was obtained from 3cm till plots from sites Woodlands and AR6. The legend indicates levels of increasing soil disturbance treatments. The site AR5 is a Serrated Tussock dominated site and no Chilean Needle grass seed was detected.

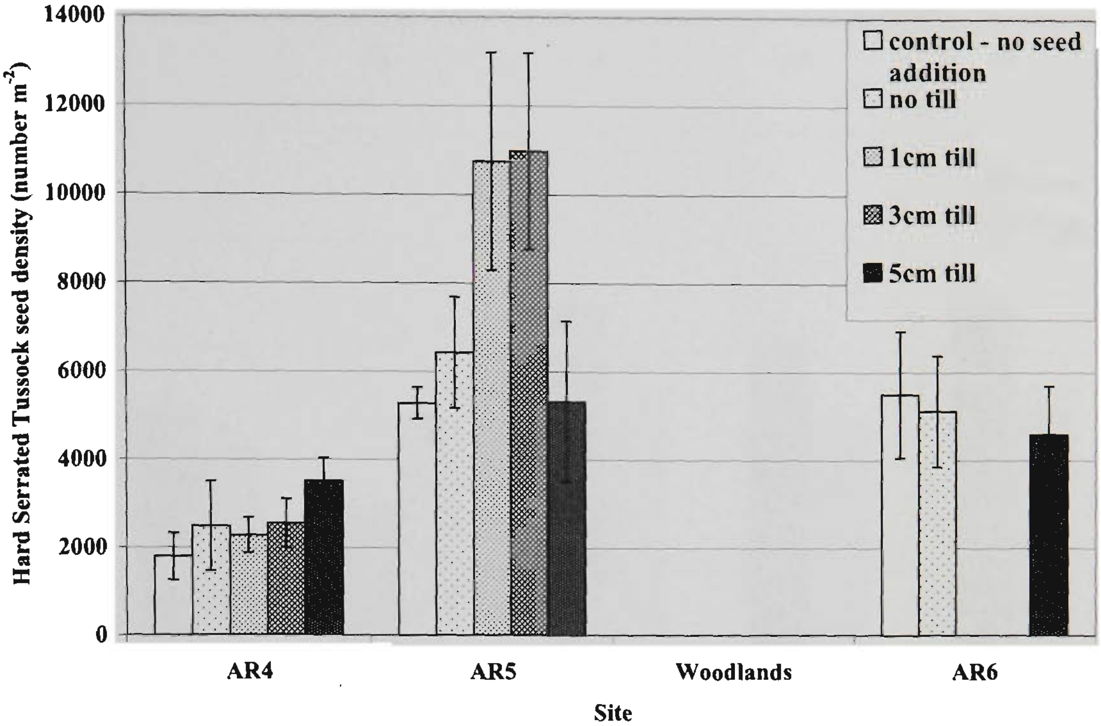

Figure 4.3: Average Serrated Tussock hard seed in soil seedbank associated with soil disturbance on four sites. Bars indicate standard error (n=8). No significant differences were found between controls and treatments or within treatments. Site AR6 did not have measurements taken for the 3 cm till treatment. The legend indicates levels of increasing soil disturbance. The site Woodlands was dominated with Chilean Needle grass and no Serrated Tussock seed was detected.

Soil Physical properties

Dry bulk density

Average Dry bulk density results for soil samples are shown in Figure 4.4. Fresh tilled soil was significantly denser in the 2-5 cm depth sample than in the 0-2 cm depth sample. Soil sampled one, two and three years after tilling showed the opposite trend where the 0-2 cm soil profile is significantly denser than the 2-5 cm soil profile. No till treatments exhibited a similar trend in bulk densities to the older disturbed soil profiles.

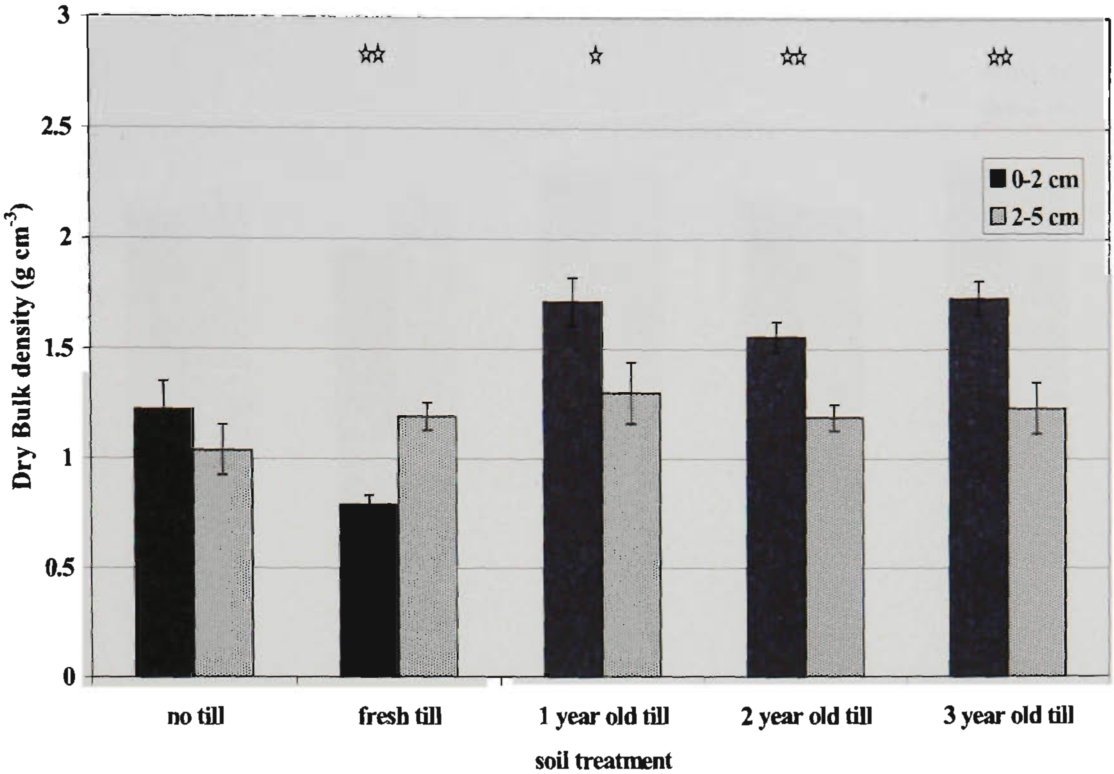

Figure 4.4: Average Dry Bulk Density associated with soil depth and age since soil disturbance tested in March 2003 at re-establishment site AR6. Bars indicate standard error (n=8), Key: * indicates a significant difference ($p<0.05$), and ** indicates a highly significant difference ($p<0.01$) between depths for individual soil disturbance results.

Soil particle density

No significant differences ($p>0.05$) were found between depths of soil profile for soil particle density (see figure 4.5).

Porosity

Porosity in freshly tilled soil was significantly ($p<0.01$) higher in the 0-2 cm soil profile when compared to the 2-5 cm profile (Figure 4.6). No till, one, two and three years since till all showed the trend of the 0-2 cm soil profile being less porous than the 2-5 cm soil profile although this difference was only significant ($p<0.05$) in the two year since till samples.

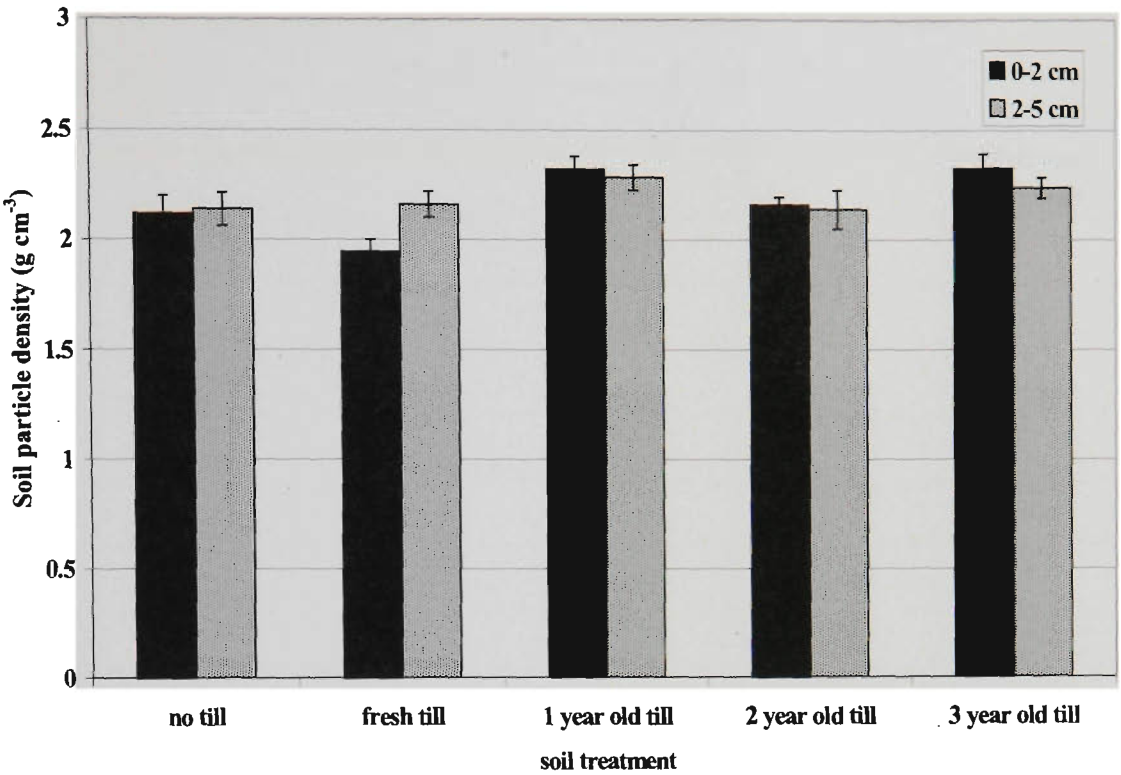

Figure 4.5: Average Soil Particle Density associated with soil depth and age since soil disturbance tested in March 2003 at re-establishment site AR6. Bars indicate standard error (n=8).

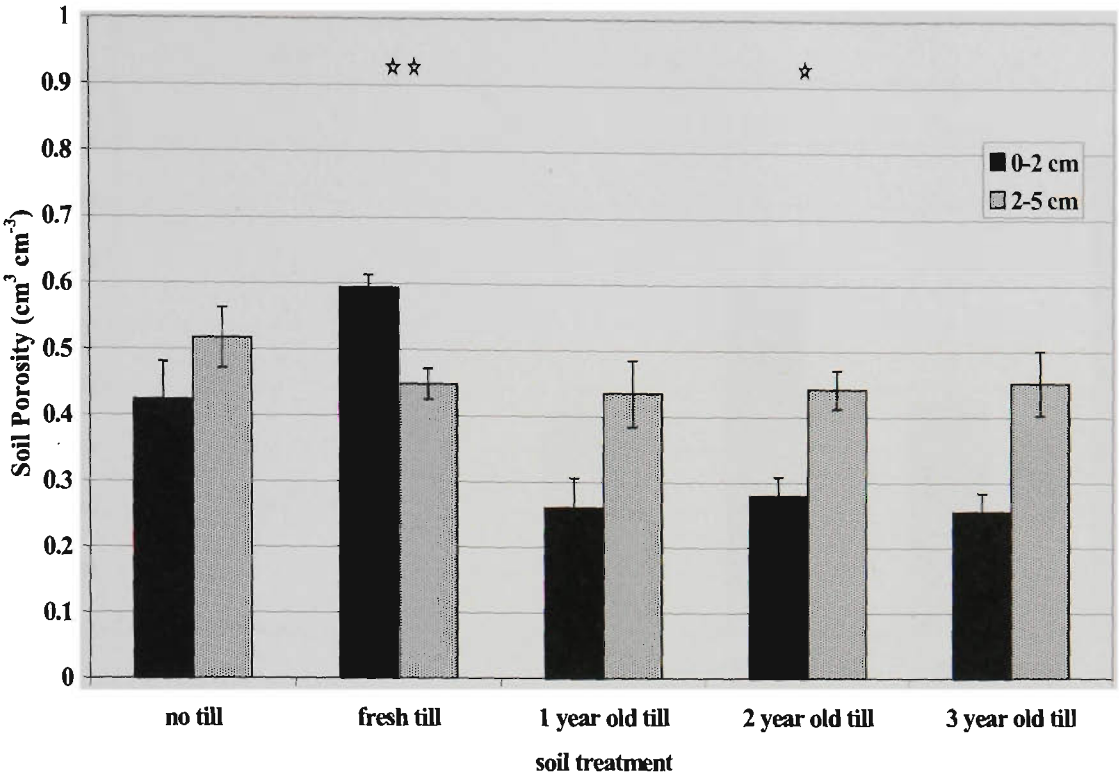

Figure 4.6: Average Soil Porosity associated with soil depth and age since soil disturbance tested in March 2003 at re-establishment site AR6. Bars indicate standard error (n=8). Key: * indicates a significant difference ($p<0.05$), and ** indicates a highly significant difference ($p<0.01$) between depths for individual soil disturbance results.

