An investigation into the effects of understorey modification on woodland eucalypt recruitment

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To Kath and Frank
Still a source of inspiration
Declaration of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Chapters 4 is taken directly from a manuscript authored by Alison Skinner, Ian D. Lunt, Sue McIntyre, Peter G. Spooner and Sandra Lavorel, published in the journal *Austral Ecology* (2009). This study was developed from ideas by the candidate, with Ian D. Lunt, Sue McIntyre, Peter G. Spooner and Sandra Lavorel contributing ideas and advice during the development of the experimental design. The establishment and maintenance of the experimental site, collection and analysis of samples, and data analysis was undertaken solely by the candidate, excepting chemical analyses of soil samples which were carried out by the Environmental and Analytical Laboratories at CSU, Wagga Wagga. The manuscript was written entirely by the candidate, with editorial advice from Ian D. Lunt, Sue McIntyre, Peter Spooner, Sandra Lavorel and two anonymous reviewers.

Chapter 6 is taken directly from a manuscript authored by Alison Skinner, Ian D. Lunt, Sue McIntyre, Peter G. Spooner and Sandra Lavorel. The ideas for this study were those of the candidate (with inspiration from a study by Bassett et al. (2005), with Ian D. Lunt providing some advice on experimental design. All experimental work including setup, maintenance, sample collection and processing were carried out by the candidate. The manuscript was written entirely by the candidate, with editorial advice from Ian D. Lunt, Peter Spooner, Sue McIntyre, Sandra Lavorel and two anonymous reviewers.
Published works submitted as part of this thesis


Other published works by the author relevant to this thesis

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Abstract

Widespread tree clearing, grazing, cultivation and fertiliser use have had lasting effects on the understorey of grassy woodlands of south-eastern Australia, creating novel systems where resource availability has been altered and ecosystem processes such as tree recruitment may now be impaired. While there is increasing emphasis within management agencies on using natural regeneration to achieve catchment revegetation targets in agricultural landscapes, the effects of understorey modification on woodland eucalypt recruitment are not well known.

I compared the potential for tree recruitment in a range of variously modified grassland states based on a state and transition model of woodland vegetation change in agricultural landscapes. Specifically I investigated the effects on seedling germination, growth and survival of changes in species composition of the grass layer, biomass buildup following grazing exclusion, soil nutrient enrichment, and soil compaction in a series of field and laboratory experiments.

A landscape-scale germination trial across five pasture states in the Goulburn-Broken catchment of central-northern Victoria found that germination of two woodland trees *Eucalyptus albens* and *Eucalyptus microcarpa* was higher in a pasture dominated by the exotic annual grasses *Bromus molliformis* and *Hordeum leporinum*, than a native perennial, or exotic perennial-dominated states. No seedlings survived beyond spring due to severe drought, highlighting that extreme environmental conditions override the influences of understorey modification.

In an experiment aimed at testing the effects on eucalypt recruitment of soil nitrogen and phosphorus enrichment resulting from fertiliser use, I hypothesised that enrichment would increase the growth of both exotic pasture species and woodland eucalypt seedlings growing in isolation, but change the balance of competitive advantage when growing together. Soil nutrients increased pasture biomass, but had no measurable effect on tree growth. Establishment of *E. albens* and *E. microcarpa* seedlings was severely restricted by pasture biomass as low as 1.5 t ha\(^{-1}\). Increased soil fertility resulted in a competitive advantage to the pasture, and did not improve tree seedling establishment when grown either with or without exotic herbaceous pasture (grassy understorey) species.
The mechanisms limiting woodland eucalypt recruitment in three different understorey (pasture) states – dominated by native perennials, exotic annuals or an exotic perennial – were investigated in simulated low and high rainfall years by manipulating light and water availability. An assessment of germination and survival of seed-sown and planted nursery-raised seedlings of *Eucalyptus microcarpa* found that soil moisture availability was the most limiting factor in all three states, and light availability did not affect recruitment. Contrary to expectations, recruitment was highest in the exotic perennial pasture state, and lowest in the native perennial pasture state, though recruitment was very low where pastures were intact. Increased water supply produced a greater response from seedlings in the exotic perennial and exotic annual pastures than in the native perennial pasture. Large differences in recruitment between the states were attributed to improved soil water relations under the exotic pastures compared with the native pasture. Soil properties had a larger impact on recruitment across the three states than differences in species composition *per se*.

In a laboratory experiment, I found that soil compaction from a bulk density of 1.0 to 1.4 Mg m$^{-3}$ at a soil water content of 20% decreased the rooting depth of six week-old *E. albens* seedlings by 75%. An exotic annual grass weed *Vulpia myuros* showed a similar reduction with increased soil compaction. Results suggest that young seedlings of both *E. albens* and *V. myuros* will be more susceptible to surface drying in compacted than uncompacted soils and therefore face a greater risk of desiccation during the critical months following germination. Any competitive advantage that *V. myuros* may have over *E. albens* is not evident in differential response to soil compaction.

Soil moisture availability was repeatedly identified as the mechanism limiting seedling germination, growth and survival in these experiments. The findings of this research and knowledge from the literature are combined in a conceptual model of the impacts of understorey modification on recruitment as facilitated by changes in soil water availability.
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Chapter 1: Introduction

1.1 The importance of natural regeneration

In south eastern Australia large scale clearing of agricultural areas has proceeded for over 200 years since European settlement, reducing biodiversity and ecosystem function. In response, natural resource management agencies have adopted ambitious revegetation targets at catchment scales. Increases in tree and shrub cover are usually achieved through ‘active re-vegetation’ such as the planting of nursery grown seedlings or ‘direct seeding’ into prepared seedbeds. Whilst highly effective, both of these activities are expensive. The cost of achieving catchment-scale targets through ‘active re-vegetation’ alone has been calculated to be many times greater than the resources currently available (Bryan et al. 2005).

It is recognised that natural regeneration from existing seed-bearing trees can contribute to catchment re-vegetation targets and may in some instances be a cost-effective alternative to active re-vegetation. However, the lost-opportunity costs (e.g. if stock are removed for a set period to allow recruitment to take place) may also be large. Actual costs will depend on both the productivity of the farming enterprise and the likelihood of regeneration in any given year, which is predicted to vary greatly across the landscape (Dorrough et al. 2008).

In this thesis I investigate some of the key knowledge gaps raised in predictive models of tree recruitment; namely the causes of spatial and temporal uncertainty. The literature provides ample evidence that land management practices modify understorey floristic composition. My central question is: how does modification of the grass layer due to land management practices affect tree recruitment?

Reliance on natural regeneration as a re-vegetation option in the short to medium term requires a greater understanding of where, and over what time periods, regeneration is most likely to occur, or conversely, where regeneration is unlikely and therefore where more intensive management intervention may be necessary.

The view that tree recruitment events are sufficiently common to be able to contribute to re-vegetation targets is questionable since a lack of recruitment in many agricultural areas of south eastern Australia is evident and has been noted as a major

Recruitment can occur incrementally or, when stimulated by an environmental ‘event’ such as fire or high rainfall, as a discrete pulse. The latter reflects mass germination and seedling establishment driven by release from interspecific competition. Recruitment events in grassy woodlands are naturally episodic and infrequent so discerning where management affects recruitment, and where recruitment is limited by the timing of natural processes is not straightforward. Even where limitations to recruitment are known, devising management interventions that can overcome them in our current agricultural systems is itself highly challenging. For example, in the 200 years since European settlement, widespread tree clearing and tree decline have affected ecosystem function through development of dryland salinity, increase in erosion, and reduced carbon sequestration. Recruitment is needed to reverse these impacts and to replace the aging and often isolated paddock trees which provide important habitat for woodland fauna in agricultural areas (Gibbons et al. 2008). Several existing models that examine changes to woodland condition and the presence of barriers to tree recruitment (Spooner & Allcock 2006, Standish et al. 2009) are examined in chapter 2. These identify exotic weed dominance, increased soil nutrient load and insufficient (or absent) seed supply as the major barriers to recruitment in woodlands. There are significant gaps in our understanding of the degree to which these factors reduce or prevent recruitment, and how they can be managed.

1.2 Defining recruitment success

A recruitment event is described as the mass germination of a large cohort of seedlings, typically associated with environmental ‘events’ which provide release from inter-specific competition (Watson et al. 1997). Newly emerged eucalypt seedlings (‘recruits’) may be present in large numbers but they are rarely observed in the field until well after emergence, typically when they are large enough to protrude above the grass layer. Consequently, mass emergence may go unnoticed if subsequent mortality
is very high. In following chapters, the term ‘germinant’ is used to describe newly emerged seedling recruits in order to distinguish them from the planted (nursery raised) seedlings.

‘Established’ seedlings are defined as those that survive their first summer, since sapling mortality is generally low. A “successful” recruitment event is in practice defined by the presence of one or more established seedlings. Self-thinning, which occurs at later stages, is influenced less by management, and is consequently not addressed in this thesis.

Eucalypt recruitment is highly variable is space in time, owing to the interaction of numerous biotic and abiotic influences. The frequency of recruitment has important consequences for the persistence of tree populations at both local and landscape scales, and is the response variable of most interest in regards to land restoration. The size of a cohort at germination increases the probability of some seedling establishing in that cohort. Most importantly, changes in germination and survival rates reflect changes in the probability of seedling establishment under a given set of conditions, and therefore the predicted inter-annual frequency of recruitment owing to climatic variability.

In their simulation model of tree decline and replacement, Gibbons et al. (2008) found that the strongest influence on the number of mature trees in a landscape was the number of new recruits per adult tree, followed by frequency of recruitment relative to lifespan, tree mortality and the relative diameter of the initial stand (reflecting stand age). Growth rate of seedlings was less important in determining their survival to maturity. However, as the growth rate of young seedlings determines how long they are susceptible to herbivory by livestock, both seedling growth and seedling survival are considered in my experimental work.

1.3 Thesis structure

Chapter 2

The international and Australian literature on barriers to tree recruitment in grassy woodlands is reviewed with a focus on changes caused by management. I describe some of the grasslands states that are representative of common land use histories and describe the reproductive biology of the genus *Eucalyptus* which underpins some
of the natural limitations and coincident events that determine the frequency and extent of tree recruitment. The barriers to tree recruitment are: seed limitation due to widespread tree-clearing over the last century; intensive livestock grazing across much of the landscape resulting in seedlings being trampled or eaten; and insufficient rainfall. A commonly cited explanation for the absence of recruitment in areas where a seed source is present and where grazing has been removed is the impact of increased competition from exotic grasses and forbs. Consequently a series of experiments was designed (Chapters 3 to 6) to test the commonly-stated hypothesis that recruitment is limited by increased competition from exotic grasses.

Chapter 3

A landscape-scale germination trial was established in north-central Victoria to assess the impacts of land use history on potential for tree recruitment. The effect on recruitment of livestock exclusion (by fencing for several years) was studied using five grassland states that are typical for the region.

Chapter 4

The effects of soil fertility on tree establishment were separated from the effects on herbaceous competitors. Soil nutrients and herbaceous biomass were manipulated in a plot trial to test the hypothesis that there is a cross-over point along a gradient of soil fertility where the benefit to eucalypts of improved nutrition is balanced by the cost of increased competition from herbaceous species.

Chapter 5

I tested whether water deficiency is the main limitation to tree recruitment in most locations in most years, or whether other mechanisms still prevent recruitment when moisture is adequate. The effects of moisture and light in limiting eucalypt recruitment were examined in years of simulated average and high rainfall for three distinct (and unreplicated) grassland states using a factorial design in plot trials. Germination and seedling growth in for each grassland state were compared to test the hypothesis that the mechanisms limiting recruitment differ between states.
Chapter 6

The hypothesis that land use may have lasting impacts on recruitment potential through its effects on soil structure was tested in a laboratory experiment where the root growth of two woodland eucalypt species was compared across five levels of soil compaction.

Chapter 7

Results from previous chapters are synthesised to provide an overall assessment of the potential for woodland eucalypt recruitment in agricultural areas. Finally, a conceptual model is developed to show how land use changes, episodic events, and multiple natural barriers affect eucalypt recruitment processes.
Chapter 1: Introduction
- Why is natural regeneration of woodland eucalypts important?
- Key research question: How does modification of the grass layer affect eucalypt recruitment?

Chapter 2: Literature review
- How have grassy woodlands been changed by land management?
- How do State and Transition models describe vegetation change?
- What are the main barriers to tree recruitment?
- How are vegetation changes caused by land management practices predicted to affect tree recruitment?
- How does seed supply vary in space and time due to natural processes and management influences?

Chapter 3: Assessing the potential for natural tree regeneration between grassland states - a landscape-scale germination trial
- How does eucalypt germination and survival differ between pasture states?
- Does the duration of livestock exclusion affect recruitment potential?

Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility
- How does soil nutrient enrichment influence tree recruitment in an exotic pasture?
  - Do eucalypts and exotic pasture species both respond positively to nutrient additions when competitors are excluded?
  - Does increased pasture growth reduce tree growth?

Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia
- What are the mechanisms limiting tree recruitment in high and low rainfall years?
- How do light, water and herbaceous biomass manipulations affect tree seedling germination, growth and survival?

Chapter 6: The effects of soil compaction on germination and early growth of Eucalyptus albens and an exotic annual grass
- Is eucalypt germination and growth inhibited in highly compacted soils?
- Is Eucalyptus albens more sensitive to soil compaction than a common grass weed, Vulpia myuros?

Chapter 7: Summary and synthesis
- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 2: Literature review

This review comprises two parts:

Part A (sections 2.1 to 2.5) addresses the changes to grassy woodlands since European settlement in Australia; the use of state and transition models to describe land use impacts on understorey characteristics and current knowledge of the main barriers to tree recruitment in tree-grass systems. This information underpins the main theme of my thesis, the impacts of understorey modification on tree recruitment in Australia. Known and hypothesised impacts are summarized in section 2.6.

Part B (section 2.7) provides an overview of eucalypt biology relevant to seed production and dispersal. These topics were not explicitly investigated as a part of my research, but informed the chosen experimental methodology and the interpretation of results.

PART A

2.1 The nature of grassy woodlands

Grassy woodlands are differentiated from other vegetation types in Australia by their open canopy structure (less than 30% canopy cover) and their grassy understorey, where shrubs – if present – are not dominant (Specht 1970). They are associated with fertile clay-loam soils in the 500 – 900mm average annual rainfall zone (Keith 2004). Prior to European arrival, their understorey was dominated by tall, perennial tussock grasses such as Kangaroo Grass, *Themeda triandra*, interspersed with perennial forbs (Prober & Brown 1994, McIntyre et al. 2002a). The overstorey is generally comprised of several co-dominant species of the genus *Eucalyptus*, though *Callitris* and *Casuarina* trees co-occur in some areas. The most common overstorey species are *Eucalyptus blakelyi* (Blakely’s Red Gum), *Eucalyptus albans* (White Box), *Eucalyptus melliodora* (Yellow Box), *Eucalyptus polyanthemos* (Red Box), *Eucalyptus microcarpa* (Grey Box) and *Eucalyptus macrorhyncha* (Red Stringybark). Grassy woodlands once occupied millions of hectares, in a belt stretching from southern Queensland south-west to the Victorian Tablelands, encompassing the NSW tablelands and western slopes (Prober et al. 2002, Keith 2004; Fig. 2.1, Fig. 2.2).
The association of grassy woodlands with prime grazing land led to rapid tree clearing for agricultural development, so that today, most of the area once occupied by grassy woodlands has been highly modified, and woodland remnants with an intact native understorey are rare (Prober & Thiele 1993).

2.2 Historical land use impacts on grassy woodlands

In south-eastern Australia, the years 1800 to 1950 were associated with pastoral exploitation of native grasslands and grassy woodlands, while 1950 to 1970 was a period of intensive pasture improvement with superphosphate fertiliser and the introduction of exotic species (Wolfe & Dear 2001). At the time, there was limited knowledge of the effects of these changes on soil health and catchment hydrology (Wolfe & Dear 2001) and only in the last two decades have adverse impacts of understorey changes on recruitment been identified as a concern.

Past land management practices have an overwhelming influence on current vegetation composition; not only in Australia (e.g. McIvor 2001, Lunt & Spooner 2005), but in areas used for agriculture worldwide (e.g. Russia – Mikhailova 2000; France – Andrieu et al. 2007, Quetier et al. 2007; North America – Brown & Boutin 2009). Changes to understorey composition in grassy woodlands have been driven primarily by cultivation, grazing and fertiliser addition (in combination with grazing) rather than tree removal per se (McIntyre & Lavorel 1994, Wolfe & Dear 2001, Clarke 2003).

Cultivation for cropping and for the establishment of ‘improved’ pastures has resulted in rapid and sometimes irreversible changes to species composition in Australia through direct removal of native species (McIntyre et al. 2002b, Lewis et al. 2008). Cultivation has also led to substantial reductions in soil organic carbon worldwide (Potter et al. 1999, Yadav & Malanson 2008), with the follow-on effect of altering the availability of soil nutrients - particularly phosphorus – due to mixing of the soil profile (Short et al. 2007).

The sensitivity of Australian native grasslands to grazing is attributed to their having evolved in the absence of heavy grazing by large herbivores (Lunt et al. 2007); prior to European settlement, grassy woodlands were subject to light grazing only by soft-footed native marsupials (Garden & Bolger 2001). Some studies have suggested that composition remains fairly stable under grazing in the absence of fertiliser and
Fig. 2.1: Pre-1750 distribution of Box-Gum Grassy Woodlands synonymous with *Eucalyptus albens* (White Box) Grassy Woodlands (adapted from Prober & Thiele 1995).

Fig. 2.2: Distribution of eucalypt woodlands in Australia (includes both shrubby and grassy woodlands), showing change in extent since European settlement (DEWHA 2009).
that purported “grazing effects” are strongly tied to elevated soil phosphorus (McIntyre et al. 2002, Garden et al. 2003, Dorrough et al. 2006). The influence of grazing and fertiliser interactions on pasture composition are more marked in the southern, winter-dominant rainfall regions of Australia than the north (Garden & Bolger 2001).

The direct and widespread effects of increased grazing pressure and fertiliser use on species composition of grassy woodlands in southern Australia are: (i) an increase in the abundance of annual life-forms, corresponding to an increase in exotic herbs (Pettit et al. 1995, McIntyre & Lavorel 2001, Wolfe & Dear 2001, McIntyre et al. 2002b, Clarke 2003, Garden et al. 2003, Prober and Thiele 1995, Lunt et al. 2007); (ii) a decrease in the abundance of highly palatable native perennial forbs (Pettit et al. 1995, Prober & Thiele 1995); (iii) a decrease in native species richness with increasing disturbance (Pettit et al. 1995, Chalmers et al. 2005, Dorrough et al. 2006); (iv) a shift towards dominance by plants with traits of higher specific leaf area and decreased leaf dry matter content (McIntyre & Lavorel 2007, McIntyre 2008); and (v) a shift towards shorter plants, particularly stoloniferous- or rosette-forming architectures over tussock structures (Diaz et al. 2007).

While fertiliser use has had a direct and lasting impact on soil chemical composition, grazing has indirectly altered soil nutrients by accelerating nutrient cycling during decomposition. This is due to the influence of grazing on resource allocation within plants, which can increase the rate of mineralisation (Semmartin & Ghersa 2006), and increased abundance of annual species with increased grazing which can lead to self-sustaining positive feedback loops between N-cycling and understorey composition (Prober et al. 2002b, Lenz et al. 2003, Prober et al. 2005).

In addition to the direct and indirect effects of grazing on understorey condition through changes to botanical composition, grazing can damage biological soil crusts and increase erosion risk (Tongway et al. 2003), compact soil (Greenwood & McKenzie 2001, Wilson et al. 2008) and reduce plant litter and soil organic matter, which is an important determinant of soil moisture holding capacity (Wilson et al. 2008). Grazing regimes can moderate these effects, and “rotational” or “time-controlled” grazing has far less impact on soil properties than continuous grazing (Sanjari et al. 2008).

Other impacts on Australian ecosystems brought about by land management changes are related to the reduction in native vertebrate fauna following the
introduction of predators such as foxes (Bauer & Goldney 2000, Martin 2003). Digging by native fauna created patchy topsoil disturbance that helped to maintain soil health (Martin 2003) and provided gaps in the grassy layer that may have benefited tree recruitment by providing a release from competition. While the introduction of rabbits to Australia has resulted in extended periods of widespread soil disturbance (Eldridge & Kwok 2008), the devastation to native flora through intense herbivory negated any possible benefit to tree recruitment.

Fire is not common in woodlands today, due to active suppression in agricultural areas and around townships. Woodland communities have historically experienced a low fire frequency compared to forest communities of the higher rainfall zones, due to lower understorey fuel loads and litter harvest by termites (Hobbs 2002). Lamont & Downe (1979) suggest that following European settlement, fire frequency may have increased in woodland areas from an average 45 year return period to seven years, but there is little historical information on fire regimes in woodlands (Hobbs 2002).

In general, agricultural development of former Grassy Woodlands of south-eastern Australia has taken place on more fertile soils of the lower slopes and plains. However, within these zones, native vegetation remains in areas where administrative boundaries rather than land capability have dictated their management (Lunt & Spooner 2005). Hence in these zones there is a weak relationship between conservation status and soil fertility. While there is a broad landscape bias in agricultural development toward valley floors and lower slopes, both administrative history and land-use change interact to determine current vegetation composition.

2.3 State and Transition models of vegetation change

State and transition models (STM) provide a useful framework for describing ecosystems, by generalising the effects of key events or processes on vegetation composition and soil properties. Within STMs, “states” represent a relative stable and persistent community assembly that can vary to a certain degree within space and time, and “transitions” are triggered by a change in a particular factor or combination of factors, for example grazing pressure, extreme rainfall events, or nutrient enrichment (Westoby et al. 1989). It is important to note that State and Transition
models are heuristic models, whose primary purpose is as teaching tools, and they are not intended to provide analytically derived models (Westoby et al. 1989).

STMs differ from traditional continuum models of plant succession in that change need not occur in a linear fashion. Instead, the effects of stochastic events, the presence of threshold responses to environmental change and the potential for biotic interactions or limitations on vegetation change can be explicitly incorporated into the model (Suding et al. 2004). The more recently developed “alternative stable state models” (Suding et al. 2004) are a special subset of STMs that focus on the identification of degradation thresholds, whereby return from a degraded state to a more desirable state may be restricted by increased resilience in the degraded state. This can occur due to balancing feedbacks, such as changes to soil nutrient cycling that benefit the persistence of the dominant species in the degraded state, or biotic limitations such as localised extinctions or lack of a seed source (Yates & Hobbs 1997b, Spooner & Allcock 2006).

Vegetation states are most often characterised by a combination of vegetation structure and the abundance of exotic species (e.g. Westoby et al. 1989, Spooner & Allcock 2006, Standish et al. 2009). They can also be characterised in terms of plant life histories, dominant plant traits, resource levels (McIntyre & Lavorel 2007), productivity (Wolfe & Dear 2001) or other characteristic of interest, termed “system state variables” (Suding et al. 2004).

The factors that drive transitions between states, termed “controlling variables” usually represent continuous environmental variables, for example increasing nutrient enrichment, disturbance frequency or abiotic stress. However, the potential resilience of states to small changes in the controlling variable means that transitions may require a threshold level of the controlling variable to be reached before a transition occurs (Suding & Hobbs 2009).

In practise, most STMs define the transitions by specific management actions, such as grazing, fertiliser application, cultivation, in isolation or in combination, or natural events (e.g. Westoby et al. 1989, Yates & Hobbs 1997b, Spooner & Allcock 2006, McIntyre & Lavorel 2007). In Australian woodland ecosystems, the use of qualitative rather than quantitative controlling variables partly reflects the sensitivity of native understorey species to even low levels of grazing by livestock and nutrient
enrichment (Dorrough et al. 2008, Lunt et al. 2007), since changes to the original vegetation state occurred rapidly following livestock grazing and fertiliser application.

The emergence of states whose composition is new to that biome and which are the result of human action, have been coined “novel ecosystems” (Hobbs et al. 2006). In essence, these are a collection of human-induced “states”, the stability of which is not well known (Cramer et al. 2007).

Many STMs attempt to identify thresholds in resource or management gradients which, if exceeded, will result in a rapid (and often not easily reversible) transition between one state and another (Yates et al. 1997b, Spooner & Allcock 2006). Thresholds could represent any environmental variable, for example level of soil nutrient, grazing intensity, salinity (Yates & Hobbs 1997a) or abundance of exotic groundcover. The presence of a threshold between states is often identified by a visible shift in vegetation composition across a relatively small change in environmental condition. In practice, thresholds cannot be experimentally quantified due to their interaction with and dependence on a large number of other environmental variables, as noted by Standish et al. (2009). An example of hypothesised thresholds to tree recruitment is shown in Fig. 2.4 (Spooner & Allcock 2006).

Several STMs of vegetation change have been developed for woodland ecosystems in Australia, and these show subtle differences in the way system state variables and controlling variables are defined. A STM of shrubby eucalypt woodlands of south-western Australia by Standish et al. (2009, revised from a model developed by Yates & Hobbs 1997b) defines five broad states by vegetation composition; primarily in terms of the degree of modification away from an original “undegraded” state (Fig. 2.3). Transitions between states (not shown in Fig. 2.3) are qualitatively defined as driven by management actions, such as clearing, overgrazing, and changes in soil fertility. Standish et al. (2009) note that the controlling variable can rarely be quantified, due to interacting abiotic and biotic factors.