Available soil moisture

In control and soil disturbance treatments, available soil moisture was significantly higher ($p<0.05$) in the 2-5 cm soil profile than in the 0-2 cm soil profile (Figure 4.7). Tilled with Kangaroo grass re-establishment treatments had the highest percentage moisture (16%) in the 2-5 cm soil profile when compared with the other treatments. No till plus Kangaroo grass re-establishment had the next highest soil moisture in the 2-5 cm soil profile at approximately 11 percent (Figure 4.7).

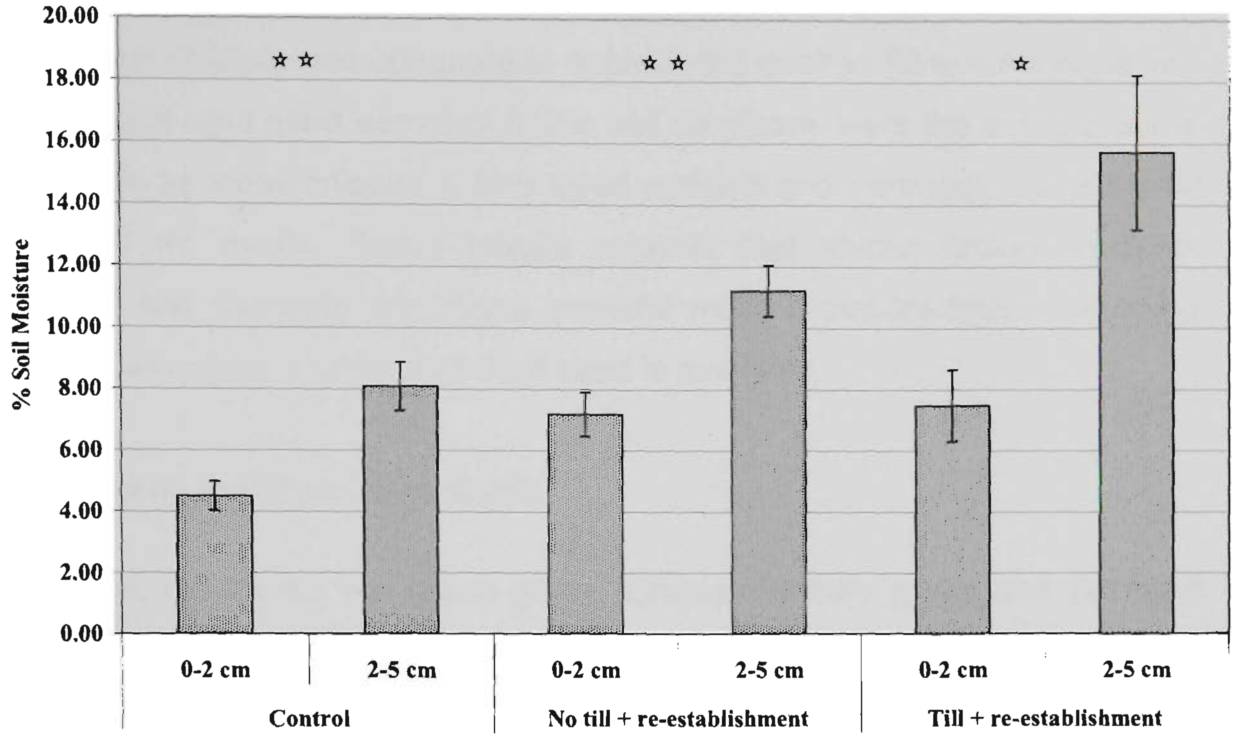

Figure 4.7: Average percentage soil moisture with depth of soil profile and Kangaroo grass re-establishment method tested in March 2003 at re-establishment site AR6. Bars indicate standard error (n=6). Key: * indicates a significant difference (p<0.05), and ** indicates a highly significant difference (p<0.01) between depths for individual soil disturbance results.

Discussion

Kangaroo grass seed addition

Theoretical values of expected soil seed content of Kangaroo grass, based on the amount of Kangaroo grass seed applied to re-establishment treatments were an underestimate of the actual amount of seed found in the soil seedbank after seed addition for all sites. This could possibly be due to the large variation in seed found in individual plants and therefore within Kangaroo grass hay used for revegetation. Greybox and Grasslands Indigenous Nursery mixed the hay thoroughly before placing in woolsacks. However this mixing and the subsequent sampling of the woolsacs to determine an estimate or average of the amount of seeds per woolsac does not appear to be a good estimate. Further sampling of wool sac other than the nine samples per woolsack used in these trials may be warranted. Germination results from this trial are reported in Chapter 3. However, it was considered that the density of Kangaroo grass seed applied to re-establishment plots was adequate to achieve the desired 60 seedlings per m^2. Overall total hard seed densities in the soil seedbank were the same order of magnitude as those calculated from seed addition and were well above the 60 seed per m^2 levels. These results suggest that abiotic factors such as moisture and microsite conditions become more important factors affecting seedling emergence when sufficient seed is available.

Other seeds in the soil seedbank

Seedbank results for Kangaroo grass, Chilean Needle grass and Serrated Tussock were the main species examined. High densities of the exotic geophyte *Romulea rosea* were also found but are not commented on further in the data presented here.

Kangaroo grass

Sites with re-establishment trials were almost completely dominated prior to re-establishment of Kangaroo grass by a mixture of Serrated Tussock and Chilean Needle grass. Visual surveys of vegetation indicated that a mixture of Kangaroo grass and *Austrostipa* spp. dominated the re-establishment sites eight years prior to trial start dates (Hocking personal communication). No *Austrostipa* seeds were found in any seedbank cores (data not presented). Only minimal densities (average of 105 $\pm$ 105 per m^2) of Kangaroo grass seed were found in un-seeded controls. This represents the back ground level of seeds and therefore can be used as an estimate of how long Kangaroo grass seeds remain in the soil seedbank. These figures had a large error associated with them. Even so they had a statistically lower density of Kangaroo grass seed than treatments with seed addition as expected. Eight years of minimal vegetation management accompanied with large-scale weed invasion most likely have contributed to a depleted soil seedbank of native grass seed to a level that is unable to respond to favourable germination conditions. Scarlet & Parsons (1990) noted the lack of reinstatement of native grassland species with long inactive periods of vegetation management. Lunt (1995) comments on the Scarlet & Parsons (1990) observation and suggested that the low level of native diversity that re-establishes after a long period without active management could be because many native species seed do not survive in soil seedbanks for a long period. Results from Chapter 2 of this thesis on the germination potential of Kangaroo grass stored over time indicate that a significant reduction in the germination potential occurs after five years of storage at room temperature. The equivalent change in Kangaroo grass seed viability in the field is unknown. These results suggest that active vegetation management to encourage flowering and seed set in Kangaroo grass dominated grasslands on the Western basalt Plains should be carried out at least every four years for adequate incorporation of native seed into the soil seedbank. How this might be achieved is beyond the scope of this study.

A general trend of increasing Kangaroo grass average seed density in the soil seedbank occurs with increasing depth of soil disturbance to five cm in depth. However this trend was not statistically significant ($p>0.05$) except at site AR5 between no till plus seed Kangaroo grass addition treatments and five cm till depth Kangaroo grass seed addition treatments. Kangaroo grass seedling establishment results for these trials (Chapter three) showed no statistically significant ($p>0.05$) increase in density compared with increasing depth of soil disturbance during a close to average precipitation year (Chapter three). By comparison, during the drought year a significant ($p<0.05$) increase in Kangaroo grass emergence with soil disturbance was found. This suggests that the higher seed density in the soil seedbank of tilled compared with non-tilled re-establishment treatments may be part of the reason for increased germination in tilled plots during drought situations but not in average rainfall years. It may be possible that seed when directly broadcast on the soil surface is subject to greater predation than seed broadcast on to disturbed soil. The disturbed soil creates greater areas for the seed tip and awn to act as a corkscrew and enter the soil. Rates of seed entering the soil were not investigated in this study.

Chilean Needle grass

Previous research carried out on seedbank density of Chilean Needle grass on the Northern Tablelands of NSW Australia indicates average densities of around 4600 firm seeds per m^2 (Gardener 1998). Chilean Needle grass has additional cleistogamous seed in nodes and the root system of plants (Connor *et al.* 1993; Gardener 1998) that could contribute to the soil seedbank if plants are cultivated into the soil (Gardener 1998). Gardener (1998) extrapolated data to fit a decay curve of seed longevity in the soil seedbank since time of input. On this basis the seedbank of Chilean Needle grass was predicted to reach a density of 10 seeds per m^2 after 12 years with a half-life equalling 1.31 years (Gardener 1998). Further field investigation is needed to determine the amount of decay for Chilean Needle grass seed. Emergence of Chilean Needle grass seedlings recorded in chapter 3 indicates maximum densities below 18 seedlings per m^2 in all except one site and treatment. This germinating density in the soil seedbank of Chilean Needle grass is very low in comparison with the amount of seed available to germinate. One site at Woodlands in the no till control treatment recorded a Chilean Needle grass density of 83 seedlings per m^2 (Chapter three). This is the same site and treatment that recorded the highest density of Chilean Needle grass seed in the soil seedbank (Figure 4.2). The reasons for this generally low conversion of Chilean Needle grass seed recruitment from the seedbank could be due to competition factors and the time at which vegetation was assessed (late March 2002 & 2003). Other explanations include: the seedlings emerging slowly; the seeds may not be viable; there could be dormancy mechanisms operating or; there was not enough soil moisture to imbibe the seed.

Serrated Tussock

No obvious trends were found in the soil seedbank of Serrated Tussock within Kangaroo grass re-establishment trials. Densities of over 10,000 hard seeds per m^2 were found in one cm and three cm soil disturbance plus Kangaroo grass seed addition treatments. This could mean that problems arising from

Serrated Tussock re-establishment may occur in these treatments. However, no Serrated Tussock seedlings were reported in these treatments (Chapter three). Only mature Serrated Tussock plant regrowth at minimal levels was recorded 18 months after first Kangaroo grass seedling emergence. The lack of Serrated Tussock recruitment from the soil seedbank could be due to competition factors and the time at which vegetation was assessed (late March 2002 & 2003). Other explanations for low seedling emergence would be similar to those listed for Chilean Needle grass in the section above.

Soil Physical Properties

Soil properties were investigated to explain the trend of greater establishment of Kangaroo grass in five cm deep soil disturbance treatments compared with no soil disturbance treatments during low precipitation (drought) years. The five cm deep till treatments are similar to fallowing methods used by agriculturalists (Nie *et al.* 1999). That is, the soil is slightly disturbed once and then left alone.

Soil dry bulk density was significantly higher in the 0-2 cm soil profile compared with the 2-5 cm soil profile in one, two and three years since soil disturbance treatments (Figure 4.4). The one-year since soil disturbance represents the soil status at time of vegetation assessment. The two and three year since soil disturbance treatments represent the projected soil properties under aging Kangaroo grass re-establishment areas. Areas that had been subjected to soil disturbance at least one year prior to soil analysis had formed a dense layer at the soil surface. The denser layer at the soil surface in the soil disturbance plots may contribute to the amount of evaporation of soil water form the soil surface. Rowell (1994) indicates that some soils can form a "crust" on the surface helping reduce evaporation. This crust is most likely an area of reduced soil porosity and organic carbon. Freshly tilled soil had a significantly denser layer at 2-5 cm soil profile when compared with the 0-2 cm soil profile. This result can sometimes be due to machinery traffic (Stenberg 2000) and compaction where the cultivator blade contacts the underlying soil (Rowell 1994), which may be the case here.

Soil particle density was not significantly different in the 0-2 cm soil profile when compared with the 2-5 cm soil profile for any age of soil disturbance treatments. This indicates a uniformity of soil particle density within the top five cm soil profile and is not further discussed.

Soil porosity was greater in the 2-5 cm soil profile when compared with the 0-2 cm soil profile for all except fresh soil disturbance treatments. Treatments that had soil disturbance (except the fresh till treatment) had a greater difference between porosity of the 0-2 and 2-5 cm soil profile when compared with no till treatments. However this was only statistically significant in the two years since soil disturbance treatments. Rowell (1994) proposed that an increase in soil porosity should be accompanied with a decrease in soil bulk density. This is the same trend as observed by comparing figures four and six. At field capacity a greater porosity can be accompanied also by an increase in available soil water (Rowell 1994).

Available soil moisture expressed as a percentage was measured at two soil depths for control, no till plus Kangaroo grass seed addition and five cm deep till and Kangaroo grass seed addition treatments. The soil profile at 2-5 cm deep had a significantly greater percentage of available soil water when compared with the 0-2 cm soil profile for all treatments. Treatments incorporating soil disturbance had the greatest average percentage of available soil water (approx.16 percent) in the 2-5 cm deep soil profile compared to no disturbance treatments (approx.11 percent). This is almost five percent more moisture available for plant imbibition, germination and growth in the soil disturbance treatments measured during a drought period.

Using shallow depth soil disturbance for competitively replacing Chilean Needle grass and Serrated Tussock with Kangaroo grass was beneficial to Kangaroo grass establishment during below average rainfall years. Soil disturbance positively influenced the amount of Kangaroo grass seed in the soil seedbank during re-establishment trials. The once only nature of the soil disturbance used in these trials also resulted in increased available soil moisture at the 2-5 cm soil depth compared with no soil disturbance treatments. A greater level of soil moisture in the 2-5 cm samples may be contributed to greater pore spaces, reduced evaporation potential and more rapid infiltration.