A similar STM of vegetation change in Eucalyptus albens (White Box) Grassy Woodlands has been developed (Fig. 2.4) by Spooner & Allcock (2006). Again, the model defines alterative states a priori by vegetation composition, relating to their difference from an original “pristine” state. The transitions in this model are defined by
combinations of grazing, introduction of exotic species, fertiliser application, tree death, and recruitment or planting of trees, and represent known or hypothesised causes of change in state. In this model, recruitment drives a transition between states, whereas it is the outcome of the transition in other models, demonstrating that the boundaries between controlling variables, transitions and system state variables are sometimes blurred.

Another way of defining vegetation states is demonstrated by McIntyre & Lavorel (2007) in their STM of temperate eucalypt grassy woodlands of south-eastern Australia. Their model places greater emphasis on recognised land uses and associated management practises, such that the transitions form the basis of the model, and the states become the inevitable outcome of the specified combinations of management actions. In their model, states take on a “Land Systems” (Christian 1958) approach whereby both vegetation structure and soil properties are defining features of the state. This differs somewhat from other STMs in that states are defined by particular levels of soil fertility, which is itself presented as a controlling variable in most other models. This model is described in more detail in Chapter 3.

The identification of distinct and persistent understorey states — hereafter pasture states — in former grassy woodlands in Australia provides a basis for comparing their capacity to support eucalypt regeneration. In assessing their regeneration potential, I focus solely on the potential for eucalypt recruits to germinate and grow in these states, rather than recruitment of herbaceous native understorey species or other woody native species such as Callitris or Allocasuarina species which may be present in grassy woodlands. Both the recruitment of trees and recruitment of understorey species could imply a transition to a new state which may or may not be more resistant to change than the former state, but this is not the focus of my research.

In this thesis, the term pasture is used to represent the herbaceous layer, both in the context of landscapes cleared of trees, and as the understorey component of woodland ecosystems that have been subject to grazing. My experimental work assumes adequate tree seed supply for the purpose of comparing recruitment potential between states. However it is highly likely that seed supply and pasture state are correlated at landscape scales; effects of land use on seed production are described in section 2.7.5.
Fig. 2.3: A state and transition model of vegetation change in shrubby eucalypt woodlands in south-western Australia (Standish et al. 2009), modified from an earlier model by Yates et al. (1997b). The boxes represent stable states maintained by feedback loops, in contrast to transient states, such as old-fields. Transitions towards more “degraded” states – or a shift away from the desired original woodland state – are shown by the solid arrows, and transitions that shift vegetation to less degraded states are shown by dashed arrows.
Fig. 2.4: A state and transition model of vegetation change for *Eucalyptus albens* (White Box) Grassy Woodlands by Spooner & Allcock (2006). The seven states are identified by vegetation structure and species composition, and the transitions between alternative states are driven by combinations of management actions or biotic changes such as tree death (T9), tree recruitment (T5), or growth and self-thinning. B1 and B2 represent barriers that prevent or greatly restrict the transition of states back towards the original grassy woodland state.

**Transitions**

T1: grazing of pristine woodland causes reduction or loss of grazing sensitive species and shift to dominance of short grasses.

T2: grazing and clearing removes trees and causes shift to dominance of short grasses; may be reversible early in transition (see T4).

T3: concurrent clearing, grazing, fertilization, and introduction of exotics move pristine woodland directly to mixed pasture.

T4: removal of grazing early in transition from S1 to S2 may permit recovery to S1, but T4 is very unlikely.

T5: recruitment of trees into native grassland, a rare event, or clearing of trees in native woodland.

T6: addition of fertilizer, sown pasture species, and invasive exotic species moves native grassland to mixed grassland.

T7: tree clearing, addition of fertilizer and pasture species, and invasion of exotic species move S2 to S5. Recruitment or planting of trees in S5 moves to S2 (rare).

T8: fertilization, stock camps, and invasion of noxious weeds move S2 to S4.

T9: tree death moves S4 to S6.

T10: continued heavy grazing, erosion, and invasion of noxious weeds moves S5 to S6.

T11: tree death moves S4 to S5.

T12: prompt management such as de-stocking and rabbit control (seen at Burrendong in 1960’s) can return S6 to S5.

T13: management attempts to move from S6 to S5 can also result in transition to S7 if propagules available (seen at Burrendong).

T14: growth and self-thinning of S7 creates S8, a previously unrecorded state for woodlands in the Burrendong area.
Barriers to tree recruitment

While an adult eucalypt has the potential to produce in excess of two million seeds in a single year under ideal conditions (Vesk et al. in press), this is not typical of all trees in a population, nor does seed supply occur at high levels every year. Even in years of high seed fall, abiotic and biotic influences on each stage of the lifecycle (often termed environmental ‘barriers’ or ‘thresholds’) reduce or prevent effective recruitment. The primary stages in the eucalypt lifecycle and the environmental influences upon each of these stages have been thoroughly reviewed by Semple (1997). The following section is a review of the key influences on recruitment during the two critical early lifestages; germination and seedling survival through the first summer.

Seed supply

Seed supply has an overriding influence on tree recruitment patterns at the local scale, though environmental filters influence finer scale patterns (Gardescu & Marks 2004, Garcia & Houle 2005, Standish et al. 2007, Fischer et al. 2009). Eucalypts do not have a specialised dispersal mechanism, and the vast majority of seeds fall within 1.5 times the height of the canopy. As eucalypts do not have a persistent soil seed bank, recruitment in areas devoid of trees relies on management intervention to reintroduce seed or provide a future seed source through planting of seedlings. Natural regeneration is not a revegetation option in these areas.

Seed supply varies between trees and years according to tree age, tree density and natural cycles of seed production. Tree clearing has massively reduced seed supply at a landscape scale by removing seed-bearing trees, and decreased production per tree by increasing tree isolation, leading to higher levels of self-fertilisation in remaining trees (Potts & Wiltshire 1997).

Viability of the seed store

Most eucalypt species do not maintain a soil seed store (Andersen 1989), though canopy storage of a small portion of the seed crop for up to four years (where release is gradual) can buffer against germination failure following mass seed release (Yates et al. 1994a, Bassett 2002). Eucalypt seeds have no innate year-to-year dormancy (Gill 1997), and although seeds of the Western Australian woodland gum E. salmonophloia
were observed to resist germination in sub-optimal temperatures (Yates et al. 1996), most eucalypt seeds do not remain viable for more than one year post-dispersal (Grose 1960 cited in Gill 1997, Yates et al. 1995). The viability of buried seed of *Eucalyptus victrix*, a woodland tree of northern Western Australia, dropped by 50% in three months and no viable seeds remained after five months (Florentine & Fox 2002), though this is unlikely to be directly comparable to eucalypts in temperate regions. The timing of seedfall therefore has a large influence on emergence rates, since seeds that do not germinate soon after seedfall are quickly lost from the seed pool (Yates et al. 1995).

2.4.3 Seed predation

Post-dispersal seed predation, primarily by ants, is a major limitation to recruitment, and can reduce the available seed pool by more than 70%. In a mallee eucalypt community in southern Australia with a low level of herbaceous cover, up to 98% of seed was collected by ants and removed from the viable seed bank (Andersen 1989), while in a native grassy understorey in the Northern Tablelands of NSW, ants removed 73% of an *E. blakelyi* crop within one week (Li et al. 2003). Isolated mallee eucalypt seeds in southern Australia were found to have a half life of about five days due to removal by ants (Wellington & Noble 1985). The proportion of seeds lost to predators can vary considerably according to seasonal climatic conditions, the nature of the grassy understorey and size of the seed crop (Andersen 1989).

Predation by ants is temporarily reduced by fire and cultivation (Majer et al. 1997), and predation was found to be lower under sheep grazing than in ungrazed areas in river red gum forests (Meeson et al. 2002). Bare ground and litter cover have been shown to have variable effects on seed predation (Mittelbach & Gross 1984, Facelli 1994).

2.4.4 Factors affecting germination

Soil moisture has a strong influence on both germination and seedling survival. Germination in eucalypts is sensitive to soil matric potential; a laboratory study of four species observed 100% germination at -0.1 MPa, 70% germination at -0.5 MPa, and complete inhibition of germination at -1.0 MPa (Schütz et al. 2002).

Effects of neighbouring plants on germination are most often attributed to changes in soil moisture. In a North American study, field germination rates for
hardwood trees ranged from 0% to 10% in a pasture of *Festuca elatior* and *Trifolium campestre* (de Steven 1991a). Only one of the six species tested, *Liriodendron tulipifera*, showed higher emergence in bare plots than pasture plots. The positive emergence response to vegetative cover in the other species was attributed to a moister germination microsite.

Fire is commonly associated with large tree recruitment events due to its ability to stimulate mass seed release and temporarily remove competition from grasses (Gill 1997), however it is not a prerequisite for seedling emergence in grassy systems (Gill 1997, Clarke & Davison 2001).

Germination rates of eucalypts within intact grassy understoreys vary greatly according to species and environmental conditions. A relatively high germination rate of 35% was found for the West and South Australian mallee eucalypt *E. incrassata* in a pasture dominated by exotic forbs (Hastwell & Facelli 2000), but germination rates under 10% are more typical for eucalypts within a grassy understorey.

Germination rates of around 2% and 4% at 10 weeks were found for surface sown seeds of the south-eastern Australian woodland trees *E. melliodora*, and *E. blakelyi* respectively within an unspecified pasture (Clarke & Davison 2001). Month of sowing had a strong influence on germination of *E. melliodora* and another widely distributed south-eastern Australian woodland tree *E. albens*; April to July (autumn/winter) was the optimal sowing period and resulted in 4 to 11% germination of viable seed from July to November, compared with less than 1% germination from sowings in the other months (Windsor 1998). This was attributed to a combination of reduced ant activity and higher soil moisture during the autumn/winter period.

The requirement of light for germination (or lack thereof) varies across eucalypt species (Clarke *et al.* 2000, Clarke & Davison 2001) and is influenced by soil temperature (Li *et al.* 2003). Seed burial almost doubled seedling emergence in several woodland eucalypt species (Clarke & Davison 2001), while exposure to light increased germination of *E. blakelyi*, though this effect disappeared when seed was sown at its optimal germination temperature of 25°C (Li *et al.* 2003).

Litter has been shown to have variable effects on eucalypt emergence. Leaf litter did not affect emergence of the mallee eucalypt *E. incrassata* in winter or spring when
establishment typically occurs (Hastwell & Facelli 2000), but resulted in significant increases in emergence of the south-eastern Australian tall forest tree *E. obliqua* due to increased soil water content (Hastwell & Facelli 2000). Conversely, litter was found to reduce recruitment of pines in Spain, by preventing seed contact with the soil (Castro *et al.* 2002).

**2.4.5 Factors affecting seedling survival**

Competition for soil water by the grass layer is noted as the major reason for high seedling mortality in savannas, grassy woodlands and old fields (Fensham & Kirkpatrick 1992; Davis *et al.* 1999, 2005; Smit & Olff 1998; Gordon & Rice 2000; Rey Benayas *et al.* 2003). Curtis (1990) suggests that above average rainfall for the first three months of seedling establishment is needed to overcome competition and allow successful eucalypt recruitment.

Clarke (2002) found a seedling survival rate of 4% for the woodland eucalypts *E. blakelyi* and *E. melliodora* twelve months after emergence in a native pasture dominated by the native perennial grasses *Aristida* and *Austrodanthonia* spp., though the level of ‘background mortality’ in competition free plots was not known. Survival dropped slightly further in the following four years. Following germination, 50% mortality of seedlings occurred in the first six months, attributed to frost, insect damage and water limitation. Combined with emergence data, this equated to an establishment rate of around 0.1% for *E. blakelyi* and *E. melliodora* in above average rainfall (Clarke 2002). This is much higher than the estimated establishment rate of less than 0.001% in arid environments (Jacobs 1955).

Moxham & Dorrrough (2008) observed 100% mortality of seedlings of *Eucalyptus strzeleckii* - a forest tree of the Gippsland region of Victoria - in an undisturbed exotic grass-dominant pasture, and found that soil disturbance greatly improved seedling survival (to around 10%), but not more than removal of pasture competition with herbicide. Although the exotic annual dominant pasture was shown to inhibit recruitment, no comparison with a native dominant pasture was possible.

Facilitative rather than competitive effects of herbaceous neighbours on seedling survival and growth have been noted by Holmgren *et al.* (1997), Davis *et al.* (1999) and Suding & Goldberg (1999) in studies in North America, but in all cases these were attributed to improved soil water relations.
Due to the relatively low germination rates that are expected using direct seeding into pastures in comparison to sowings into prepared seedbeds (either sprayed, cultivated or both), details about the nature of the pastures used in seeding trials are rarely recorded, and studies comparing the effects of different neighbour species are few.