Conclusion

Mechanisms were investigated whereby low-level soil disturbance significantly increased establishment of Kangaroo grass during a drought year in comparison with no soil disturbance. A strong association was found between progressive increases in depth of soil disturbance to a depth of five cm, and increased Kangaroo grass seed density (seed per m^2) in the soil seedbank. A weaker trend was also found between increased depths of tilling and decreased Chilean Needle grass seed density in the soil seedbank. No relationship was found between Serrated Tussock soil seedbank densities and depth of soil disturbance. Soil seedbank densities were high for both Chilean Needle grass and Serrated Tussock but at the time of vegetation assessment eight months after tilling this had not translated into emerging seedlings in the presence of Kangaroo grass seedlings. Soil physical properties of dry bulk density, particle density, porosity and available soil water with increasing depth of soil disturbance were also investigated. Soil disturbance to a depth of five cm was found to produce a dense layer (crust) on the soil surface in tilled treatments when compared with no till treatments, approximately nine months after soil disturbance. Soil disturbed treatments had the greatest percentage of soil moisture of all treatments in the 2-5 cm soil profile when measured nine months after soil disturbance (March 2003). It is suggested that small scale soil disturbance during dry years will benefit Kangaroo grass seed imbibition, germination and establishment through an increase in pore spaces, greater infiltration and reduced evaporation of soil moisture. An increased incorporation of Kangaroo grass seed into the soil of disturbed treatments compared with no soil disturbance treatments during drier than average spring-summer, may also promote Kangaroo grass establishment.

Chapter Five

Soil nitrogen and moisture availability associated with re-establishment over *Nassella* species

Introduction

Soil disturbance in grassland ecosystems has been linked in the past to increased weed invasion into these systems (Craigie & Stuwe 1992; Hobbs & Huenneke 1992; McIntyre *et al.* 1995; Wijesuriya 1999). Increased weed invasion with soil disturbance has more precisely been linked with increasing availability of soil nutrients and water (Moore 1970; Hobbs & Atkins 1988; Wijesuriya 1999). However, the soil seedbank would also have a major affect on type and distribution of any species invading a disturbed site. Research in to Australia's native ecosystem recruitment processes has indicated that similar mechanisms of invasion work for most species. Small-scale soil disturbance in native Tasmanian woodland habitats by native animals has shown to increase the amount of native species recruitment (Pyrke 1994). Robinson (2003) also found that soil disturbance followed by addition of native forb seed increased the proportion of native forbs that emerged compared with non-disturbed sites if sufficient viable native forb seed was present. Sindel *et al.* (1993) found that a rough soil surface increases incorporation of Kangaroo grass seed into the soil seedbank when investigated in glasshouse experiments.

Availability of soil nutrients and water has been linked with increased growth rates of most vegetation types in Australia. In studies of exotic species (including: *Medicago* spp.; *Trifolium* spp.; *Phalaris* spp.; *Lolium* spp.; *Dactilis* spp.) used commercially in Australia, uptake of soil nutrients started to occur within a few days of germination (McWilliam *et al.* 1969). McWilliam *et al.* (1969) found that plants absorbed nitrogen in greater amounts than phosphorus. Phosphorus is suggested to be limiting in most Australian soils. Kelley *et al.* (1983) and Turner & Lambert (1985) suggested that measurements in the past for phosphorus have been concentrated on documenting inorganic forms of phosphorus whereas organic forms of phosphorus may best reflect P availability. Studies of weed invasion in White Box woodlands have shown that exotics have a greater positive response to additional soil phosphorus than native species tested, including Kangaroo grass (Allcock 2002). King & Buckney (2002), in studies of nutrient enriched urban bushland, in Sydney, Australia indicated a trend of overall nutrient enrichment with species composition, rather than with phosphorus enrichment alone. Moretto & Distel (2002) found that there was higher available soil N under more palatable grasses (*Poa ligularis*) than unpalatable grasses (*Stipa tenuissima*) in laboratory incubated experiments. However, *in situ* estimates of net N mineralisation showed the opposite trend (Moretto & Distel 2002).

Rates of soil nutrient mineralization change with season and vegetation change (Joffre 1990). To obtain a representation of the potential mineralizable soil N available for plant growth, mineralization should be measured during growth seasons, e.g. autumn and spring for cool season grasses. A standard incubation time of 30 days in situ and in laboratory trials is the conventional time frame used to measure rates of mineralisation (Moretto & Distel 2002; Wijesuriya 1999). However, results by Wijesuriya (1999) indicated a continuation of mineralization after 70 days field incubation. Field mineralization studies presented in this thesis track *in situ* mineralization until a reduction in available total N is observed. This procedure allows levels of mineralization to be obtained that approach a maximum. Mineralization rates are presented for soils under alive Chilean Needle grass in autumn and spring. Total available N levels found in soil mineralization tubes are also presented for till and no-till Kangaroo grass re-establishment treatments during the Kangaroo grass emergence period. The likely links between soil N mineralization and optimum Kangaroo grass re-establishment conditions are then considered.

A major influence of burning vegetation is an increase in soil respiration rate linked to the addition of ash and a subsequent increase in N mineralisation rate (Raison & McGarity 1980; Khanna *et al.* 1994). Raison & McGarity (1980) suggested that incorporation of fresh ash increases soil pH, which is a major factor in increasing metabolic activity in podsolic soils. Moritsuka *et al.* (2001) in rhizosphere pot trials found that after soil was heated to 150°C for three hours, more cations (Ca^+, Mg $^+$ $NH4^+$) were available when compared with unheated soil. Inhibition of soil nitrification was noted with a decrease in soil pH in a trial by Moritsuka *et al.* (2001). Actual addition of nitrogen to soil from ash has been reported to be minor (Van de Vijver *et al.* 1999) in comparison with chemical soil effects of ash addition and the effects of heating on soil pH and respiration. The effects of burning Wheat (*Triticum aestivum*) thatch used in Kangaroo grass re-establishment methods on availability of soil nutrients were investigated in this thesis. Influences of burning alive, aboveground vegetation of Chilean Needle grass on availability of soil nutrients are also reported in this thesis.

Methods

Site Location

Kangaroo grass competitive replacement trials were established at the Iramoo grassland Reserve associated with Victoria University of Technology located in the suburb of St Albans, Melbourne Victoria. Further site descriptions are covered in Chapter three. Soil mineralization investigations were undertaken in treatments associated with the Kangaroo grass re-establishment project. Dominant vegetation at the Iramoo grassland Reserve includes Kangaroo grass, *Austrostipa* spp., Chilean Needle grass and Serrated Tussock (*N. trichotoma*). The underlying soil type is basaltic cracking clay.

Soil sampling

Soil cores were taken in replicates of six, one per re-establishment treatment replicate. Core depth was to five cm and diameter of four cm. The depth of core was chosen because it appears from previous studies that this is the most active zone for soil nutrient availability to plant roots (Dormaar 1992; Wijesuriya 1999). Cores were placed on ice for transportation. Average time of transportation was 15 minutes. Upon arrival at the laboratory, soil samples were immediately homogenised and any plant roots, rocks and large invertebrates removed. Soil moisture, nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen and phosphate phosphorus were all extracted on the day of sampling and analysed within 48 hours of collection using the methods below.

Soil analysis

Soil moisture

Soil moisture was calculated gravimetrically by drying at 105°C ± 2 °C until constant weight of soil was achieved. Estimated dry weight of soil was used in calculation of nutrient concentrations. Multiplying fresh weight of soil by 100 minus the moisture content and then dividing by 100 gave an estimate of the percentage dry weight of the soil.

Soil available N

Nitrate-nitrogen, nitrite-nitrogen and ammonia-nitrogen were each determined and added together to estimate the total availability of N in soil. Each of these forms of available N were extracted from soil using a KCl solution (Adams *et al.* 1989; Wijesuriya 1999). A 0.5 M KCl solution was used instead of the recommended 1M KCl solution because it was found that background variability was reduced without affecting extraction efficiency. Samples were also shaken for one hour before centrifugation as extraction efficiency was increased using this treatment (data not shown). Flow injection analysis was used to determine concentrations of nitrate-nitrogen and nitrite-nitrogen. The Flow Injection Analyser used was a Tecator Aquatec®. Methods provided by Tecator were used for the nitrate and nitrite analysis. The method of ammonia-nitrogen analysis used followed Strictland & Parsons (1977). In this method a colourimetric reaction was undertaken followed by absorbance measurement at 640 nm using a UV-Visible spectrophotometer. Extraction efficiency was tested for every batch of samples using controls and spiked samples.

Soil available P

The method described by Olsen & Sommers (1982) was used for available phosphate extraction and analysis. A 0.5 M $NaHCO_3$ solution was used as the extractant. Soil samples were shaken for one hour before centrifugation and analysis. Concentration was determined colourimetrically at 880 nm using a UV-Visible spectrophotometer. Extraction efficiency was tested for every batch of samples using controls and spiked samples.

Soil *in situ* net mineralization

Analysis of soil net mineralization under alive (herbicide untreated) and dead (herbicide treated) Chilean Needle grass involved the application of atrazine as Nufarm Nutrazine® at a rate of 8.4 kg active ingredient per ha to selected Chilean Needle grass treatments. Application of herbicide was in late April 2002; mineralization tubes were placed in the ground after one month.

Mineralization associated with field soils was estimated using the tube incubation technique (Raison *et al.* 1987). Closed top PVC tubes were placed into the soil to a depth of five cm. Tubes were 15 cm long and had a diameter of four cm. At the time of incorporation of tubes into the soil, a soil sample was also taken for calculation of initial levels of available nutrients. Tubes were incubated for 15 days, 45 days, 75 days and 120 days in replicates of six during autumn mineralization experiments. Tubes were incubated for one, two and three months in replicates of six during spring mineralization experiments. Net N mineralization was determined by estimation of total available nitrogen concentration (NO_3-N + NO_2-N + NH_4-N) found in the tubes. This method was used for both autumn and spring mineralization analysis.

Statistical analysis

Statistical analysis was carried out using SPSS v 11.0. All data were tested for normality using histograms and analysis of skewness and kurtosis. Transformations were conducted according to Zar (1984) if required. General

Linear Model univariate analyses with or without posthoc tests were used for simple comparisons. General Linear Model multivariate analyses with or without posthoc tests were used to test significance between subject effects.

Results

Soil net N mineralization of *Nassella* invaded patches in May 2002 (Autumn)

Soil net N mineralization was measured under alive and recently sprayed (with herbicide) Chilean Needle grass plants (Figure 5.1). Maximum N mineralization was reached after 15 days *in situ* tube incubation in soil underneath alive Chilean Needle grass (approx. 6 μg per g) and recently sprayed with herbicide treatments (approx. 8 μg per g). Soil N mineralization under alive and recently sprayed Chilean Needle grass was significantly higher ($p<0.01$) after 15 days when compared with time initial (Table 5.1). Nitrogen mineralisation at day 15 for *in situ* incubation tube samples was significantly greater ($p<0.01$) in alive and recently sprayed treatments when compared with time periods of 75 and 120 days (Table 5.1). Available soil moisture was found to fluctuate (Table 5.2) during the autumn mineralization experiment. However, these fluctuations were not significant ($P>0.05$) between treatments at each time period.

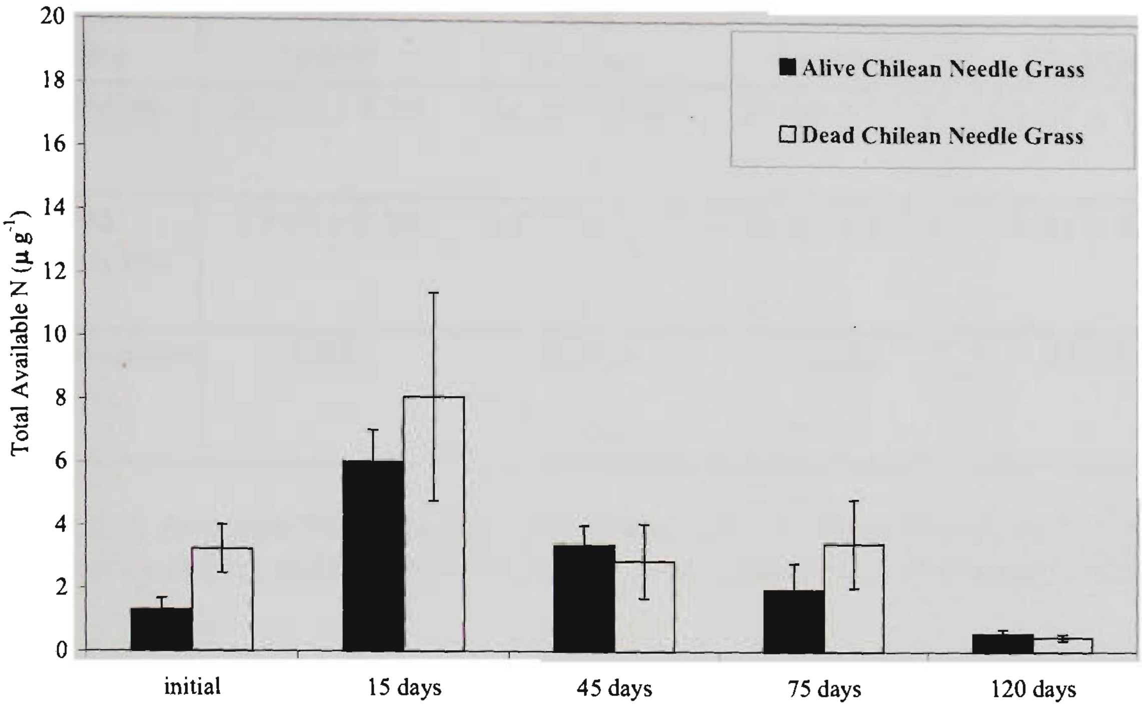

Figure 5.1: Mineralizable N expressed as average total available nitrogen associated with soil in incubation tubes underneath alive and recently dead Chilean Needle grass plants in Autumn 2002; Bars indicate standard error (n=6).