Results from a study of _E. melliodora_ regeneration from seed found that seedling establishment was highest in pastures dominated by native species, and seedling establishment declined as the proportion of exotics in the pasture increased (Lawrence _et al._ 1998), however growth of established seedlings in the same study was greater in exotic dominated pastures. Survival of transplanted _E. melliodora_ seedlings was lower in an exotic perennial pasture (dominated by _Phalaris aquatica_) than an exotic annual pasture for spring sowings (Semple & Koen 2003), and yet survival after one year was relatively high (around 20%). In the same study, seedling growth did not differ between the exotic perennial and exotic annual pastures.

While fire can increase seedling emergence, it is also a potential source of mortality for eucalypts, though most mature trees will re-sprout after being burnt (Gill 1997). Fire frequency and intensity have been shown to influence the dynamics of tree-grass systems worldwide (Jeltsch _et al._ 1996, Sankaran _et al._ 2008), including in savannas in northern Australia, where tree cover was found to be negatively correlated with fire frequency over long periods (Lehmann _et al._ 2008, Lehmann _et al._ 2009). Conversely, fire and grazing were not significant explanatory variables in a model of woody vegetation change in central Queensland (Fensham _et al._ 2005), rather annual rainfall was the best determinant of changes in tree cover.

### 2.4.6 Herbivory
Grazing by livestock, native or pest animals has an adverse impact on seedling survival and growth. Grazing by livestock did not greatly affect emergence of woodland eucalypts in intact pasture in south-eastern Australia (Clarke & Davison 2001), but strong inverse relationships between grazing pressure and probability of observing recruitment have been found by Spooner _et al._ (2002), Dorrough & Moxham (2005), and Fischer _et al._ (2009) in the same region. The influence of sheep grazing history on tree recruitment in South Australia was shown to be greater than the effects of
herbivory by other animals, such as rabbits, goats and kangaroos (Tiver & Andrew 1997).

Increased growth rates can directly influence eucalypt seedling survival. Faster root elongation and production of fine roots improves access to soil moisture and nutrients (Fabião et al. 1995, O’Grady et al. 2006), and rate of increase in shoot height determines the period that seedlings are at risk of being eaten by livestock (Semple & Koen 2001, Allcock & Hik 2004, Vesk & Dorrough 2006). Semple & Koen (2001) found that two woodland eucalypts E. melliodora and E. blakelyi grew less than 20cm a year, and four years after the removal of grazing had not yet reached the 1m ‘escape height’ for herbivory by sheep. Allcock & Hik (2004) found that transplanted E. albens seedlings exceeded 1m height within 18 – 24 months in the absence of livestock grazing, but seedlings had grown to only 50cm after 35 months when subject to livestock grazing. Overall, seedlings reached escape height faster in grasslands than woodlands across all grazing treatments (Allcock & Hik 2004).

2.5 Effects of understorey modification on eucalypt recruitment

Observations of mass regeneration of eucalypts following disturbances such as fire, cultivation, scalping or herbicide application, have led to the widely held belief that recruitment cannot occur in the presence of competition from herbaceous species. Nevertheless, seedlings can establish in undisturbed grassy understoreys – albeit at much reduced levels – as demonstrated by the presence of eucalypt regeneration in grasses on roadside reserves freed from grazing pressure, and by studies in pastures (Clarke 2001, Semple & Koen 2003). Venning (1985) noted that eucalypt regeneration occurred in grassy woodlands without fire in years of above-average rainfall, where understorey was native dominant, not fertilized, and subject to low levels of grazing.

In my experimental work, grassland states reflect both changes to species composition and soil properties, as previously described by McIntyre & Lavorel (2007). I hypothesise that grassland states will influence tree recruitment primarily by altering competition for resources during seedling emergence, survival and early growth (section 2.4). Changes to above and below ground biomass or the physical structure of the grass sward are expected to influence the amount of resources available for tree seedling establishment. Changes in available resources are predicted to be the result
of differences in soil water uptake between species, altered litter production and nutrient cycling, and altered soil water storage through changes to soil structure and organic carbon content, in accord with previous studies by Southwell et al. (2008), Prober et al. (2005), Sanjari et al. (2008), and Wilson et al. (2008). Changes to the sward are also predicted to affect emergence due to altered light interception and microclimate, including changes in wind speed and temperature at the soil surface (Battaglia et al. 2000, Yates Norton & Hobbs 2000).

2.6 Summary and hypotheses

State and transition models are used to describe ecosystems and their responses to environmental change, including land management practices. A variety of State and Transition Models (STMs) exist that describe and explain changes to Grassy Woodlands. In this study, the STM of McIntyre & Lavorel (1997) was selected because this model focuses explicitly on management-derived grassland states, and the goal of this research was to assess the influence of grassland states on the potential for tree recruitment.

Impacts of management actions on the understorey, and predicted impacts of understorey modification on tree recruitment are summarised in Table 2.1. Highlighted topics are further investigated in my experimental work.

Recruitment is strongly influenced by the availability of seed, and competition from herbaceous species. This review has shown that there are numerous studies that demonstrate the adverse impact of the herbaceous layer on tree seedlings, both in Australia and overseas. However, very few studies have considered the impacts of understorey modification on tree recruitment, beyond comparing the presence and absence of herbaceous species.

While dominance of the herbaceous layer by exotic annual grasses has been linked to inhibition or reduction of eucalypt recruitment in Australia in landscape-scale surveys, there is less information about mechanisms underpinning barriers to recruitment in modified grasslands and pastures.

The following chapters aim to address this knowledge gap by testing recruitment success in a range of grassland states within a landscape-scale germination trial, and by investigating which mechanisms are acting upon seedlings within controlled field
Table 2.1: Summary of predicted direct and indirect influences of different management practices on seedling emergence and survival of *Eucalyptus* spp. in grassy woodlands, based on current literature. Topics in bold are further investigated in my experimental work. Hypothesised impacts are displayed in *italics*, while demonstrated impacts are in normal text.

<table>
<thead>
<tr>
<th>Management action</th>
<th>Direct impacts/changes</th>
<th>Indirect impacts/changes</th>
<th>Hypothesised or demonstrated impact on seedling emergence or survival</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widespread tree clearing for pasture development</td>
<td>Fewer seed bearing trees, reduction in seed production at landscape scale</td>
<td>Isolation of trees; reduced seed set and increased potential for inbreeding depression (reduced ‘fitness’ of offspring)</td>
<td>Reduced fitness (growth and survival) may be apparent in second generation seedlings</td>
<td>Young <em>et al.</em> 1996; Burrows 2000; Butcher <em>et al.</em> 2005</td>
</tr>
<tr>
<td>Increasing grazing pressure</td>
<td>High levels of seedling mortality due to trampling and herbivory</td>
<td>Shift in composition of the grass layer towards grazing tolerant and/or less palatable species; altered nutrient cycles. Possible soil compaction, particularly at ‘stock camps’ – commonly around isolated paddock trees. Changes to soil surface microclimate</td>
<td>Changes to water uptake by the grass layer. A reduction in plant available water in cases of extreme compaction. Physical restriction to root growth likely to have the biggest impact on access to soil water. Reduced emergence and seedling survival due to higher levels of seedling desiccation</td>
<td>Clarke 2002; Prober <em>et al.</em> 2005; Close <em>et al.</em> 2008. Bassett <em>et al.</em> 2005; Wilson <em>et al.</em> 2008</td>
</tr>
<tr>
<td>Removal of grazing</td>
<td>Reduced seedling mortality from trampling and herbivory</td>
<td>Build-up of standing pasture biomass and removal of soil surface disturbance</td>
<td>May reduce light available to newly emerged seedlings or provide a physical barrier to seed soil contact resulting in reduced emergence. Overall increases in recruitment with reduced grazing, due to lower seedling mortality</td>
<td>Yates Norton &amp; Hobbs 2000</td>
</tr>
<tr>
<td>Fertiliser application</td>
<td>Increased tree ‘dieback’ caused by defoliation of adult trees by insects, attributed to increases in concentration of foliar nutrients</td>
<td>Reduced seed set where dieback is severe.</td>
<td>Lower emergence due to fewer seeds</td>
<td>Curtis 1990; Reid &amp; Landsberg 2000; Close <em>et al.</em> 2008. Yates &amp; Hobbs 1997; Semple &amp; Koen 2003; Prober <em>et al.</em> 2005; Cramer <em>et al.</em> 2007</td>
</tr>
<tr>
<td>Cultivation</td>
<td>Effective removal of all seedlings</td>
<td>‘Homogenisation’ of the structure of the grass layer and removal of germination microsites</td>
<td>Possible reduction in water available for tree seedling establishment</td>
<td>Dorrough &amp; Moxham 2005; Cramer <em>et al.</em> 2007</td>
</tr>
<tr>
<td>Fire</td>
<td>Stimulates eucalypt capsule opening and seed release. Death of young seedlings and removal of adult tree biomass</td>
<td>Temporary removal of the herbaceous layer; reduced competition for light and water</td>
<td>Pulse recruitment event following fire, with higher than average seedling survival</td>
<td>Gill 1997; Lehmann <em>et al.</em> 2008</td>
</tr>
</tbody>
</table>
plot trials and a laboratory experiment. The study species used in my experimental work were *Eucalyptus albans* (White Box) and *Eucalyptus macrocarpa* (Grey Box), which are common grassy woodland overstorey species with a wide distribution through the grassy woodland belt of south-eastern Australia (see Figs. 2.1 and 2.2). These species were chosen because they are have a wide distribution, are often present in remnants where regeneration is sought, and they commonly co-occur with other woodland eucalypts such as *E. blakelyi* (Blakely's Red Gum), *E. polyanthemos* (Red Box), *E. melliodora* (Yellow Box), *E. macrorhyncha* (Red Stringybark). Therefore they were considered to be broadly representative of other grassy woodland eucalypts, though there are substantial differences in seed size between *E. albans* and *E. microcarpa* (see Section 2.7.5) and within the group of species listed above.

I hypothesise that recruitment will be highest in the least-modified, native-dominated grassland states, and will decrease with increasing modification of the understorey. I also hypothesise that the increase in herbaceous biomass within each grassland state following removal of grazing reduces the potential for tree recruitment. This is tested in Chapter 3 using a landscape-scale germination trial across five grass states and two fencing treatments.

In chapter 4, I test the hypothesis that both *Eucalyptus* species and exotic pasture grasses both show a positive growth response to soil nutrient additions when competitors are excluded, and that increased growth of exotic grasses reduces *Eucalyptus* species' seedling growth when grown in competition.

In chapter 5, I hypothesise that the mechanisms limiting tree recruitment differ between low and high rainfall years, and between grassland states. Specifically, I hypothesise that that native perennial pastures will support more tree recruitment than exotic annual pastures, and exotic annual pastures more tree recruitment than exotic perennial pastures. I also hypothesise that grassland state, light, and water supply will have an interactive effect on tree seedlings, with light having a greater influence than water in high-biomass exotic perennial pastures, and water availability having a greater influence in lower biomass exotic annual and native perennial pastures.

Finally, I investigate the impacts of soil compaction on *Eucalyptus* recruitment. I hypothesise that *Eucalyptus* germination and growth is inhibited in highly compacted
soils and that *Eucalyptus albens* is more sensitive to soil compaction than a common grass weed, *Vulpia myuros*.

Due to the importance of seed availability in determining the occurrence of eucalypt recruitment in time and space, I next provide a review of the eucalypt biology literature, describing the limitations and influences on seed production and dispersal. Concurrent research on seed production and seedfall in woodland eucalypts in agricultural areas by Vesk *et al.* (in press) was foreshadowed at the start of this project. Hence, I chose not to undertake experimental work to assess seed fall, and instead rely on this review to inform the methodology and interpretation of subsequent experiments.

**PART B**

### 2.7 Reproductive biology of *Eucalyptus* spp.

The following section addresses the key biological processes relevant to the timing, duration and quantity of seed produced by eucalypts. I draw heavily from work on woodland eucalypts by Semple (1997) and Windsor (1998), from the production forestry literature (especially Florence 1996) and from a general review of eucalypt biology by House (1997). The relative importance of environmental sieves in reducing the potential for tree recruitment in the context of modified grassy woodlands is discussed.

#### 2.7.1 Time to reproductive maturity

Variation in the time to reproductive maturity in eucalypts exists between species, but also within species, due to environmental influences on early growth, and genetic variation between individuals in a population and between provenances of a species. While it is possible for some seed set to occur within five years from establishment under favourable conditions (Boland *et al.* 1980, Moncur & Hassan 1994), significant seed crops do not usually occur until trees have reached an age of 10 to 20 years for smaller eucalypts, or 20 to 40 years for most species (Jacobs 1955, Gill 1997).

#### 2.7.2 Floral sequence

The ‘floral sequence’ encompasses the formation of the inflorescence, shedding of involucral bracts, bud development, flowering, capsule maturation and opening of
capsule valves, necessary for seed release (Florence 1996). Sequence length influences, but is not equivalent to, frequency of flowering or frequency of seedset (addressed in 2.7.4). ‘Gums’ and ‘boxes’ of the Eucalyptus subgenus Symphyomyrtus (which include the woodland trees Eucalyptus blakelyi, E. melliodora, and E. bridgesiana of south-eastern Australia) generally have a relatively short sequence length of three to four years from bud initiation to seedfall (Curtis 1990, Florence 1996), compared to the ‘ashes’ and ‘stringybarks’ of Monocalyptus with sequence lengths of five to six years (Florence 1996).

Variation in the length of the sequence due to genetic and environmental influences may occur within a species between provenances, between stands, between individuals in each stand, and even between different ‘sides’ of the one tree (Florence 1996). On any individual, shaded parts of the canopy may flower up to several weeks later than exposed parts (Eldridge & Griffin 1983). Long, dry summers and warmer climates are associated with shorter flowering sequences, and in the case of the Western Australian forest tree, E. diversicolor, the length of the sequence can vary between 3 and 5 years (Florence 1996).

2.7.3 Time of Flowering
The majority of eucalypt species flower over a period of 3-5 months, commencing from early in spring to early summer (Boland et al. 2006). Time of flowering is linked to day-length, daily temperature, soil moisture during the current and preceding seasons, and solar radiation (House 1997).

Variation in the length and timing of flowering largely reflects climatic differences between locations. Species found in high altitude, high rainfall environments with short summers are associated with short, late flowering periods, while coastal and inland species subject to milder climates tend to commence flowering earlier, and flower for longer (Semple 1997). Mugga Ironbark (Eucalyptus sideroxylon) occurs across a wide geographic and climatic range in southern-Australia, and flowers in all months of the year across Victoria, and for periods of between one and 12 months (Porter 1978 cited by Semple 1997). Climatic variation may lead to significant variation in commencement and duration of flowering between years at the one locality (Semple 1997).
2.7.4 Frequency of flowering and flower output

While it is possible for mature trees to produce some flowers each year, for most species, heavy flowering events (resulting in ‘good seed years’) are interspersed with several years of low or negligible flower production (Florence 1996, Burrows and Burrows 1992). This cyclic pattern is presumed to reflect a reduction in resources available for flowering as resources are directed towards maturing fruit (Jacobs 1955), but Florence (1996) notes that this regular cycle of heavy flowering may be upset by climatic and biological (disease and predator) fluctuations, so that ‘bumper’ crops may only be produced at ‘wide and irregular intervals’.

Species in the subgenus *Monocalyptus* typically display multiple stages of floral development at any one time reflecting their longer sequence lengths, while species in the subgenus *Symphyomyrtus* are less likely to have overlapping seed crops (Florence 1996).

High levels of bud abortion prior to flowering in the south-east Australian forest trees *E. regnans* (Ashton 1975) and *E. pilularis* (Florence 1964) and the Western Australian *E. diversicolor* (Breidahl 1983 cited in Bassett 2002) were linked with drought. Insect predation has also been found to be a major cause of bud loss in some species (e.g. for the mallee *E. baxteri* of southern Australia; Andersen 1989).

Flowering can also vary between trees and within the canopy, and is determined primarily by tree age, canopy size and environmental conditions preceding and during floral development (Florence 1996). High levels of bud abortion prior to flowering were linked to drought in *E. diversicolor* (Florence 1996). Observed synchronisation of seed peaks of individuals in some populations was previously thought to be under genetic control (see House 1997), but may be better explained by a combination of density-dependent pollination limitation and environmental influences on a plant’s ‘resource budget’ (Crone et al. in press). Variation in flowering and seeding patterns have important consequences on recruitment potential, as species that have overlapping floral sequence and that release seed over several years can buffer low seed production years and reduce their chance of inbreeding depression caused by selfing (Bassett 2002).
2.7.5 Seed production and viability

Seed production is determined by the size of the preceding flowering event and post-flowering losses, including abortion of sterile capsules immediately after flowering (Florence 1996, House 1997), and pre-dispersal seed predation (Bassett 2002). Like flowering, seed set is cyclic in nature, and a 25 to 65-fold difference in seed set between good and poor seed years is not unusual (Florence 1996).

Capsules (and seed) of most species mature between 3 and 10 months after flowering, with the woodland trees *E. blakelyi*, *E. melliodora* and *E. bridgesiana* having fruit maturation times of 3-5, 4-7, and 7-9 months respectively (Curtis 1990). At maturity, capsules contain a mixture of fertile seed and ‘chaff’ – unfertilised ovules, which may make up to 95% of capsule contents by weight (Pryor 1976).

The number of seeds per capsule is commonly between 4 and 10, but increases to over 30 under some circumstances (Pryor 1976). An average of 2 seeds per capsule was found in natural populations of the south-eastern woodland species *Eucalyptus albens* (Burrows 1995) and five seeds per capsule in *E. microcarpa* (Vesk et al. in press).

There is a large range in the size of seeds across species, and due to the difficulty of distinguishing and sorting seed from chaff in smallest-seeded species, seed ‘viabilities’ are defined as the number of germinants per unit weight of seeds and chaff combined. The smallest-seeded species may have in excess of 15 000 seeds per 10g of capsule contents and the largest-seeded less than 100 seeds per 10g, but the majority of species have mean seed viabilities of between 1500 and 6000 per 10g (Boland et al. 1980). Within grassy woodlands, common box-barked species including *E. albens* (White Box), *E. melliodora* (Yellow Box), *E. microcarpa* (Grey Box), *E. bridgesiana* (Apple Box) and *E. polyanthemos* (Red Box) have mean seed viabilities of around 1400, 3630, 8500, 2500, and 5750 seeds per 10g respectively, while *E. blakelyi* (Blakely’s Red Gum) has on average 6870 viable seeds per 10g, and *E. macrorrhyncha* (Red Stringybark) 530 viable seeds per 10g (ATSC 2006). Viability can vary greatly across populations of the one species. Viabilities as low as 66 seeds per gram (Nolan pers. comm. to Windsor 1998) and as high as 476 seeds per gram (Turnbull & Doran 1987) have been found for *E. albens*.

Eucalypts have a mixed mating system; they are self-compatible but show preferential outcrossing. Multiple pre-dispersal barriers to selfing regulate the degree
of self-incompatibility (i.e. through abortion of capsules), but inbreeding depression is common and is expressed by a reduction in seed set or seed viability (compared to cross-pollinated flowers) or as reduced vigour and reproductive output of offspring. Selfing resulted in an 11 – 96 % reduction in seed set per capsule across thirteen *Eucalyptus* spp. (Potts & Wiltshire 1997).

In the field, tree isolation was associated with reduced seed set and viability in the widespread woodland tree *E. melliodora* (Burrows 2000) and in the endangered forest tree *E. benthamii* (Butcher *et al.* 2005), with around a 50% reduction in the mean number of seeds per capsule on isolated trees compared with trees in remnant populations for both species, and reductions in seed viabilities of up to 14% and 30% respectively, though estimates of capsule abortion were not made. In *E. benthamii*, the genetic diversity of the progeny from isolated trees was significantly reduced, which has implications for seedling vigour and viability of future generations (Butcher *et al.* 2005, Broadhurst & Young 2007). However, maintenance of or even increases in pollen-flow (out-crossing) with increasing fragmentation and isolation of *E. wandoo* remnants in Western Australia was attributed to changes in pollinator, that allowed for long distance pollen dispersal (Byrne *et al.* 2008).

There are few estimates of total seed crop per tree, with the majority of studies applying to commercial forestry species. Accepted estimates of the seed crop requirements to reach target forestry plantation densities of 5000 trees/ha are around 0.5 to 1.3 kg (Jacobs 1955), though yields of up to 13kg of seed have been reported from a single tree (Pryor 1959). Bassett *et al.* (2002) calculated seed crops of the south-eastern Australian forest tree *E. sieberi* to be between 27,600 and 138,000 viable seeds per tree. Seed production by *E. microcarpa* (Grey Box) in modified grassy woodlands ranged from around 1000 seeds per tree to more than 28 million, with environmental variables explaining only 26% of modeled variation (Vesk *et al.* in press).

Estimates of the seed crop size by hectare are most relevant to uniform forest stands or woodlands of known density, and as such are less applicable to heavily cleared agricultural landscapes where trees exist as isolated individuals or in remnants of variable density. In eucalypt forests, the canopy dominance of the tree is the most important determinant of seed production; dominant and co-dominant individuals contribute more than 90% of a population’s seed crop, with seed production greatly
suppressed in trees under canopy shading (Jacobs 1955). Seed production is greatest at maximum canopy spread, and trees will continue to produce seed in quantity as they age, while canopy size and health are maintained (Jacobs 1955). Seed crops of 1-2.3 million seeds/ha were estimated for the Western Australian tall open-forest species E. diversicolor (Loneragan 1979), while estimates of around 1 million to 8 million seeds/ha applied to mallee eucalypts in Victoria (Wellington & Noble 1985), around 30 million seeds/ha in E. wandoo woodlands of Western Australia (Burrows et al. 1990), and up to 40 million seeds/ha for woodlands in north-eastern Australia (Burrows and Burrows 1992). Seed production of E. microcorys varied from 52 000 to 580 000 viable seeds/ha/year over 3-4 years (van Loon 1966 cited in Florence 1996). Estimates of maximum seedfall near the dripline are more relevant to open woodlands; six months prior to a recruitment event in the northern tablelands of NSW, 100-200 seeds m-2 were found under E. caliginosa (Curtis 1990). Yates et al. (1994a) found that in a remnant woodland, seed fall under a canopy of the Western Australian gum E. salmonophloia was 233 seeds m2, while in gaps seed fall was 120 seeds m2 (Yates et al. 1994a). Due to widespread tree clearing and modification of the herbaceous layer associated with agricultural development, the abundance of adult, seed-producing trees is now likely to be highly confounded with grassland state in much of south-eastern Australia. Less intensively used grassland states such as native-grass dominated pastures typically are more likely to contain woodland remnants than broad-acre improved pastures, though there are sufficient exceptions such as borders with roadside vegetation and isolated paddock trees that justify an investigation of recruitment potential across multiple grassland states. Managers now have limited opportunity to intervene to maximise seed set, beyond ensuring that adult trees (such as isolated paddock trees) are not cleared.

2.7.6 Pre-dispersal seed predation

Seed predation by birds was observed within stands of E. sieberi, which were also linked to a peak in seedfall caused by spillage of seed during feeding (Bassett 2002). Predation by insects caused losses of up to 20% (0.6 seeds per capsule) in E. delegatensis (Boland & Martensz 1981 in Bassett 2002).
2.7.7 Time of seedfall

Following the maturation of seeds within the capsule, seedfall can only occur once the capsule has dried sufficiently to cause the opening of capsule valves. This usually begins in early summer, and will occur gradually over many months, but can occur very rapidly following fire or consecutive days of hot, dry weather (Gill 1997).

Capsules of *E. blakelyi* (Blakely’s Red Gum) generally mature in April/May, and seedfall may commence soon after, though it is usually promoted by warm, dry conditions (Curtis 1990). Seedfall continues to increase throughout the year with the drying of more capsules through spring, and a major peak in seedfall occurs in early to mid-Summer with a second, smaller peak occurring in the second summer after capsule maturation. By the second autumn, the majority of capsules will have fallen from the tree (Curtis 1990).

Similarly, *E. melliodora* shows two seedfall peaks with the highest in the first summer after capsule maturation, though capsules of this species may not mature until May/June and seedfall does not usually start until early Spring (Curtis 1990).

An intensive study of seedfall in *E. albens* in the Cowra region of NSW over two years showed commencement of seedfall in September, with a major peak in seedfall between December and January, and a much smaller peak in the following Summer (Semple 1997).

2.7.8 Dispersal mechanisms and spatial patterns of seedfall

Eucalypts seeds undergo passive dispersal by wind, and do not have specialised seed dispersal structures to aid dispersal (Cremer 1965). Consequently the majority of seed falls within a distance 1.5 times the height of the parent tree from the canopy edge, though dispersal distances could potentially be many times greater in high winds (Lawrence et al. 1998). In a controlled test of seed dispersal distance for 15 eucalypt species of differing seed weight, Cremer (1977) found that dispersal ranged from 20 to 50 m when dropped from 40 m height at a wind speed of 10 km/h.

As seed is not ejected from the capsules, any seed which remains in the capsule after it has fallen from the canopy is likely to rot or to be taken by predators, as it is unable to germinate within it.

Asymmetrical patterns of regeneration around *E. melliodora* and *E. albens* trees was found by Lawrence et al. (1998), where seedling recruits were more common on
the southern side of parent trees. This was attributed to more favourable topsoil moisture content and increased protection due to shading, though potential differences in seed fall due to aspect (and its effect on flower production and fertilisation) were not addressed. There were insufficient data to link seedfall data to wind data, though dry northerlies may have increased seedfall on the southern side of trees.

2.7.9 Summary

Seed dispersal greatly restricts the speed at which natural regeneration can be used to restore trees to large, cleared areas, since multiple recruitment events will be needed for recruits to move more than several hundred metres from the original seed-bearing tree, and this could potentially take several decades.