Time	Results of Tukey Post Hoc Test, p value	
	Alive Chilean Needle grass	Dead Chilean Needle grass
Initial vs. 15 days	0.000**	0.002**
Initial vs. 45 days	0.156	0.998
Initial vs. 75 days	0.934	1.000
Initial vs. 120 days	0.927	0.13
15 days vs. 45 days	0.044*	0.002**
15 days vs. 75 days	0.001**	0.006**
15 days vs. 120 days	0.000**	0.000**
45 days vs. 75 days	0.516	0.991
45 days vs. 120 days	0.029*	0.129
75 days vs. 120 days	0.521	0.129

Table 5.1: Statistical significance of average soil total available nitrogen (tube incubation samples) from mineralization experiments in May 2002 for treatments with alive Chilean Needle grass or Chilean Needle grass sprayed with herbicide.

Date Time	May Initial	May 15 days	June 45 days	July 75 days
Herbicide	20.85 ± 1.31	14.39 ± 0.86	21.48 ± 1.23	21.37 ± 1.56
No Herbicide	21.61± 0.84	15.71 ± 1.12	18.60 ± 0.93	19.01 ± 1.25
Significance (p)	0.632	0.375	0.083	0.258

Table 5.2: Average Soil Moisture (%) in the 0-5 cm soil profile in weed control treatments during autumn mineralization experiment, standard error (n=8).

Soil N mineralization during a Kangaroo grass re-establishment germination and growth period

Maximum levels of mineralised (net) soil N were achieved in all re-establishment treatments after two months of field incubation during spring-summer 2002 (Figure 5.2). The level of soil N mineralization at two months for till and no-till re-establishment treatments were significantly ($p<0.01$) greater than results obtained at one and three months (Table 5.3). Maximum mineralization of soil N within incubation tubes under alive Chilean Needle grass plants (control treatments) in spring also occurred at the two-month period. However, maximum soil N mineralization under alive Chilean Needle grass was significantly ($p<0.01$) lower when compared to both till and no-till re-establishment treatments at the two-month soil incubation. Signs of initial soil N immobilization occurred at the third month of field tube incubation in both no till and till revegetation treatments.

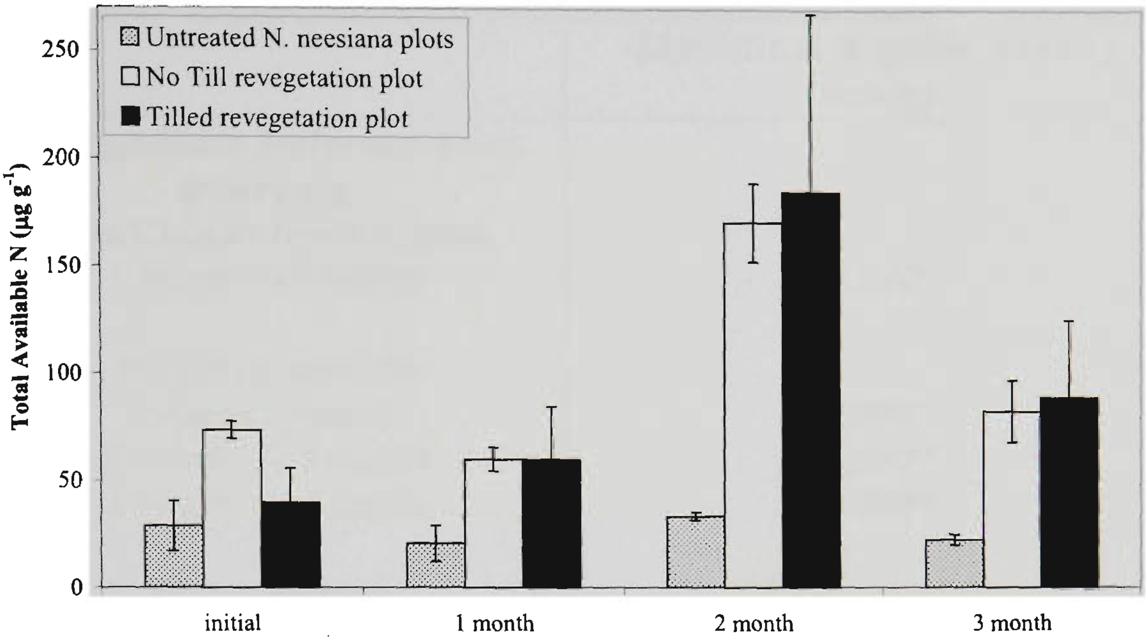

Figure 5.2: Mineralizable N expressed as average total available nitrogen associated with soil in incubation tubes in three Kangaroo grass re-establishment techniques, trial started in October 2002 Bars indicate standard error (n=6). See Table 5.3 for results of statistical comparison.

Statistical comparison of total available N tested in incubation tube soil samples in October 2002 between revegetation treatments and between incubation time periods are presented in Table 5.3. Only statistically significant results are presented in this table.

	Statistical Significance (p value)
Comparison between time intervals	
Alive Chilean Needle grass	
1 month vs 2 month	0.039*
No Till treatments	
Initial vs. 2 month	0.000**
2 month vs. 1 month	0.000**
2 month vs. 3 month	0.000**
Till treatments	
2 month vs. initial	0.000**
2 month vs. 1 month	0.000**
2 month vs. 3 month	0.001**
Comparison between treatments	
Initial	
Alive Chilean Needle grass vs. no till	0.000**
No till vs. Till	0.000**
1 month	
Alive Chilean Needle grass vs. no till	0.000**
Alive Chilean Needle grass vs. no till	0.000**
2 month	
Alive Chilean Needle grass vs. no till	0.003**
Alive Chilean Needle grass vs. no till	0.007**
3 month	
Alive Chilean Needle grass vs. no till	0.020*
Alive Chilean Needle grass vs. no till	0.011*

Table 5.3: Statistical significance of average soil total available nitrogen (tube incubation samples) from mineralization experiments in October 2002 for treatments with alive Chilean Needle grass; No till revegetation treatments; and Till revegetation treatments. Only comparisons with a statistically significant result are shown. Key: * indicates a statistically significant result at $p<0.05$, ** indicates a statistically significant result at $p<0.01$.

Soil moisture (Table 5.4) for mineralization experiments during spring showed a significantly ($p<0.01$) higher amount of water available in soils of both till and no-till re-establishment treatments compared to the control treatment at the initial and one month stages of the trial. Available soil moisture dropped in all spring mineralization treatments in the third and fourth months of the trial. No significant ($p>0.05$) differences were found in soil moisture availability between treatments at this time.

	October 2002	November 2002	December 2002	January 2003
Till & Revegetation	20.85 ± 0.47	7.86 ± 1.21	4.76 ± 0.56	3.12 ± 0.49
No Till & Revegetation	23.26 ± 0.80	6.04 ± 0.52	5.86 ± 0.65	3.29 ± 0.41
Chilean Needle grass Control	13.51 ± 0.80	2.25 ± 0.19	4.21 ± 0.49	2.48 ± 0.39
Significance				
Till x No till	0.094	0.275	0.394	0.942
Till x Control	0.000**	0.000**	0.750	0.545
No Till x control	0.000**	0.001**	0.129	0.374

Table 5.4: Average Soil Moisture (%) in the 0-5 cm soil profile in revegetation treatments during spring mineralization experiment, standard error (n=8). Key: ** Indicates a significant ($p<0.01$) difference or interaction between revegetation treatments at selected months.

Availability of soil nutrients before and after a Kangaroo grass re-establishment thatch burn

No significant differences ($p>0.05$) were noted in soil nutrients in till re-establishment treatments after a thatch burn compared with initial levels (Table 5.5). An increase in nitrate-nitrogen and a decrease in ammonia-nitrogen were noted when before burn levels were compared with after burn levels for till treatments. Total available N slightly increased in till re-establishment treatment soil after a thatch burn but this increase was not significant ($p>0.05$).

	Percentage Moisture	Available Phosphate	Nitrate Nitrogen	Nitrite Nitrogen	Ammonia Nitrogen	Total available Nitrogen
Till plus revegetation						
Before burn	23.06 ± 0.48	6.04 ± 0.55	12.06 ± 2.88	0.05 ± 0.01	36.79 ± 6.38	48.90 ± 9.26
After burn	20.85 ± 0.47	6.16 ± 0.49	26.94 ± 14.39	0.06 ± 0.01	28.64 ± 4.08	55.63 ± 18.45
Significance (p)		0.976	0.205	0.413	0.160	0.196
No Till plus revegetation						
Before burn	22.31 ± 0.49	4.05 ± 0.18	15.31 ± 3.09	0.06 ± 0.01	26.14 ± 3.26	41.51 ± 6.37
After burn	23.63 ± 0.79	7.34 ± 0.67	15.33 ± 1.47	0.11 ± 0.04	59.03 ± 4.03	74.47 ± 5.54
Significance (p)		0.001**	0.692	0.196	0.003**	0.008**
Chilean Needle grass control						
Before burn	13.04 ± 0.60	3.72 ± 0.40	1.20 ± 0.20	0.08 ± 0.01	22 ± 0.02	23.28 ± 0.23
After burn	13.51± 0.80	5.39 ± 0.74	1.97 ± 0.23	0.11 ± 0.02	27.19 ± 3.70	29.26 ± 3.95
Significance (p)		0.067	0.014*	0.177	0.031*	0.716

Table 5.5: Average concentration (μg per g) of soil parameter associated with three Kangaroo grass re-establishment techniques before and after thatch burn. Measurements taken in October 2002, standard error indicated (n=8). Key: * indicates a significant difference ($p<0.05$), ** indicates a significant difference ($p<0.01$) for average soil parameter levels before and after a Kangaroo grass re-establishment thatch burn.

In no-till re-establishment treatments a highly significant increase ($p<0.01$) in phosphate-phosphorus, ammonia-nitrogen and total available N was noted in soil after a thatch burn. The greatest increase in total available N was noted after a burn in no-till re-establishment treatments reaching a concentration of 74.47 ± 5.54 µg per g dry weight.

In soil under the control with alive Chilean needle grass plants, both nitrate-nitrogen and ammonia-nitrogen significantly increased ($p<0.05$) in concentration after above ground vegetation was burnt.

Soil moisture did not significantly change ($p>0.05$) in any treatment when before fire soil moisture was compared with after burn soil moisture.

Discussion

Soil Nutrient Availability

May 2002 Mineralization

The maximum level of soil N mineralization in incubation tubes placed under alive and recently sprayed (with the herbicide atrazine) Chilean Needle grass plants in autumn was found to occur within 15 days. This is a similar time frame as that noted by Moretto & Distel (2002) for soil N mineralization in autumn and spring under *Poa ligularis* (approx. 60 µg per g) and *Stipa tenuissima* (approx. 20 µg per g) related to *N. neesiana* and *N. trichotoma* in Central Argentina. Maximum levels of soil N mineralization under Chilean Needle grass in autumn were much lower than those reported by Moretto & Distel (2002). Levels of soil N mineralization under Kangaroo grass plants in similar soils to the study reported here at Derrimut grassland reserve, Melbourne Victoria showed a concentration of 10 µg per g after 70 days (Wijesuriya 1999). In Wijesuriya (1999), soil N was still increasing at 70 days. Mineralization of soil N under Chilean Needle grass appears to be low in comparison with other similar species. This may mean that Chilean needle grass is efficient at storage of available nutrients in plant tissue giving it a competitive benefit. More research is needed to fully investigate any benefits

Chilean Needle grass might gain by storage of available soil nutrients in comparison with native species

Application of atrazine to growing plants of Chilean Needle grass in autumn appears to slightly increase soil N mineralization rates in comparison to soil N mineralization rates under alive Chilean Needle grass (roots of Chilean Needle grass are severed at the time when tubes are placed in the ground). Soil N mineralization was slowly increasing with time up to 15 days under dead Chilean Needle grass. Further investigation is required to obtain the maximum level of soil N mineralization under dead Chilean Needle grass. The slightly increased rate of soil N mineralization could be due partially to the presence of atrazine in the soil. Atrazine induced mineralization has been reported to be at a greater rate in the topsoil than sub-soil (Sparling *et al.* 1998). Sparling *et al.* (1998) reported that the greatest rate of atrazine induced mineralization was slow even in incubated standard conditions with 2-36 percent degradation to CO_2 after 96 days and 4-41 percent degradation to CO_2 after 263 days. Enhanced temperature has been reported to increase the rate of atrazine induced mineralization; a doubling in rate with every increase in temperature of $10^{\circ}C$ (Sparling *et al.* 1998). However, Klint *et al.* (1993) report that temperature did not enhance atrazine mineralization. An increase in number of atrazine degrading microbes and also aerobic conditions are needed for efficient atrazine degradation (Sparling *et al.* 1998). Hayar *et al.* (1997) found 90 percent of atrazine residues remained bound to loamy clay soil after 63 days of incubation. It is possible that the slow and uncompleted mineralization of soil N in incubation tubes under alive and dead Chilean Needle grass was partially affected by low soil temperatures and possibly not due to the presence of atrazine slowing mineralization rates. Soil temperatures were not monitored in this experiment.