Woodland eucalypts are capable of producing a large number of seeds in some years, yet germination rates and survival are highly variable and potentially very low. My experimental methods were developed on the principle that more seeds improve the likelihood of recruitment. Hence I have chosen to use the highest possible sowing rate based on the seed available for purchase. By sowing eucalypt seed at rates several magnitudes larger than those found for natural seed fall by Curtis (1990) and ten times higher than sowing rates used by Clarke & Davison (2001), I hope to pick up relative changes in recruitment success that would not otherwise be apparent with few seeds and high levels of seedling mortality.
Chapter 1: Introduction
- Why is natural regeneration of woodland eucalypts important?
- Key research question: How does modification of the grass layer affect eucalypt recruitment?

Chapter 2: Literature review
- How have grassy woodlands been changed by land management?
- How do State and Transition models describe vegetation change?
- What are the main barriers to tree recruitment?
- How are vegetation changes caused by land management practices predicted to affect tree recruitment?
- How does seed supply vary in space and time due to natural processes and management influences?

Chapter 3: Assessing the potential for natural tree regeneration between grassland states - a landscape-scale germination trial
- How does eucalypt germination and survival differ between pasture states?
- Does the duration of livestock exclusion affect recruitment potential?

Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility
- How does soil nutrient enrichment influence tree recruitment in an exotic pasture?
  - Do eucalypts and exotic pasture species both respond positively to nutrient additions when competitors are excluded?
  - Does increased pasture growth reduce tree growth?

Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia
- What are the mechanisms limiting tree recruitment in high and low rainfall years?
- How do light, water and herbaceous biomass manipulations affect tree seedling germination, growth and survival?

Chapter 6: The effects of soil compaction on germination and early growth of Eucalyptus albens and an exotic annual grass
- Is eucalypt germination and growth inhibited in highly compacted soils?
- Is Eucalyptus albens more sensitive to soil compaction than a common grass weed, Vulpia myuros?

Chapter 7: Summary and synthesis
- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 3: Assessing the potential for natural tree regeneration between grassland states: a landscape-scale germination trial

3.1 Introduction

The lack of tree recruitment in the highly cleared agricultural regions of southern Australia has been identified as a major threat to woodland biodiversity. Many of the remaining trees that provide critical habitat for hollow-dependent woodland fauna pre-date European settlement and are approaching the end of their lives (Gibbons et al. 2008). Where seed supply is still sufficient, the primary barrier to recruitment across most of this landscape is considered to be continuous grazing (set-stocking) by livestock (Reid & Landsberg 2000, Saunders et al. 2003, Dorrough & Moxham 2005). However, reduction or exclusion of grazing for long periods does not always result in successful tree recruitment, as shown in sites that have been fenced for conservation purposes for up to a decade (Spooner & Briggs 2008). In these instances it is generally thought that tree recruitment is prevented by increased competition from exotic grasses and forbs (Yates & Hobbs 1997, Semple & Koen 2003, Dorrough & Moxham 2005).

Common trajectories of change to understorey composition in temperate grassy woodlands of south-eastern Australia have been described in a STM by McIntyre & Lavorel (2007; Fig. 3.1). Their model was developed from a review of floristic data from many agricultural and ecological studies, with the intention of creating a model that is generalisable to rural landscapes globally. It focuses on the key processes that drive transitions between states, being grazing, fertiliser application and cultivation combined with the sowing of exotic legumes and grasses. System state variables in their model are a combination of the level of soil fertility and degree of disturbance, rather than states determined a priori by vegetation structure or functional groups. Five broad states are identified: (1) reference grassland (now rare; Prober et al. 2002a), having low soil P and soil N; (2) native pasture, having low soil P and medium soil N; (3) fertilised pasture, having high soil P and N, but still low levels of soil disturbance; (4) sown pasture, having high soil P and N, and high soil disturbance; and (5) enriched pasture, having medium soil P and N, and little sward and soil disturbance from grazing. The shift from state (1) to state (4) represents a gradient of increasing
land use intensity, soil nutrient enrichment, and understorey modification, while state (5) is the outcome of removing livestock grazing from fertilised and sown pastures (McIntyre & Lavorel 2007). Recognition of the negative impact of heavy grazing on tree recruitment means that “fencing off” restoration areas to exclude livestock is common practice, so investigation of state (5) is particularly relevant to restoration efforts.

I used the STM framework developed by McIntyre & Lavorel (2007) as a basis for classifying understorey modification in a rural landscape, and used this classification to compare the recruitment potential of different grassland states.

The aim of this study was to investigate the impacts of land use history on eucalypt germination and survival following the removal of grazing using a landscape-scale germination trial in north-central Victoria.

I also tested whether the potential for regeneration is greater immediately after removal of grazing than after several years of “resting” from grazing, leading to biomass build-up.

I hypothesised that recruitment would be highest in the least disturbed grassland states (dominated by native pasture) and would decrease with increasing land use intensity, also reflecting increased understorey modification. I also predicted that recruitment would be higher immediately after the removal of grazing, than after several years of resting and biomass build-up.
Fig. 3.1: State and transition model of vegetation change in temperate eucalypt grassy woodlands of south-eastern Australia, describing five land use types. The states (numbered 1–5) are described in terms of their associations with grazing, soil disturbance and soil fertility. Circular arrows depict the management associated with the maintenance of the state and straight arrows describe the transitions between states (McIntyre & Lavorel 2007; Fig. 1).
3.2 Methods

3.2.1 Study region

The Goulburn-Broken catchment of north central Victoria (Fig. 3.2) was selected for the study because: (1) it contains a diversity of land uses and landforms (including grazing and cropping, slopes and plain; (2) it has a history of extensive tree clearing and consequently a need for re-vegetation to restore biodiversity and landscape function; (3) natural resource managers are encouraging natural regeneration as a potentially cost-effective method of landscape-scale re-vegetation through incentive programs such as ‘Bush Returns’ (where landholders are offered financial incentives to remove livestock grazing from designated regeneration areas); and (4) it is similar to much of the wheat-sheep belt in south-eastern Australia (formerly dominated by grassy woodlands) in its diversity of land uses.

In this region, grassy woodlands are dominated by the overstorey species *E. microcarpa* (Grey Box) and are now mostly restricted to crown land (e.g. reservations or roadside linear strips) or occur on private property as small remnants or isolated paddock trees. The predominant land use is livestock (sheep or cattle) production on grazed pastures, which include both “improved” pastures and “native” pastures - a term commonly used to describe pastures that have not been sown with exotic pasture species, but which are rarely free of exotic species. Cropping of winter-growing annuals also occurs on the plains in the region, most often in rotation with pastures.

3.2.2 Identification of common grassland states

A focus group of experts were engaged to identify and classify the most common land uses in the region and the dominant ground layer species that are associated with these land uses (reflecting alternative “Grassland States”). The focus group included three natural resource managers from the local Catchment Management Authority, two District Agronomists and one local ecologist, all of whom had an intimate knowledge of past and current land uses in the region.
Figure 3.2: Location of experimental plots within the north-central region of Victoria. Grassland state treatments are distinguished by symbol colour as follows; green = Austrodanthonia/Austrostipa, red = Bromus/Hordeum, black = Phalaris/Dactylis, orange = Lolium, blue = Medicago. The fencing treatment is shown by solid (not fenced) and hollow (fenced) symbols.

Three major “land uses” were identified in the Goulburn-Broken region. These were: (1) areas under continuous cropping; (2) areas recently cultivated for pasture establishment (“Cultivated”); and (3) areas not cultivated within the last 20 years (“Uncultivated”).

As areas under continuous cropping are unlikely to be set aside for tree regeneration under current social and economic conditions, I excluded land that was currently being cropped from the study in order to concentrate on areas where regeneration is more likely to occur and where it is more likely to be viewed as acceptable by landowners.

Plots included in the “Cultivated” grassland states were restricted to paddocks that (a) had been cultivated at least once in the last 5 years (i.e. normally subject to continuous cropping or less than 5 years into a pasture phase if farmed with cropping
and pasture phases) and (b) were still in production (must have been cropped or grazed in the previous year).

Plots included in the “Un-cultivated” grassland states were restricted to paddocks that (a) had never been cultivated (noting this is rare and hard to prove) or if pasture had been sown, had not been cultivated in the previous 20 years; (b) were still in production (must have been subject to continuous/rotational grazing in the previous year); and (c) had not been fertilised or sprayed in the last 5 years, in order to restrict the impacts of elevated soil nutrient to long term effects, rather than short term effects. Further classification of these land use treatments into alternative grassland states was based on divergence in composition due to fertiliser history and pasture species sown (Table 3.1). Thus the grassland states selected for investigation in this experiment were defined by their management history, but identified using dominant pasture species as determined by local expertise.

The typical dominant species identified for each land use, representing “Grassland States” of different management history, are shown in Table 3.1.

### 3.2.3 Experimental design

Each of the five grassland states identified (dominated by (i) *Medicago*, (ii) *Lolium*, (iii) *Phalaris/Dactylis*, (iv) *Bromus/Hordeum*, (v) *Austrodanthonia/Austrostipa*) were further divided into two fencing treatments; those being plots which were currently grazed by livestock (“Not fenced” plots), and plots that had already been fenced to exclude livestock for between 3-10 years for conservation purposes (“Fenced” plots). Both of these “fencing” treatments were then fenced at the start of the experiment to exclude livestock, replicating the actions typically taken by managers when trying to encourage natural regeneration. Landholders provided anecdotal evidence about the management history of each plot, so I was confident “Not fenced” and “Fenced” plots of the same grassland state had matching management and species dominance prior to livestock exclusion by fencing. Additional fencing of “Fenced” plots within areas where livestock were already excluded was necessary to ensure that the impacts of kangaroo and rabbit/hare grazing were similar across all treatments. It should be noted that both “Not fenced” and “Fenced” plots were fenced for the duration of the experiment.
Table 3.1: Common grassland states found in the Goulburn-Broken catchment of Victoria as defined by land use history and dominant herbaceous species. States increase in land use intensity and vegetation modification from top to bottom. Column 3 identifies the equivalent Grassland State as defined by McIntyre & Lavorel (2007).

<table>
<thead>
<tr>
<th>Grassland State</th>
<th>Representative Dominant Species</th>
<th>Lifeform/origin of dominant species</th>
<th>Landuse</th>
<th>Typical management history</th>
<th>Associated pasture and soil characteristics</th>
<th>Plots fenced &gt; 3 years prior to experiment (n)</th>
<th>Plots fenced at sowing (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Pasture (perennial)</td>
<td>Austrodanthonia spp. / Austrostipa spp. (Wallaby Grasses / Spear Grasses)</td>
<td>Medium to tall perennial native tussock-forming grasses (C₃ photosynthetic pathway)</td>
<td>“Un-Cultivated”</td>
<td>Not recently cultivated or fertilised, though may have a long grazing history.</td>
<td>Low to moderate biomass, associated with low-moderate nutrient soils.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Fertilised Pasture (annual)</td>
<td>Bromus molliformis / Hordeum leporinum (Soft Brome / Barley Grass)</td>
<td>Short to medium annual exotic grasses (C₃ photosynthetic pathway).</td>
<td>Not recently cultivated, but history of heavy grazing and fertiliser additions</td>
<td>Often associated with high nitrogen loads due to stock camping, but can persist in low nutrient, compacted soils, where other species cannot.</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Fertilised Pasture – (perennial)/ Enriched Grassland</td>
<td>Phalaris aquatic / Dactylis glomeratum (Phalaris / Cocksfoot)</td>
<td>Tall perennial exotic grasses (C₃ photosynthetic pathway).</td>
<td>Not recently cultivated, history of regular fertiliser additions.</td>
<td>High biomass; requires moderate to high soil P, and moisture</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Sown Pasture (annual)</td>
<td>Lolium rigidum (Annual Ryegrass)</td>
<td>Medium height annual exotic grass (C₃ photosynthetic pathway).</td>
<td>“Cultivated”</td>
<td>Recent history of cultivation and fertiliser use, not sown to ‘improved pasture species’ or sown, but not maintained.</td>
<td>Will persist in most soils, but thrives in soils of higher nutrient.</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sown Pasture (perennial)</td>
<td>Medicago sativa (Lucerne)</td>
<td>Medium to tall perennial exotic forb.</td>
<td>Cultivated for pasture establishment, herbicide removal of annual grasses. Commonly top-sprayed to remove grasses.</td>
<td>High water use pasture grown on more fertile, deeper soils of valley floor and lower slopes. N-fixing.</td>
<td>0 (not applicable to this state)</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

**Total** | 21 | 32
Fig. 3.3: A cultivated, sown pasture state dominated by the exotic annual forb *Medicago sativa* (Lucerne – “L”).

Fig. 3.4: A cultivated, sown pasture state dominated by the exotic annual grass *Lolium rigidum* (Annual Ryegrass = “AR”).
Fig. 3.5: An un-cultivated, fertilised pasture state, dominated by the exotic perennial grasses *Phalaris aquatica* (Phalaris) and *Dactylis glomeratum* (Cocksfoot; “PD”). Some *Vulpia myuros* and *Trifolium subterraneum* is also present in this pasture.

Fig. 3.6: An un-cultivated, unfertilised native pasture state dominated by the native perennial grasses *Austrostipa* spp. and *Austrodanthonia* spp. (“AA”).
**Fig. 3.7:** An un-cultivated, frequently disturbed fertilised pasture, dominated by the exotic annual grasses *Bromus molliformis* (Soft Brome) and *Hordeum leporinum* (Barley Grass; “BB”). The yellow-flowered *Arctotheca calendula* (Capeweed) which was present at most plots is visible here.

**Fig. 3.8:** Fence-line contrast of Phalaris dominated pasture state. The area to the left of the fence is regularly grazed by cattle (“Not fenced”), the area to the right has been freed from grazing for more than five years (“Fenced”), and shows a large build up of standing biomass, typical of an “Enriched Grassland” state.
I aimed to locate six independent replicate plots for each of the five grassland states by fencing treatment combinations, giving a total of $5 \times 2 \times 6 = 60$ plots. Logistic restrictions and rarity of some treatments meant that I fell short of these targets, and there were less “Fenced” plots than “Not fenced” plots. The experiment proceeded with an unbalanced design comprising 53 plots (Table 3.1). These were spread across 33 independently-managed locations (i.e. on 33 different farms). “Fenced” and “Not fenced” plots were often adjacent (on the same farm), sharing their management histories prior to the time when fenced to exclude livestock, while replicates of the different grassland states were always located on different properties and separated by at least 100m (Fig. 3.2).

![Diagram of experimental setup]

Fig. 3.9: Layout of E. albens and E. microcarpa germination subplots within experimental plots at each of 53 plots.

Each of the 53 plots was 1.8m by 1.8m in area, and was fenced from livestock grazing using 1.2 m high galvanized steel mesh panels. Within each plot, 16 quadrats were established, 15cm by 15cm in area, in which eucalypt seed was sown (Fig. 3.9); eight for each of two common woodland eucalypts, Eucalyptus albens and Eucalyptus microcarpa. For each of the species 4 plots were sown in autumn and 4 in spring. These species are widely distributed across the grassy woodland belt in New South Wales and Victoria and were chosen to be broadly representative of woodland
eucalypts. Quadrats were placed at a regular spacing of 20cm apart to reduce the likelihood of seed movement between quadrats and to cover variation in the sward at the local scale. Species treatments assigned to quadrats were aligned in rows for ease of assessment, since within-plot gradients were considered to be unlikely.

Quadrats were sown with 1.5 g of *Eucalyptus albens* seed (and chaff) or 1g of *Eucalyptus microcarpa* seed in spring (late September 2005), and again in new quadrats adjacent to spring sowing (Fig. 3.9) after the late autumn break of rain (June) the following year. Seeds were evenly scattered within each quadrat from a height just above the top of the grass sward without disturbance to the soil surface or vegetation to imitate natural seedfall. Germination tests in the laboratory showed the quantities of seed sown were equivalent to roughly 160 and 500 viable seeds per quadrat (about 640 and 2000 seeds per plot) for *E. albens* and *E. microcarpa* respectively.

*Eucalyptus albens* has the larger and heavier seed of the two species, with around 250 viable seeds $g^{-1}$ (of seed and chaff combined) compared with 800 or more seeds $g^{-1}$ for *E. microcarpa* (Boland *et al.* 1980), though differences in average and maximum seed production between the species are not well known. Due to the very small size of eucalypt seeds and the similarity of their appearance to infertile ovules (chaff), convention dictates that the ‘viability’ of eucalypt seedlots is defined as the number of seeds to germinate in test conditions. I assessed the viability of the Grey Box (*E. microcarpa*) seedlot used in this experiment using standard test conditions (ATSC 2009). Seeds were placed on filter paper over moist vermiculite at 25 degree Celsius, and a final germination count was carried out at 14 days. My assessment indicated a viability of 8000 viable seeds per 10g, typical of *E. microcarpa* seedlots (ATSC 2009).

The quantity of seed sown in each quadrat was limited by the availability of seed for purchase in the case of *E. albens* and by the physical volume of seed (and chaff) that could be spread across a 15cm square quadrat without creating a visible layer of chaff for both species. Germination typically occurs within seven to 14 days after sowing in ideal conditions (ATSC 2009). In this experiment, germination counts were carried out between eight and ten weeks after sowing and survival checked at five months after sowing. This was due to the logistical constraints of travel between sites and to allow seeds to respond to a large rain event after sowing. Since seeds do not maintain viability beyond a few months once they have fallen from the canopy (see
section 2.4.2), no further germination counts were carried out after this time. Viable seeds that failed to germinate were not distinguished from those that germinated and failed to survive, as neither group of seeds would be expected to contribute to recruitment in future years (Florentine & Fox 2002).

Further monitoring of seedling survival was not undertaken, as no seedlings survived beyond five months in either the spring or autumn sowing, reflecting the severe drought.

Three samples of above-ground herbaceous biomass were collected from each plot in late spring by cutting all shoot material within a 30cm square at three randomly chosen points in the other half of the fenced plot from the quadrats. Live biomass was separated from litter at the time of collection, but litter was not used in the final assessment as the difficulty of separating sticks and heavy materials from other litter bulk rendered the weight of litter samples an inadequate measure of litter effects on seedlings. These samples were dried at 75°C for 3 days prior to weighing, and were pooled to obtain plot averages. Ground cover was assessed for the eight within-plot quadrats. Light intensity below grass swards was not measured for logistic reasons.

Three soil samples per plot were collected at the same time at the points where above-ground grass biomass had been removed, using metal 20mm diameter metal cores driven to 10cm depth. Soil samples were kept refrigerated prior to being sent to the Environmental and Analytical Laboratories (Charles Sturt University, Wagga Wagga) for analysis of extractable phosphorus and nitrate. Extractable P was assessed by the Colwell method, using a 1:100 ratio of soil: 0.5M NaHCO$_3$ mixed for 16 hours, filtered/centrifuged, with colourimetric finish. Nitrate-N (NO$_3$-N) was extracted using 1:10 ratio of soil:2M KCl, mixed for 1 hour, filtered/centrifuged and then analysed on a segmented flow auto-analyser.

3.2.4 Analysis

Germination counts for each species were pooled to plot level, representing the sum of the four quadrats and count data were adjusted to account for differences in sowing rates and seed viability prior to analysis. *Eucalyptus albens* count data were multiplied by three and *E. microcarpa* count data were unchanged.
The relationship of “Grassland State” (fixed) and “Fencing” (fixed) to frequency of eucalypt germinants of each species was fitted to a generalized linear model of poisson distribution (log-link). As data were overdispersed, the model was re-run using quasi errors (log-link, mean-squared variance) and both chi-square and F-tests to check model significance within an analysis of deviance; the latter using an empirical scale parameter, rather than assuming a scale parameter of 1.0 (Crawley 2002). Paired-plot analysis was not carried out because the unbalanced nature of the design. This resulted in a reduced power to detect treatment differences, hence a conservative analysis.

The effects of pasture biomass and soil fertility indicators on germination were tested by including them separately (one at a time) as covariates in the model alongside treatment effects. Linear convergence could not be achieved in the model when multiple covariates were included due to overdispersion of the data.

Differences in pasture biomass and soil fertility indicators between pasture states and fencing treatments were tested within an ANOVA. Bonferroni pair-wise comparisons of treatments were used where significant differences were identified.

### 3.3 Results

No germinants were recorded from the spring sowing, either in the year of sowing or during observations in the following year.

A total of 931 germinants (671 E. albens and 260 E. microcarpa were recorded from the autumn sowing. Germination exhibited a poisson distribution; the number of plots with zero counts greatly exceeded those where germination occurred (Fig. 3.10).

Of the 53 experimental plots in the study spread across 33 independently-managed locations, 19 plots (36%) and 9 locations (27%) exhibited no germinants at all, while 18 plots (34%) and 13 locations (39%) recorded less than 10 germinants (i.e. germination of less than 0.25 %).

Mean germination across all grassland states was low (17.6 germinants/plot). Median values for each plot (species combined) ranged from zero for the Phalaris/Dactylis state to 20 for the Bromus/Hordeum grassland state.

None of the 931 germinants from the autumn sowing survived to the following summer.
**Figure 3.10:** Frequency distribution histogram of the total germination observed in plots across all grassland states and fencing treatments. Bars show germination classes 0-9, 10-19, 20-29, etc. “Germination counts per plot” reflects the sum of *E. albens* and *E. microcarpa* germination. A majority of plots showed no germination.

The analysis of germination showed that “Grassland State” was a significant explanatory variable when modeled using quasi-likelihood to account for data overdispersion (*F* = 5.66, *P* < 0.001), while “Fencing” was not (*F* = 0.52, *P* > 0.05; Table 3.2). While mean values and standard errors are not very useful for comparing treatments within poisson-distributed data, Fig. 3.11 shows that median levels of germination were highest in the “Un-cultivated” *Bromus/Hordeum* annual-grass dominated pasture, followed by the “Un-cultivated” *Austrodanthonia/Austrostipa* native perennial dominated pasture. Germination was very low in all other states. There was no significant difference in eucalypt germination between “Fenced” plots (mean 14 seedlings/plot, 0.35% germination) and “Not fenced” plots (9.4 seedlings/plot, 0.24% germination, Fig. 3.12), and no interaction between “Grassland State” and “Grazing” was found (Table 3.2).
Table 3.2: Analysis of deviance for generalised linear model of plot germination by species with quasi-likelihood family of errors (log-link, variance = mean squared, dispersion parameter = 4.7).

<table>
<thead>
<tr>
<th>Model</th>
<th>d.f.</th>
<th>Deviance</th>
<th>Residual d.f.</th>
<th>Residual Deviance</th>
<th>F value</th>
<th>P(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>105</td>
<td>386.6</td>
<td>104</td>
<td>290.9</td>
<td>20.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>95.8</td>
<td>104</td>
<td>290.9</td>
<td>20.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grassland State</td>
<td>4</td>
<td>106.1</td>
<td>100</td>
<td>184.7</td>
<td>5.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fencing</td>
<td>1</td>
<td>2.5</td>
<td>99</td>
<td>182.3</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>Species*Grassland State</td>
<td>4</td>
<td>24.0</td>
<td>95</td>
<td>158.3</td>
<td>1.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Grassland State*Fencing</td>
<td>3</td>
<td>24.5</td>
<td>92</td>
<td>134.0</td>
<td>1.74</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Number of Fisher scoring iterations = 1172.

Fig. 3.11: Box and whisker plot of plot total germination (E. albens and E. microcarpa combined) by grassland state, showing median values (white bar), quartiles and outliers. Bar labels for grassland states are as follows: L= Lolium rigidum - Sown pasture (annual); BH= Bromus molliformis/Hordeum leporinum - Fertilised pasture (annual); AA= Austrodanthonia spp./Austrostipa spp. - Native pasture; M=Medicago sativa - Sown pasture (perennial); PD = Phalaris aquatica/Dactylis glomeratum - Fertilised pasture (perennial)/Enriched grassland. “Grassland State” was a significant factor in the analysis of deviance ($F = 5.66$, $P < 0.001$).
**Fig. 3.12:** Box and whisker plot of plot level germination (*E. albens* and *E. microcarpa* counts combined) by fencing treatment, showing median values (white bar), quartiles and outliers. (F = “Fenced” (fenced for 3-10 years), G = “Not fenced” (grazed until the start of the experiment)). “Fencing” was not a significant factor in the analysis of deviance (F= 0.52, P >0.05).

Species differences were significant (F= 20.5, P <0.001) and explained more of the model deviance than “Grassland State” (Table 3.2), though “Species” did not interact with “Grassland State” or “Fencing” treatments. The larger-seeded *Eucalyptus albens* showed consistently higher germination rates than *E. microcarpa* (Fig. 3.13).

**Fig. 3.13:** Box and whisker plot of plot level germination by tree species, showing median values (white bar), quartiles and outliers (straight bars). ‘Species’ was a significant factor in the analysis of deviance (F = 20.44, P <0.001).
The influence of “Grassland State” was not explained by plot above-ground herbaceous biomass, which was not a significant factor ($F = 1.95, P >0.05$), and was consequently removed from the model in Table 3.2.

There was no significant difference in above-ground pasture biomass between Grassland States ($F = 2.3, P > 0.05$), but “Fenced” plots had significantly higher biomass than “Not fenced” plots ($F = 10.8, P < 0.01$, Table 3.3) and there was a Grassland State*Fencing treatment interaction ($F = 3.9, P <0.05$) reflecting the increase in biomass in the “Fenced” Phalaris state (Table 3.3).