Availability of Soil Nutrients during Kangaroo grass Growth

Soil total available nitrogen during the germination period of Kangaroo grass in October 2002 was higher in revegetation areas that had Chilean Needle grass removed compared with areas with established Chilean Needle grass. This result was anticipated, as there was no vegetation to utilise any soil-stored nutrients. Research by Wijesuriya (1999) indicated that when soil is disturbed an increase could be found in available soil nutrients. If vegetation removal is classified as a component of soil disturbance the results shown in Table 5.5 correspond to Wijesuriya (1999) results. Mechanical soil disturbance reported in this Chapter did not increase the availability of total available nitrogen or available phosphate compared with vegetation removal.

The increased amount of soil total available nitrogen in revegetation treatments with cleared vegetation compared with areas of established vegetation indicates that revegetation carried out in the cleared areas will have more total available nitrogen for growth. More nitrogen will allow germinating Kangaroo grass seedlings to increase in biomass (size above and below the soil layer) and therefore become more competitive than if germinating in an area with established vegetation.

Spring 2002 mineralization

Mineralization experiments in October (spring) 2002 were developed to assess the maximum amount of soil total available nitrogen that could be released during the Kangaroo grass germination period. Mineralization of soil N in Kangaroo grass re-establishment trials in spring was at a maximum after two months in tilled, non-tilled and control treatments. Till re-establishment treatments had significantly ($p<0.01$) greater amount of total available N after two months when compared with no-till re-establishment treatments and control treatments. No-till re-establishment treatments also had a significantly ($p<0.01$) greater amount of total available N after two months when compared with control treatments. Both re-establishment treatments had all vegetation killed on the site by application of atrazine approximately five months before the spring mineralization experiment started. During this period, Kangaroo grass seed and wheat thatch were laid on the treatment areas, except controls. The thatch layer was removed in October using a controlled burn initiated by a gas flamethrower as source of ignition. The presence of a thatch layer kept soil available moisture at a consistent 20 percent. After the thatch was removed available soil moisture still remained significantly ($p<0.01$) higher in both re-establishment treatments when compared to the control treatment for October and November. Mineralization rates are known to be sensitive with both soil water content and temperature (Stanford & Epstein 1974; Stanford *et al.* 1974; Pakrou & Dillon 2000). Wijesuriya (1999) noted an increase in soil N mineralization in tilled treatments compared with no-till treatments. Both soil moisture and soil disturbance are likely to have contributed to the pattern in soil N mineralization produced in these results. Unkovich *et al.* (1998) suggested that an increase in mineral N availability will result in an increase in plant growth. It is likely that Kangaroo grass seedlings in till re-establishment treatments will have access to more available mineral N than no-till re-establishment treatments. As a result, Kangaroo grass seedlings in till re-establishment treatments might be expected to have a greater biomass than Kangaroo grass seedlings in no-till re-establishment treatments (Chapter six).

The effects of fertilizer addition on soil mineralization and leaching were studied by Cookson *et al.* (2000) on the Canterbury plains of New Zealand. No significant effects from fertilizer addition were noted on clay-fixed N, anaerobically mineralizable N and total N at time of harvest (Cookson *et al.* 2000). Cookson *et al.* (2000) suggested that fertilizer addition and cultivation in the autumn can contribute to loss of nitrate by leaching in winter. Rates of fertilizer addition of 200 kg N per ha resulted in a soil total N content of 18.5 µg N per g (Cookson *et al.* 2000). This level of N available in the soil in the Cookson *et al.* (2000) study is very low in comparison to results found in this study. This suggests that any fertilizer addition to the type of re-establishment treatments discussed in this study may in fact lead to an increase in leaching of N into the water table and may not lead to increases in biomass of Kangaroo grass seedlings.

Fire and soil nutrients

The burning of the thatch over Kangaroo grass tilled and un-tilled re-establishment treatments was found to have different effects on available soil nutrients depending upon soil treatment. Both re-establishment treatments had a greater amount of available soil water compared to control treatments regardless of whether there was a burn. The greater amount of available soil moisture is thought to partially influence the available levels of soil nutrients measured as these nutrient may have been in an aqueous form. In till re-establishment treatments available soil nitrate-nitrogen was found to increase in soil after the thatch burn. Ammonia-nitrogen in this treatment reduced in availability after the thatch burn. Total available N slightly increased after the burn in till re-establishment treatments contributed largely by an increase in nitrate-nitrogen although the difference was not statistically significant. In summary burning of thatch over till re-establishment treatments had no significant impact on the soil nutrients examined (N & P).

Re-establishment treatments that were not tilled exhibited a significant ($p<0.01$) increase in available phosphate-phosphorus, ammonia-nitrogen and total available nitrogen after a thatch burn compared with before the thatch was burnt. No-till re-establishment treatments initially had a lower concentration of total available soil N when compared with till re-establishment treatments before the thatch burn. After the thatch burn, soil available N concentration was higher (by approx. 19 µg per g) in the no-till re-establishment treatments when compared with the till re-establishment treatments. The higher level of available soil N in no-till re-establishment plots did not appear to influence the proportion of soil N mineralised in these soils as shown in Figure 5.2.

The control plots with alive Chilean Needle grass had a much lower level of all soil nutrients and water measured before and after foliage burn in comparison with the re-establishment treatments. Possibly due to the activity of growing plants. A significant ($p<0.05$) increase in the amount of both soil nitrate-nitrogen and ammonia-nitrogen were noted in soil after Chilean Needle grass foliage was burnt in comparison to before foliage burn levels.

It is likely that increased available soil nutrients after a thatch and foliage burn could be partially attributed to ash reincorporation into the soil which induce changes in soil pH and respiration rates due to changes in soil chemistry by ash incorporation and by heating of the soil matrix (Raison & McGarity 1980; Giardina *et al.* 2000). However these previous reports were associated with Eucalypt forests and may have different soil chemistry interactions from a basaltic grassland situation. Addition of nutrients by ash incorporation would be minor according to Khanna *et al.* (1994). Warm soils can also favour microbial mineralisation activity.

Implications for Management

An *in situ* incubation period of two months to assess mineralization rates (Western basalt plains grasslands in the west of Melbourne) during spring provided a better estimate of soil N mineralization than an incubation period of 30 days.

Soil N mineralization was greatest in till re-establishment treatments when compared with no-till re-establishment treatments. If Kangaroo grass seedlings access these nutrients then till re-establishment treatments have greater potential for Kangaroo grass seedlings to increase in biomass than no-till re-establishment treatments. However, an increase in biomass of invading weeds may also be likely under higher nutrient levels. Addition of fertilizer to till Kangaroo grass re-establishment trials may not significantly increase standing biomass but further investigation is required.

Burning thatch used in Kangaroo grass re-establishment trials was found to increase total available N in no-till re-establishment treatments to levels greater than in till re-establishment treatments. In Kangaroo grass re-establishment trials where soil cultivation is not used but available soil nutrients are needed to increase biomass of Kangaroo grass seedlings, burning the thatch over the re-establishment site will increase available soil N and P.

Conclusion

Potential mineralizable Nitrogen (as NO_3-N, NO_2-N, & NH_4-N) was measured in soil using the tube incubation method under an existing stand of Chilean Needle grass in May (Autumn 2002). It was noted during this experiment that the availability of total N was lower than expected. This experiment was conducted at a time when drought conditions were still prevalent and it is thought that this fact (low soil water content) and low soil temperatures reduced the ability of soil biota to mineralise soil N. Soil available nutrients were also measured during the October 2002 Kangaroo grass germination period. During this experimental period total available N was highest in no till revegetation plots (74.47 ± 5.54 µg per g) followed by till revegetation plots (55.63 ± 18.45 µg per g) and finally in established Chilean Needle grass control plots (29.26 ± 3.95 µg per g). The only statistically significant difference for total available N was between no till revegetation plots and established Chilean Needle grass plots with $p = 0.006$. Available Phosphate measured at the start of Kangaroo grass germination period was not statistically different ($p>0.05$) between soil treatments and the established Chilean Needle grass control. Soil moisture was statistically greater ($p<0.01$) when both till and no till revegetation treatments were compared with the established Chilean Needle grass control during the start of the Kangaroo grass germination period, October 2002. There was no statistical difference between till and no till revegetation treatments for soil moisture during the start of the Kangaroo grass germination period, October 2002. The full potential of mineralizable total N was then investigated during the germination period for Kangaroo grass in October 2002. In incubation tubes placed into the soil of revegetation and control plots a statistically greater ($p<0.01$) amount of total available N was found in both till (185.15 ± 82.80 µg per g) and no till (170.95 ± 18.27 µg per g) revegetation treatments compared with established Chilean Needle grass controls (33.60 ± 1.96 µg per g) after two months of incubation. No statistical difference ($p>0.05$) occurred between till and no till revegetation treatments at the two-month tube incubation analysis. This suggests there is a large potential of mineralizable total available N in both till and no till revegetation treatments at the time of Kangaroo grass germination. Soil available nutrients were also compared before and after a revegetation thatch burn in October 2002. It was found that processes attributed to the burn, significantly increased ($p<0.01$) the amount of total available N (from an average of 41.51 ± 6.37 µg per g to 74.47 ± 5.54 µg per g) and available phosphate (from an average of 4.05 ± 0.18 µg per g to 7.34 ± 0.67 µg per g) in no till revegetation treatments. Non-significant ($p>0.05$) increases in total available N and available phosphate occurred in till revegetation treatments and Chilean Needle grass controls in soil samples compared before and after a revegetation thatch burn.

Chapter Six

Effects of nutrient, water and potassium-humate addition on aboveground biomass of seedlings and seed soil penetration

Introduction

Fertilization and irrigation have long been used in agricultural systems to increase productivity of cash crops. For weedy species phosphorus addition has been shown to increase the establishment potential of exotic grass species invading Bushland around Sydney (Allcock 2002). Phosphorus addition has also been shown to increase the response of pasture species after grazing in Tasmania (Reid & Richardson 1998). McWilliam *et al.* (1969) reported that adequate water, nutrients and light are all required during the early stages of seedling development. Fertilizer addition trials by Loeppky *et al.* (1999) indicated that when available soil N and P are low, the addition of fertilizer can increase yield. However, when available soil nutrients are high, addition of fertilizer does not increase yield. In a study of fynbos shrubland vegetation restoration in South Africa, fertilization was found to increase canopy cover in treatments where topsoil had been removed and in shallow topsoil treatments for alien annual species (Holmes 2001). Holmes (2001) suggested that fertilizer addition may lead to increased native vegetation cover in areas without significant topsoil. However, due to increased mortality of proteoid species during establishment on deep topsoil areas, fertilization is not recommended on deep topsoil areas for native species restoration (Holmes 2001).

Some small-scale research has been conducted on the effects of fertilization and irrigation on establishment of Australian native grasses but results are mixed. Glasshouse trials by Hagon & Chan (1977) reported that decreasing soil water availability decreased the establishment of Kangaroo grass as well as other natives and an exotic grass. Hadas & Russo (1974a) have suggested that every seed type has a particular water potential needed for germination. Once this level of water potential is reached, germination rates are not affected. Irrigation of Kangaroo grass seeds on mine establishment sites on the Central Tablelands of New South Wales resulted in an increase in germination but this increase was not significant compared with controls. McDougall (1989) trialed irrigation of Kangaroo grass re-establishment field trials and found a positive growth response at first but no other effects after the initial irrigation. McDougall (1989) applied irrigation to Kangaroo grass re-establishment areas in March for six weeks. The maximum growth rate of Kangaroo grass would not have been achieved as irrigation was applied when Kangaroo grass (a summer growing C4 photosynthetic grass) would have been slowing its metabolism in response to decreasing ambient and soil temperatures and irradiance as autumn and winter approached. A more appropriate time for irrigation of Kangaroo grass trials would be during the active germination and growth period of Kangaroo grass, spring and summer seasons.

Fertilization of Kangaroo grass has mixed results that appear to depend on the life stage of the plant, season of fertilizer addition and whether the trial is based in the field or in greenhouse trials. Nutrient addition to Kangaroo grass grown in pot trials resulted in an increase in dry shoot weight and an increase in tiller number (Groves *et al.* 1972). This has the potential to be beneficial to Kangaroo grass seedlings grown in the field as biomass may also increase and therefore aid competition between species. However, when Kangaroo grass was grown with *Poa labillardieri* (a C3 photosynthetic plant) and fertilizer added *Poa labillardieri* was able to respond to the increase in nutrient availability more quickly than Kangaroo grass (Groves *et al.* 1972). This could indicate a potential problem of weed invasion in Kangaroo grass establishment areas that are fertilized because many weedy grasses are known to respond rapidly to nutrient addition (Wijesuriya 1999). Waters *et al.* (2000) also found a potential problem with fertilizer addition after sowing of native perennials from competition with weeds. Timing of fertilizer addition appears to be crucial if weed competition is to be minimised. The application of fertilizer to Kangaroo grass during the summer season has the potential to benefit Kangaroo grass over exotic species. The amount of fertilizer addition needs to be great enough to maximize Kangaroo Growth but low enough for most of the nutrients to be utilized before the growing season of the exotic species.

As reported by O'Connor (1996) and Phillips (2000) the availability of Kangaroo grass seed has an overwhelming influence on the recruitment of Kangaroo grass. For seedling establishment of any species there needs to be sufficient seed present in the soil. Safe microsites are also needed to maximize germination (Winkel *et al.* 1991; Sindel *et al.* 1993). Safe microsites are associated with stones, soil cracks and litter (Winkel *et al.* 1991). Sindel *et al.* (1993) suggested that a soil surface high in organic matter may help with Kangaroo grass seeds absorbing water, which could lead to seeds germinating more quickly. It is possible that an increase in surface organic material may also increase the safe microsite number and hence number of locations where seeds with hygroscopic awns (such as Kangaroo grass) may enter the soil. However, McDougall (1989) found that too much large sized organic material on the soil surface could reduce the number of seedlings germinating. An incorporation of a chemical organic substance may increase the water and microsite availability without the hindrance of large organic matter. Humic acid formulations are used in agricultural systems as organic amendments and were considered to be a possible benefit in Kangaroo grass establishment.

Fertilizer and water addition were incorporated into a Kangaroo grass re-establishment field trial to test their effects on germination and yield of Kangaroo grass and an associated intractable weed Chilean Needle grass. A humic acid solution was incorporated into the trial to assess any benefits on seed penetration into the soil and germination. The trials were established in early 2002 (during close to average rainfall) and extended into a drought summer and following autumn in 2003.

Methods

Site

The field site was established on the Iramoo grassland Reserve in the West of Melbourne, Australia. General soil type was grey basaltic cracking clay.

Further description of the field sites can be found in Chapter three. A randomised factorial block design incorporating buffer zones was used for replication of field trials. Individual replicate treatments were 4 m^2 with an inner assessment area of 1 m^2 allowing for a 2 m buffer between treatments.

Biomass

Biomass of individual seedlings was determined using destructive sampling of aboveground vegetation. Individual plants were randomly selected from within re-establishment treatments. Plants were severed at ground level and placed into paper bags for transport. Sixteen seedlings, two per treatment replicate, were collected for both Kangaroo grass and Chilean Needle grass seedlings. Harvest was in late March 2003, approximately six months after seedling emergence. Samples were then oven dried at 70°C for 48 hours and then weighed on an analytical balance as per Ghannoum & Conroy (1998). Biomass is expressed as grams per seedling.

Fertilizer addition

The rate of fertilizer addition followed recommendations provided by the Department of Natural Resources and Environment Victoria, Agricultural Notes AG0264 (Morrow & Brown 1998) and AG0788 (Sonogan 1999). Rates chosen corresponded to soil type, average rainfall per year and land use. Using Agricultural note AG0264, the recommended rate for Grey basalt soils and establishing new pastures with an average rainfall of 500–700 mm was 50 kg N per ha per yr and approximately 20 kg P per ha per yr (equal to a ratio of 64:33 N: P kg per ha per yr). Using the list of recommended available fertilizers found in Agricultural note AG0788, Pivot 900 was determined to have a percentage of N and P at 16 percent and 8 percent respectively. Applying Pivot 900 at 234 kg per ha per yr was calculated as equivalent to 37.44 N kg per ha per yr and 18.72 P kg per ha per yr. The amount delivered by Pivot 900 was similar to the recommended rate and so was chosen as the fertilizer for addition trials. Two rates of application were included in this trial: half the recommended agricultural rate of 234 kg per ha per yr (Rate 1) and the recommended rate 468 kg per ha per yr (Rate 2) of Pivot 900. Fertilizer was applied in granular form pre weighed immediately after thatch was removed from the trial site in October 2002. Replicates of eight for each rate were incorporated into the plot design. All fertilizer addition treatments were tilled prior to addition.

Irrigation

Supplementary watering was delivered onto re-establishment sites at two rates. Rate 1 was equivalent to adding 400 ml per month per m^2 (4mm) from October 2002 until March 2003. Rate 2 was equivalent to adding 800 ml per m^2 every second month (8mm) (i.e. October, December and February). Melbourne's potable water supply was used and applied with a standard watering can before 10 am on the day of application. Melbourne's potable water supply has consistently low levels of nutrients, bacteria and additives such as chlorine. Appendix Two: Table A1 Reports a typical analysis of Melbourne's potable water supply. Replicates of eight for each rate were incorporated into the plot design. This trial was of a preliminary nature and it is recommended that further trials be undertaken.

Germination

Germination was assessed in late March 2003 on the same day as but immediately prior to biomass sampling. Individual seedlings were counted in a one-m^2 quadrant and expressed as plant density (number per m^2).

Seed penetration

Destructive sampling was used to determine the depth at which seed penetrated the soil. Ground level was marked on the emerging shoots of seedlings and the seedlings with intact roots were then removed from the soil. Seed coats were still obvious and attached to the plant. A measurement was taken from the end of the seed coat nearest the soil surface to the previously marked ground level. This distance was recorded as the seed penetration depth. Six replicate plots were used for sampling with two seedlings of each type removed per replicate plot.

Potassium-humate addition

The principal grassland researcher at Victoria University Colin Hocking obtained a concentrated solution of potassium-humate from an associate in the chemical fertilizer industry. This solution was then diluted 1:20 and applied to treatments at an equivalent of 10 ml per m^2. Ten ml of the diluted stock was added to 500 ml distilled water and added to each m^2. Potassium-humate was only used in soil seed penetration and germination experiments. Due to volume limitations, potassium-humate was not used in biomass experiments. The potassium-humate solution is still undergoing development and so derivation, formulae and specific concentration currently cannot be detailed here. Contact the authors for further information.

Statistical analysis

Statistical analysis was conducted using SPSS v 11.0. All data were tested for normality via histograms, skewness and kurtosis. Transformations were conducted according to Zar (1984) if required. General Linear Model univariate analyses with or without posthoc tests were used for simple comparisons. General Linear Model multivariate analyses with or without posthoc tests were used to examine between subject effects.

Results

Biomass

Treatments of: no-till, and till with no fertilizer and no water added had significantly ($p<0.01$) the lowest average biomass when compared with till, till plus both rates of fertilizer, and till and either rate of irrigation (Figure 6.1 and Table 6.1). Although the till plus fertilizer at rate 1 gave the highest average biomass per plant (approx.1.7 g per seedling) this was only significantly higher than the no-till treatments. No significant differences were found between till, till plus fertilizer rate 1, till plus fertilizer rate 2, till plus irrigation rate 1 and till plus irrigation rate 2 for biomass. Average biomass recorded in fertilizer at rate 1 was greater than biomass in fertilizer rate 2 treatments. Average biomass in irrigation rate 1 was less than biomass in irrigation at rate 2 treatments. Note: potassium-humate was not used in biomass experiments.

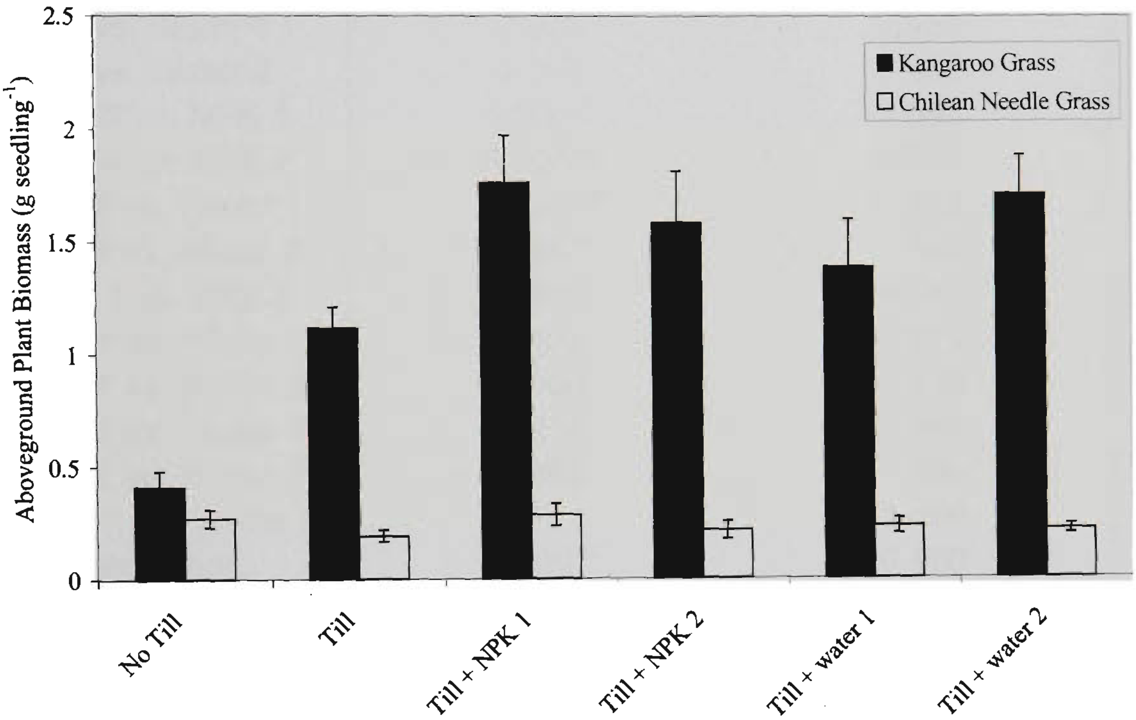

Figure 6.1: Average seedling biomass of Kangaroo grass and Chilean Needle grass in a range of re-establishment treatments measured in March 2003; Bars indicate standard error (n=6).

No statistically significant differences were found for biomass of Chilean Needle grass seedlings between treatments. However, Chilean Needle grass seedling biomass was much lower than any Kangaroo grass seedling biomass.

Seedlings	Kangaroo grass	Chilean Needle grass
Statistics (p value)		
Till vs. No Till	0.005**	0.630
Till vs. NPK 1	0.132	0.467
Till vs. NPK 2	0.472	0.998
Till vs. Water 1	0.923	0.961
Till vs. Water 2	0.165	0.995
No Till vs. NPK 1	0.000**	1.000
No Till vs. NPK 2	0.000**	0.871
No Till vs. Water 1	0.000**	0.978
No Till vs. Water 2	0.000**	0.920
NPK 1 vs. NPK 2	0.980	0.743
NPK 1 vs. Water 1	0.622	0.923
NPK 1 vs. Water 2	1.000	0.818
NPK 2 vs. Water 1	0.958	0.999
NPK 2 vs. Water 2	0.993	1.000
Water 1 vs. Water 2	0.701	1.000
Interaction	0.000**	0.450

Table 6.1: Statistical significance of seedling biomass between re-establishment treatments for Kangaroo grass and Chilean Needle grass. Key: * Indicates a significant difference at $p<0.05$, and ** indicates a significant difference at $p<0.01$. All fertilizer and water addition treatments were tilled prior to addition.

Germination

Till re-establishment treatments had a significantly ($p<0.01$) greater density of Kangaroo grass seedlings when compared with the no-till re-establishment treatments (Table 6.2 and 6.3). Till re-establishment treatments also had a significantly ($p<0.05$) greater density of Kangaroo grass seedlings when compared with till plus K-humate treatments. No statistical difference was found in Kangaroo grass seedling densities between any rate of fertilizer or irrigation addition for till and no-till re-establishment treatments.

No statistically significant differences were found for seedling density of Chilean Needle grass between any of the treatments. Fertilizer and water addition were only performed on till revegetation plots.

	Kangaroo grass	Chilean Needle grass
Seedling density (number m^{-2})		
Control	0	0.75 ± 0.31
Till	36.88 ± 4.53	8.75 ± 2.02
No Till	13.5 ± 2.90	12.38 ± 4.86
NPK 1	28.63 ± 4.99	5.5 ± 2.03
NPK 2	32.88 ± 8.39	6.25 ± 1.44
Water 1	21.5 ± 5.49	7.5 ± 1.87
Water 2	30.33 ± 6.02	8.67 ± 2.86
Till + K-humate	19.25 ± 5.12	9.63 ± 1.44
No Till + K-humate	22.75 ± 4.71	12.5 ± 3.32

Table 6.2: Average emerging seedling density for Kangaroo grass and Chilean Needle grass in re-establishment treatments, standard error indicated (n=8).

Seed soil penetration

Treatments that were tilled had significantly deeper Kangaroo grass seed ($p<0.05$) and Chilean Needle grass seed ($p<0.01$) penetration into the soil compared with no-till treatments (Table 6.4). Adding K-humate to tilled treatments did not significantly ($p>0.05$) increase the depth of seed penetration compared with till alone treatments. Adding K-humate to no-till treatments did not significantly ($p>0.05$) increase the depth of either Kangaroo grass or Chilean Needle grass seed penetration when compared with till treatments. A significant ($p<0.01$) difference was found when Kangaroo grass seed penetration in till plus K-humate treatments were compared with no-till plus K-humate treatments. Significant ($p<0.01$) interactions were found for both Kangaroo grass and Chilean needle grass seed soil penetration between treatments. So the significance of differences in within-treatment comparisons needs to be treated with caution.

	Kangaroo grass	Chilean Needle grass
Statistical Significance (p value)		
Till vs. No Till	0.003**	1.000
Till vs. NPK 1	0.969	0.962
Till vs. NPK 2	0.977	0.978
Till vs. Water 1	0.516	1.000
Till vs. Water 2	0.994	1.000
Till vs. Till + K-humate	0.024*	1.000
Till vs. No Till + K-humate	0.680	0.997
No Till vs. NPK 1	0.450	0.681
No Till vs. NPK 2	0.413	0.823
No Till vs. Water 1	0.948	0.981
No Till vs. Water 2	0.439	0.991
No Till vs. Till + K-humate	0.994	1.000
No Till vs. No Till + K-humate	0.863	1.000
NPK 1 vs. NPK 2	1.000	1.000
NPK 1 vs. Water 1	0.981	0.994
NPK 1 vs. Water 2	1.000	0.994
NPK 1 vs. Till + K-humate	0.894	0.793
NPK 1 vs. No Till + K-humate	0.997	0.561
NPK 2 vs. Water 1	0.973	0.999
NPK 2 vs. Water 2	1.000	0.999
NPK 2 vs. Till + K-humate	0.87	0.904
NPK 2 vs. No Till + K-humate	0.995	0.719
Water 1 vs. Water 2	0.966	1.000
Water 1 vs. Till + K-humate	1.000	0.995
Water 1 vs. No Till + K-humate	1.000	0.949
Water 2 vs. Till + K-humate	0.864	0.998
Water 2 vs. No Till + K-humate	0.992	0.973
Till + K-humate vs. No Till + K-humate	0.999	1.000
Interaction	0.092	0.491

Table 6.3: Statistical difference (2-way ANOVA) between re-establishment treatments for Kangaroo grass and Chilean Needle grass seedling density (n=8). Key: * Indicates a significant difference at p<0.05, and ** indicates a significant difference at p<0.01.

	Kangaroo grass Seed	Chilean Needle grass Seed
Depth of Seed (cm)		
Till	0.91 ± 0.07	1.24 ± 0.09
No Till	0.51 ± 0.10	0.64 ± 0.06
Till + K-humate	1.15 ± 0.16	1.01 ± 0.13
No Till + K-humate	0.55 ± 0.07	0.69 ± 0.06
Statistics (p value)		
Till vs. No Till	0.031*	0.000**
Till vs. Till + K-humate	0.510	0.294
Till vs. No Till + K-humate	0.060	0.001**
No Till vs. Till + K-humate	0.001**	0.022*
No Till vs. No Till + K-humate	0.988	0.969
Till + K-humate vs. No Till + K-humate	0.002**	0.061
Interaction	0.000**	0.000**

Table 6.4: Average depth of seed penetration into soil in a range of re-establishment treatments; Bars indicate standard error (n=8). Key: * Indicates a significant difference at p<0.05, and ** indicates a significant difference at p<0.01. Measurement from soil surface to lowest point of seed penetration.

Discussion

Biomass

Kangaroo grass seedlings in re-establishment areas that were tilled once to five cm in depth (similar to a pasture fallow) had a significantly greater biomass (approximately double) when compared with Kangaroo grass seedlings in no-till re-establishment areas. The most likely reason for this is the available nitrogen that is mineralised in the soil when cultivation occurred (Chapter 5). Previous work investigating soil disturbance on autumn N mineralization rates in western basalt plains grasslands have also noted a greater amount of nitrogen mineralised in till versus no-till areas (Wijesuriya 1999). Root and shoot competition is also reduced in cultivated areas (McIntyre *et al.* 1995), which will allow a greater utilization of available resources by any colonising plant. The addition of both fertilizer and irrigation increased the biomass of Kangaroo grass seedlings further when compared with standard till re-establishment plots. However, this further increase was not significant ($p>0.05$). A re-run of these trials with higher levels of replication may be worthwhile.

Fertilizer addition was at two rates. The lowest rate of 234 kg per ha per yr (Rate 1) resulted in the maximum average biomass of Kangaroo grass seedlings. The highest rate of fertilizer addition at 468 kg per ha per yr (Rate 2) resulted in a lower biomass of Kangaroo grass seedlings when compared with seedlings under rate 1. Loeppky *et al.* (1999) found that when soil available nutrients were in abundance, further addition of fertilizer did not increase yield. There is a possibility that fertilization did increase root biomass. Cookson *et al.* (2000) found a significant increase in root mass, soil mineral N and soil microbial biomass N in fertilizer addition trials to a *Lolium perenne* seed crop. The fate of N from fertilization at the higher rate could be similar to as that found by Cookson *et al* (2000). However, root and microbial biomass were not investigated here but should be the subject of further investigation.

In a literature review, Johnston *et al.* (1999) biomass increased in exotic species fertilized compared with an un-fertilized old native pasture. The improved pasture was able to support a greater stocking rate than the old native pasture. It was noted that the old native pasture, which contained significant densities of native C4 species was more nutritious during the summer phase.

From the results presented here, there is a potential to increase the standing biomass (yield) of Kangaroo grass seedlings during the summer growth season. Further research is needed to identify the maximum rate of fertilization that will significantly increase the biomass of Kangaroo grass seedlings. A more intensive range finding test of different rates of fertilization is needed. Weed invasion must be considered in any fertilization trial. The ideal situation would be for any fertilizer added to Kangaroo grass seedlings during the growth season to result in a minimal residual level in the soil at the end of the growth season. If too much fertilizer is added to re-establishment trials, problems may occur with leaching of nutrients into the water table and into water catchments, leading to eutrophication of these systems.

Fertilizer addition to Chilean Needle grass seedlings during summer at either of the rates trialed caused no significant increase in seedling biomass. The reasons for this could be a lack of sufficient moisture for growth of Chilean Needle grass (a C3 photosynthetic grass) or ambient and soil temperatures were too high for growth, or a combination. It is expected that when the temperature decreases and soil moisture increases, the Chilean Needle grass seedlings might put on more biomass than the Kangaroo grass seedlings in response to nutrient addition. Further research is needed in the growth response of Chilean Needle grass to moisture, temperature and nutrients.

Irrigated seedlings of Kangaroo grass had a greater biomass than non-irrigated seedlings and were at levels similar to the fertilized treatments. A significant ($p<0.01$) increase in Kangaroo grass seedling biomass was found in each irrigation rate treatment when compared with no till controls. Increase in general phytomass (above ground biomass) was recorded in the New England Tablelands with an increase in water availability (McIntyre *et al.* 1995). Precipitation in South African grasslands was also found to increase phytomass, and the water use efficiency of plants was mainly dependent on basal cover (O'Connor *et al.* 2001). Further increase in the rate of irrigation may further increase the biomass of Kangaroo grass seedlings, which could further increase the competitive nature of Kangaroo grass to exotics. However, weeds are also able to increase in size with an increase in available soil water although not the case with Chilean Needle grass in these trials. Reducing the soil seedbank of weed seeds prior to Kangaroo grass re-establishment may reduce the invasion of these weeds into re-establishment areas. Irrigation did not significantly increase biomass of Chilean Needle grass seedlings during summer in re-establishment areas. It is suggested that the main reason for this is the elevated temperatures during the summer period. Further research is needed to determine the responses of Chilean Needle grass biomass to irrigation across a range of seasons.

Germination

Standard till re-establishment treatments had a significantly greater density of Kangaroo grass seedlings compared with no-till re-establishment treatments ($p<0.01$) and till plus potassium-humate treatments ($p<0.05$). The addition of fertilizer, potassium-humate or irrigation to re-establishment plots made no significant difference when compared within sets of either till or no-till re-establishment treatments. Irrigation was expected to increase germination of Kangaroo grass as reported by Hagon & Chan (1977) and O'Connor (1996). Results from this study correspond more with findings by McDougall (1989) where irrigation was found to influence growth initially but emergence density was not significantly affected. Higher rates of irrigation than those investigated in this study during Kangaroo grass emergence season may further increase germination density but this needs further research to determine optimum levels of irrigation. McDougall (1989) noted an increase in the germination of broadleaf weeds (*Hypochoeris radicata*) in irrigated plots. Ongoing weed control is a necessity in most agricultural systems and is also needed in ecological re-establishment, and so the promotion of weed growth by irrigation may confound attempts to improve Kangaroo grass seedling establishment using irrigation.

The germination of Chilean Needle grass seedlings was not significantly different between any treatments but was at greatest average density in no-till re-establishment treatments. As noted in Chapter 4, the soil seedbank of Chilean Needle grass decreases with an increase in soil disturbance. The precise reason why soil disturbance decreases Chilean Needle grass soil seedbank without negative effects on seedling densities needs further research.

Chilean Needle grass was included in this trial as its an aggressive and highly invasive weed with in Melbourne's grasslands and much of the eastern coast of Australia. This weed is identified as being one of twenty Weeds of National Significance by the Australian Federal Government (WONS 2000).

Seed soil penetration

Cultivation to five cm in depth was found to significantly increase the depth of both Kangaroo grass ($p<0.05$) and Chilean Needle grass seed penetration into the soil profile when compared with no-till treatments. Cultivation increases the roughness of the soil surface and aids in safe microsite production. The hygroscopic awn of both Kangaroo grass and Chilean Needle grass are able to use the rough soil to aid in seed burial as reported by Sindel *et al.* (1993). A 10% increase in average seed soil penetration for Kangaroo grass was noted in potassium-humate addition treatments but the increase was not significant ($p>0.05$).

Weed Invasion

Weed invasion into native grasslands has been linked many times with an increase in soil nutrients (Wijesuriya 1999; Allcock 2002; King & Buckney 2002). The addition of fertilizer during Kangaroo grass re-establishment has the potential to result in increased weed invasion. Invasion by weeds was not noted during this trial but the influence of drought conditions could be part of the reason for this lack of effect. Further research of fertilizer addition and irrigation needs to be carried out during a close to average precipitation year. To reduce the impact of weed invasion preventative measures are needed. Robinson (2003) noted that two years of weed control prior to native forb establishment resulted in minimal invasion from dominant weeds once occupying the research site. McCrea *et al.* (2001) have suggested that growing a cereal crop for two years in areas to be created into native grassland habitat can help reduce soil nutrients in areas that have been eutrophied and therefore aid in natural habitat creation.

Implications for Management

If cultivation is to be used in grassland restoration or repair, the importance of not destroying any native species that is already present on the site cannot be over emphasised. All trials carried out by the author involving cultivation were in areas completely dominated by Weeds of National Significance (WONS 2000).

Tilling the soil to a depth of five cm was found to significantly increase the aboveground biomass of Kangaroo grass seedlings. Fertilizer and irrigation were found to further increase average biomass of Kangaroo grass on tilled areas although increases were not statistically significant within the experimental design imposed. Further investigation of optimising fertilizer and water addition are needed. The additional costs of irrigation and fertilizer on cost of re-establishment and impacts on the environment may outweigh any benefits. The appropriate timing of weed control, Kangaroo grass seed addition and thatch removal need to be perfected before fertilizer or water addition treatments are prescribed. The incorporation of tilling to five cm depth on a once only basis (similar to agricultural fallowing) is likely to provide adequate available soil N to aid in Kangaroo grass establishment during drought periods.

The use of potassium-humate at this stage is not worth incorporating into Kangaroo grass re-establishment on a large scale until further research clarifies whether it is useful.

Conclusion

In previous studies addition of fertilizer or water to Kangaroo grass seedling establishment areas to aid in germination and competition has had mixed results. The effects of fertilizer and water addition at various rates on germination and aboveground biomass were investigated during a drought-affected year. In no water or fertilizer addition of Kangaroo grass seed controls, a significant increase was found in Kangaroo grass seedling biomass ($p<0.01$) and germination density ($p<0.05$) in till re-establishment treatments when compared with no-till re-establishment treatments. No statistical difference ($p>0.05$) was found for Kangaroo grass seedling biomass between till re-establishment treatments with either of two rates of fertilizer or with water addition to till re-establishment treatments. In no till re-establishment treatments a significant ($p<0.01$) increase was found in all fertilizer and water addition treatments when compared with controls with no additions. No statistical difference ($p>0.05$) in Chilean Needle grass seedling biomass or density was found between treatments. A significant increase in depth of seed penetration into the soil profile was found for Kangaroo grass seed ($p<0.05$) and Chilean Needle grass seed ($p<0.01$) when till re-establishment treatments were compared with no-till re-establishment treatments. The addition of Potassium-humate at one rate on seed penetration into the soil was also investigated. A significant increase in depth of seed penetration into the soil was found when no till treatments were compared with till plus K-humate treatments for both Kangaroo grass seed ($p<0.01$) and Chilean Needle grass seed ($p<0.05$). Chilean Needle grass seed penetration was significantly less in no till plus K-humate compared with till treatments, suggesting that K-humate has a lesser impact on Chilean Needle grass seed penetration than tilling. Tilling soil of Kangaroo grass re-establishment treatments was found to increase aboveground biomass of establishing Kangaroo grass seedlings when compared with no-till treatments. Addition of fertilizer and water increased average biomass of Kangaroo grass seedlings when compared with till treatments. Tilling soil increases the depth that both Kangaroo grass and Chilean Needle grass seed can penetrate into the soil profile when compared with no till treatments.

Chapter Seven

Implications For Management of Kangaroo grass Re-establishment Research

Detailed discussions of findings were discussed in each Chapter. The following provides a summary of results and how they can be used for Kangaroo grass re-establishment in the west of Melbourne, Victoria. Prefatory Note: Misuse of the following information may result in damage to natural grassland ecosystems.

Kangaroo grass Seed

- Kangaroo grass seed should not be stored for longer than four years at room temperature before use. Percentage germination of the seed is likely to significantly reduce after this time period.
- The quantity of Kangaroo grass seed harvested in the floret per kg of material harvested using a brush header is significantly greater than harvested using a sickle bar mower or by mechanical cut and bail techniques.
- The maximum percentage germination of Kangaroo grass seed can be accurately estimated under growth cabinet at conditions of 12 hours at 20°C dark and 12 hours at 30°C light diurnal regime after seven days of incubation. It must be noted that field temperatures were not monitored. This statement is only made for germination trials *in vitro*.

Kangaroo grass Establishment

- In low precipitation growing seasons low level tilling of soil and associated dead plants (weeds) immediately prior to addition of

Kangaroo grass seed to the soil and covering with a thatch aids Kangaroo grass seedling emergence.

- Only till in areas completely dominated by weeds. Careful attention needs to be paid to not destroying any desired species, as these are difficult to establish to mature age.
- Low level tilling does not significantly increase Kangaroo grass emergence during close to average precipitation growing seasons using the spray and hay method.
- A dilute acetic acid and surfactant solution can be used to kill above ground foliage of Chilean Needle grass and Serrated Tussock. However re-growth may occur within six months under moist conditions and reapplication may be necessary.
- Acetic acid and surfactant herbicide solution has potential to be incorporated into an integrated weed control solution but requires further research.
- There appears to be no difference between Kangaroo grass hay thatch and Wheat hay thatch used in the Kangaroo grass establishment method if Kangaroo grass seed is introduced to the ground before thatching.

Seed Bank

- Low-level soil disturbance carried out before introducing Kangaroo grass seed to the ground can increase the amount of Kangaroo grass seed entering the five cm deep soil seedbank.
- Chilean Needle grass seed in the soil seedbank appears to decline in density with increasing depth of soil disturbance. Further work is needed to verify this.
- The density of Serrated Tussock seed in the soil seedbank is not significantly altered or shows a trend with the degree of soil disturbance.

Soil Moisture and Cultivation (fallowing)

- Both five cm deep cultivated areas and un-disturbed areas associated with basaltic cracking clay appear to form a thin dense layer of soil on the soil surface when compared with the 2-5 cm soil profile layer following a summer period.
- Once off low level cultivation of areas in preparation for Kangaroo grass establishment appears to result in a greater availability of soil moisture in the 2-5 cm soil profile layer when compared with un-cultivated areas. This is likely to increase availability of moisture for Kangaroo grass seedling growth in pre-cultivated areas.

Soil Nutrients Available for Growth

- Maximum *in situ* net mineralization of total available soil nitrogen under alive Chilean Needle grass was achieved in autumn after fifteen days.
- Maximum *in situ* net mineralization of total available soil nitrogen under alive Chilean Needle grass was achieved in spring after sixty days.
- Soil nutrients are potentially more available for plant growth in soil cultivated when compared with un-cultivated areas.
- There was a significant increase in total available soil nitrogen in no-till re-establishment treatments after re-establishment thatch was removed by fire.

Above ground biomass of Kangaroo grass

- Low level once off soil cultivation to five cm depth appears to result in a significant increase in subsequent biomass of newly establishing Kangaroo grass seedlings compared with biomass of seedlings in no disturbance establishment treatments. This is likely to be due to increased availability of nutrients in cultivated plots compared with un-cultivated plots.

- Addition of NPK fertilizer at the recommended agricultural rate may increase biomass of emerging Kangaroo grass seedlings compared with no addition and tilled re-establishment treatments, but the differences are small and not statistically significant in trials reported in this thesis.
- Addition of water during the Kangaroo grass growing season also increased biomass of emerging Kangaroo grass seedlings when compared with no addition and tilled re-establishment treatments, but increases were small and non-significant.
- Fertilizer and water addition combined with soil tilling can significantly increase the biomass of Kangaroo grass seedlings when compared with no-till no-addition re-establishment treatments
- Fertilizer and water addition at the rates tested appear not to be necessary for germination or growth of Kangaroo grass in tilled re-establishment treatments but might aid in increasing biomass of Kangaroo grass seedlings when establishment is in un-tilled soil.
- Fertilization and irrigation during summer does not appear to significantly increase Chilean Needle grass seedling germination or biomass.

Effects of cultivation and humate addition on seed soil penetration

- Kangaroo grass seed appear to penetrate significantly deeper into the soil profile in till re-establishment areas compared with no till re-establishment areas.
- This allows greater access of the germinating Kangaroo grass seed to the available soil moisture in the 2-5 cm soil profile.
- Addition of potassium-humate at the rate tested does not appear to significantly increase the depth of seed penetration into the soil.
- Addition of potassium-humate at the rate tested can result in a significantly lower density of kangaroo grass in potassium-humate treatments when compared with till re-establishment treatments. However this may be confounded by the till effect.

Areas of Further Research

- Mechanisation of the broadcasting of Kangaroo grass seed.
- Time needed to reduce weedy species on re-establishment sites.
- An alternative herbicide to replace atrazine for removal of Chilean Needle grass during the establishment process for Kangaroo grass.
- The effect of soil disturbance over time on intra species competition between Kangaroo grass seedlings, and interspecies competition between Kangaroo grass and weedy species.
- The most effective management of recently established native grass sites.
- Effects of soil cultivation on weed invasion from soil stored seed.
- Further investigation into the relationships between retained soil moisture and cultivation.
- The total amount of mineralizable nitrogen released from dying Chilean Needle grass and the effects of this N release on Kangaroo grass re-establishment.
- A range of Potassium-humate formulations and or soil high in uncomposed plant material and affects in increasing seed-soil penetration.
- Optimisation of irrigation and fertilization rates using the results of the range finding tests presented in Chapter six to achieve increases in density and biomass of Kangaroo grass while at the same time minimising benefits to exotics.
- Seed production and viability of maturing Kangaroo grass, Chilean Needle grass and Serrated Tussock and implications for competition and ongoing management.

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Appendix One

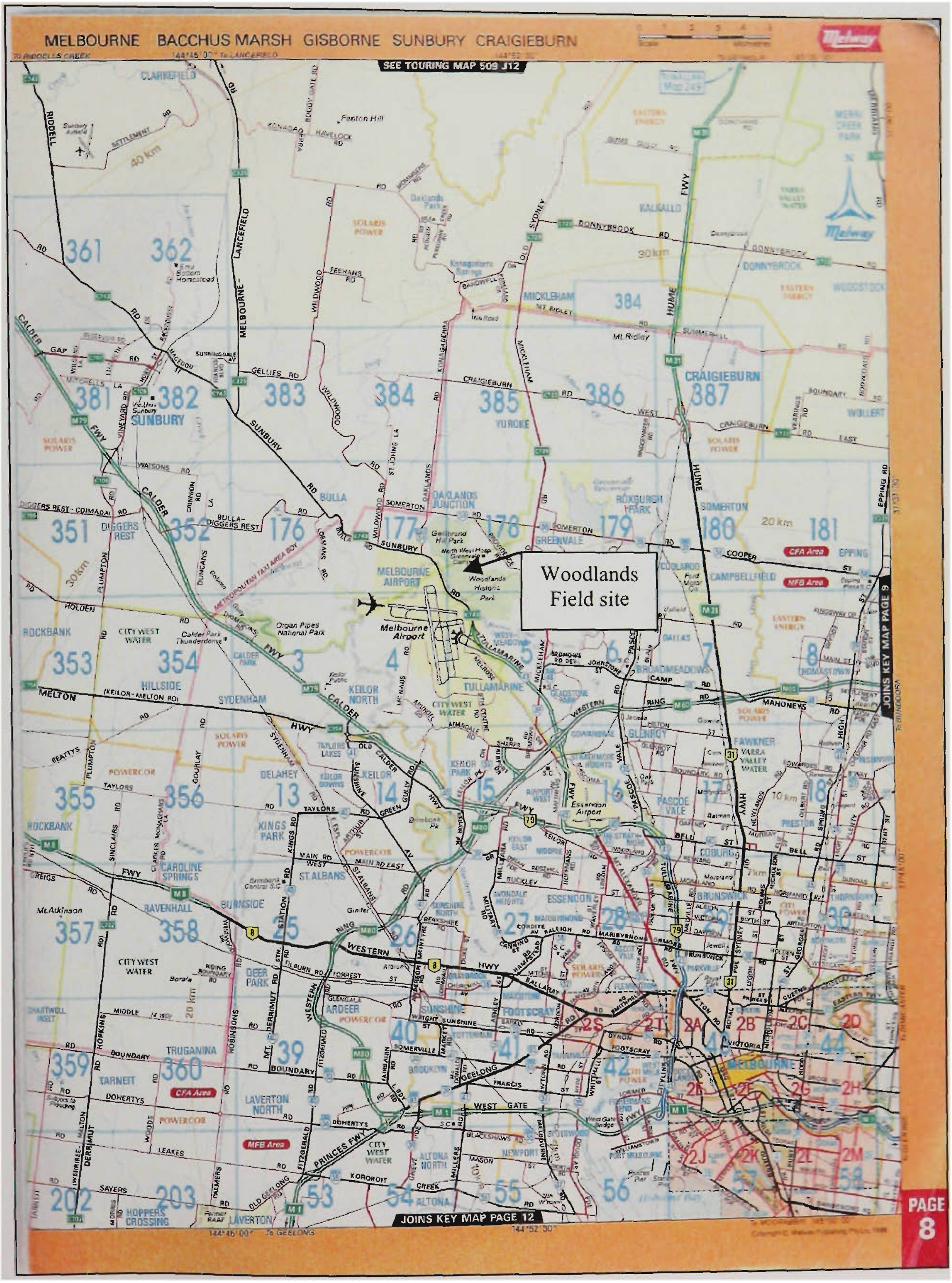

Figure A1: Map of field research sites. Map reproduced from Melway, Greater Melbourne edition 27, 2000 page 8.

Appendix Two

Parameter	Units	Silvan	Cardinia	Winneke	Greenvale	Yan Yean
pH	units	7.1 - 7.7	7.1 - 7.9	6.9 - 7.8	6.9 - 7.6	7.0 - 7.7
Colour	Pt Co	10	4	2	4	2
Turbidity	NTU	0.3 - 2.7	0.2 - 2.6	0.1 - 0.9	0.4 - 2.2	0.1 - 0.9
Specific Conductance	uS/cm	55	63	125	66	140
Iron	mg/L	0.09	0.06	0.01	0.05	0.02
Manganese	mg/L	0.009	0.005	0.004	0.005	0.005
Fluoride	mg/L	0.96	0.89	0.93	0.98	0.91
Aluminium	mg/L	0.041	0.021	0.020	0.022	0.070
Arsenic	mg/L	0.005	0.005	0.005	0.005	0.005
Cadmium	mg/L	0.0005	0.0005	0.0005	0.0005	0.0005
Calcium	mg/L	3.5	3.6	6.1	3.5	6.6
Chloride	mg/L	5.9	6.6	18.4	7.8	21.6
Chromium	mg/L	<0.005	<0.005	0.005	0.005	0.005
Copper	mg/L	0.056	0.016	0.019	0.018	0.013
Cyanide	mg/L	<0.01	0.01	<0.01	0.01	0.01
Hardness	mg/L	14	16	25	15	27
Lead	mg/L	<0.001	<0.001	0.001	0.001	0.001
Magnesium	mg/L	1.3	1.6	2.4	1.5	2.4
Mercury	mg/L	0.0001	0.0001	<0.0001	0.0001	<0.0001
Nickel	mg/L	<0.002	<0.002	<0.002	<0.002	0.002
Nitrate (N)	mg/L	0.20	0.12	0.34	0.10	0.07
Potassium	mg/L	0.4	0.5	1.2	0.6	1.9
Selenium	mg/L	<0.001	<0.001	<0.001	0.001	0.001
SILICA AS SIO2	mg/L	7.7	6.2	5.0	6.2	-
Sodium	mg/L	4.2	4.5	10.7	5.0	13.1
Sulphate	mg/L	0.9	1.1	7.0	1.1	8.5
Total Alkalinity (as CaC03)	mg/L	13.1	15.3	13.5	14.2	13.6
Total Organic Carbon	mg/L	1.9	2.8	1.7	2.6	2.3
Total Phosphorus	mg/L	0.02	0.02	0.02	0.02	0.02
Total Solids	mg/L	40	90	80	45	90
Zinc	mg/L	<0.01	<0.01	<0.01	0.01	0.01

Table A1: Typical analysis of Melbourne's potable water supply. Data provided by Melbourne Water, September 2002.