Soil characteristics did not differ significantly between grassland states (Table 3.3), though a Grassland State*Fencing interaction was found for nitrate and extractable P. This reflected the higher levels of nitrate found in the long-fenced Bromus/Hordeum state, and the higher levels of extractable P found in the long-fenced Bromus/Hordeum state and long-fenced Phalaris state compared with other states. Although soil nitrate was highly variable, neither soil nitrate nor other measured soil characteristics were significant when included as covariates in the analysis of deviance for germination (nitrate, $F = 0.68, P >0.05$; total N, $F = 1.1, P >0.05$; phosphorus, $F = 0.91, P >0.05$). Consequently, these variables were removed from the model in Table 3.2.

Table 3.3: Means of selected soil characteristics across the five ‘Grassland States’. Values within each column that do not share the same letter are significantly different ($P <0.05$).

<table>
<thead>
<tr>
<th>Grassland State (and Representative Dominant Species)</th>
<th>Fencing treatment</th>
<th>Soil nitrate-N (mg/kg)</th>
<th>Soil extractable P (mg/kg)</th>
<th>pH</th>
<th>Soil Total N (mg/kg)</th>
<th>Above-ground biomass (g/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sown pasture – perennial (Lucerne)</td>
<td>Not fenced</td>
<td>39.8$^{ab}$</td>
<td>349.0$^{cd}$</td>
<td>5.6$^e$</td>
<td>2083$^{f}$</td>
<td>28.2$^g$</td>
</tr>
<tr>
<td></td>
<td>Fenced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sown pasture - annual (Annual Ryegrass)</td>
<td>Not fenced</td>
<td>13.1$^a$</td>
<td>226.0$^c$</td>
<td>5.3$^e$</td>
<td>1914$^{f}$</td>
<td>19.6$^g$</td>
</tr>
<tr>
<td></td>
<td>Fenced</td>
<td>15.7$^{ab}$</td>
<td>288.3$^{cd}$</td>
<td>5.5$^e$</td>
<td>2370$^{f}$</td>
<td>61.7$^g$</td>
</tr>
<tr>
<td>Fertilised pasture/Enriched Grassland (Phalaris/Cocksfoot)</td>
<td>Not fenced</td>
<td>28.3$^{ab}$</td>
<td>390.4$^{c}$</td>
<td>5.4$^e$</td>
<td>3224$^{f}$</td>
<td>28.6$^g$</td>
</tr>
<tr>
<td></td>
<td>Fenced</td>
<td>27.8$^{ab}$</td>
<td>399.3$^{cd}$</td>
<td>5.3$^e$</td>
<td>3105$^{f}$</td>
<td>147.4$^h$</td>
</tr>
<tr>
<td>Fertilised pasture - annual (Brome/Barley Grasses)</td>
<td>Not fenced</td>
<td>26.8$^{ab}$</td>
<td>345.0$^{cd}$</td>
<td>5.6$^e$</td>
<td>2391$^{f}$</td>
<td>30.5$^g$</td>
</tr>
<tr>
<td></td>
<td>Fenced</td>
<td>49.8$^{b}$</td>
<td>426.7$^{d}$</td>
<td>5.6$^e$</td>
<td>3581$^{f}$</td>
<td>49.0$^g$</td>
</tr>
<tr>
<td>Native pasture (Wallaby/Spear Grasses)</td>
<td>Not fenced</td>
<td>12.6$^a$</td>
<td>333.0$^{cd}$</td>
<td>5.7$^e$</td>
<td>2313$^{f}$</td>
<td>24.2$^g$</td>
</tr>
<tr>
<td></td>
<td>Fenced</td>
<td>24.5$^{ab}$</td>
<td>294.7$^{cd}$</td>
<td>5.5$^e$</td>
<td>3247$^{f}$</td>
<td>27.4$^g$</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27.5</td>
<td>343.1</td>
<td>5.5</td>
<td>2712$^{f}$</td>
<td>59.6$^g$</td>
</tr>
</tbody>
</table>
3.4 Discussion

Despite the limitations imposed on the interpretation of results by extremely low levels of overall germination, it was important to describe outcomes of the experiment where assertions could be made. The above results demonstrated that germination can occur in highly modified, exotic-grass dominated grassland states, albeit at very low rates. The finding that germination was highest in the un-cultivated, fertilised pasture state dominated by *Bromus* and *Hordeum* was unexpected as I had hypothesized that the un-cultivated, unfertilised native pasture would support the highest levels of recruitment. The *Bromus/Hordeum* pasture state had almost 100% ground cover, with a very high density of grass seedlings. In this grassland state, many *Eucalyptus* seedlings were observed growing on decaying annual grass litter, and it was possible there was sufficient moisture in the dense swards to stimulate germination and support germinants throughout the winter and early spring, and which would have sheltered seedlings from extremes in temperatures (Facelli *et al.* 1999).

However, as they did not have soil contact, it was not surprising that all seedlings in the *Bromus/Hordeum* perished as the litter dried in summer. This was also the fate of seedlings in other states.

While a layer of grass litter (from either native or exotic species) can improve the germination niche of *Eucalyptus* spp. seeds by providing temporary protection from soil surface drying (Facelli *et al.* 1999), litter is also likely to substantially reduce the proportion of the seed crop that reaches the soil surface, particularly if litter is moist at the time of seed-fall (Facelli *et al.* 1999). In another study, seed predation by ants was higher in well-rested sites compared to newly disturbed sites (Mittelbach & Gross 1984) – a somewhat counter-intuitive finding, so overall, any beneficial effects of litter on recruitment are likely to be restricted to improved water relations. Since no seedlings survived in our experiment, I was not able to test whether favourable germination niches corresponded to favourable sites for seedling survival and growth.

Another surprising finding was the ability of eucalypt seeds to germinate amongst very dense, high biomass stands of *Phalaris aquatica* typical of ‘Enriched Grasslands’, although, as for the other grassland states, no seedlings survived through to the first summer. Although this grassland state had the lowest germination, the presence of some germinants showed that highly restricted light levels are unlikely to be a total barrier to germination if sufficient moisture is present.
That germination percentages of *E. albens* were significantly higher than *E. microcarpa* was not surprising, given the larger seed size of *E. albens*. Whilst I attempted to correct for differences in sowing rates, quantities of seed from seedfall varies greatly between species due to phylogenically determined differences in seed size and a tradeoff in size and quantity of seed produced (Venable 1992; Vesk pers. comm.). Consequently the inferior germination rate of *E. microcarpa* in this experiment may be offset by far greater seed production under natural conditions. While seedfall research is now being undertaken for several woodland eucalypt species in north-central Victoria – including *E. microcarpa* (Vesk, pers. comm.) – comparative seedfall data are not available for the two study species.

While fencing of regeneration areas to remove the impacts of livestock on seedlings is commonly the first step in restoration programs, the biomass build-up associated with fenced sites might be expected to reduce seed-soil contact. However, this experiment showed neither positive nor negative impacts of fencing. Fencing was expected to improve germination rates and the chances of establishment by reducing compaction and correlated indicators of soil health, such as permeability, organic matter and moisture holding capacity (Greenwood & McKenzie 2001, Wilson et al. 2008), though improvements in soil organic carbon content and infiltration may take decades rather than years (Webb et al. 1983). In addition to the soil properties listed in table 3.3, I speculate that soil moisture holding capacity may have explained variation in herbaceous biomass and eucalypt germination between grassland states, though this was not assessed at experimental sites.

In the following section of the discussion I discuss the likely causes of the extremely low germination rates in this experiment and observed differences between grassland states. It should be noted that this is speculative and based on observations from the experiment, since rainfall data was not able to be collected for each of the large number of experimental sites. The lack of germination from the spring sowing and the early death of germinants from the autumn sowing were attributed to the prolonged drought, as seedling desiccation was observed and there was no sign of predation, disease or physical disturbance which are other possible causes of death. The total annual rainfall at the nearest weather station with complete data records in the year of the experiment – Benalla (36.55°S, 145.97°E) – was 242mm; only 37% of the long term annual average. Monthly rainfall in the six months following sowing
from June to November at Benalla was 34mm, 63mm, 16mm, 20mm, 0.4mm and 7.6 mm; equivalent to 46%, 87%, 22%, 32%, <1% and 16% of long term monthly averages (Fig. 3.14).

Logistical constraints prevented the use and checking of rain gauges at the experimental plots. Anecdotal evidence from landholders suggested that there was significant variation in rainfall not only across the study region, but also between plots several hundred metres apart. I speculate that between-plot variation in rainfall would explain much of the variation in germination not explained by grassland state. Unfortunately I do not have the necessary plot-level rainfall data to test this.

Fig. 3.14: Monthly rainfall at Benalla during the year of the experiment and long term monthly averages and medians (BOM 2009). Missing data from November 2006 was due to opening of new station at Benalla airport, which measured 7.6 mm for November and 1.8mm in December.

My experiment did not set out to test the effects of inter-annual climate variability on eucalypt recruitment – which is thought to be a key driver of recruitment events (Curtis 1990, Watson et al. 1997, Fensham et al. 2005) – but it did highlight that climate had an over-riding influence on recruitment in the year of our experiment. This in itself does not undermine the value of comparing recruitment across grassland states; rather it suggests that, in at least some grassland states, potential differences may only be observed after severe moisture limitations have been overcome. The importance of high rainfall to tree recruitment and the observation of differences in recruitment response between grassland states are further investigated in Chapter 5.
Despite the death of all seedlings in their first year, interpretation of germination success is useful, since germination rates influence the probability of seedling establishment in years of favourable rainfall, or may offset other impacts (e.g. Beckage & Clark 2003). However, the reverse was shown by Smit et al. (2006) where the germination niche did not correspond with increased seedling survival, so caution in the interpretation of these findings is warranted. The finding that a total of 931 (0.67%) of the almost 138000 viable seeds sown in this experiment germinated demonstrates the very low probabilities of seedling establishment under harsh conditions. This compares with germination rates of up to 10% and 15% for surface sown woodland eucalypt seeds (E. melliodora and E. blakelyi) in the field under favourable conditions (Clarke 2001).

‘Grassland states’ – as categorised by dominant species (c.f. ‘transitions’ between states driven by history of grazing, cultivation, establishment of pasture species etc.) – are in most instances confounded with landscape and soil characteristics (Dorrough & Moxham 2005, McIntyre & Lavorel 2007). Consequently it is difficult to separate the effects of community composition and the physical structure of grassland states on tree recruitment from the effects of other characteristics such as soil properties that are closely tied to land use and resulting grassland states. Soil compaction was assessed for some of the experimental sites in this study, but results were not presented here as the data set was incomplete and inadequate to enable a conclusive comparison between states. Further investigation of the possible effects of soil compaction on tree recruitment from seed is investigated in Chapter 6.

A herbicide and sward removal treatment would aid the differentiation of soil and sward impacts on recruitment. Such a treatment was not logistically feasible in this experiment. This is further investigated in controlled plot trials (chapters 4 and 5) where I use irrigation to lessen the impacts of climatic extremes.

In summary, this experiment showed that if seed supply is sufficient, germination of eucalypt seeds is possible in grassland states dominated by exotic annual species. However, the death of all seedlings in all grassland states before summer suggests that the influence of climate on tree recruitment overrides other factors.
Chapter 1: Introduction
- Why is natural regeneration of woodland eucalypts important?
- Key research question: How does modification of the grass layer affect eucalypt recruitment?

Chapter 2: Literature review
- How have grassy woodlands been changed by land management?
- How do State and Transition models describe vegetation change?
- What are the main barriers to tree recruitment?
- How are vegetation changes caused by land management practices predicted to affect tree recruitment?
- How does seed supply vary in space and time due to natural processes and management influences?

Chapter 3: Assessing the potential for natural tree regeneration between grassland states - a landscape-scale germination trial
- How does eucalypt germination and survival differ between pasture states?
- Does the duration of livestock exclusion affect recruitment potential?

Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility
- How does soil nutrient enrichment influence tree recruitment in an exotic pasture?
  - Do eucalypts and exotic pasture species both respond positively to nutrient additions when competitors are excluded?
  - Does increased pasture growth reduce tree growth?

Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia
- What are the mechanisms limiting tree recruitment in high and low rainfall years?
- How do light, water and herbaceous biomass manipulations affect tree seedling germination, growth and survival?

Chapter 6: The effects of soil compaction on germination and early growth of Eucalyptus albens and an exotic annual grass
- Is eucalypt germination and growth inhibited in highly compacted soils?
- Is Eucalyptus albens more sensitive to soil compaction than a common grass weed, Vulpia myuros?

Chapter 7: Summary and synthesis
- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility

4.1 Introduction

In grassy woodlands, savannas and other woody-herbaceous ecosystems, the mechanisms of tree-grass co-existence continue to be debated (e.g. Callaway & Walker 1997, Holmgren et al. 1997, House et al. 2003, Sankaran et al. 2004). In general, competition from the herbaceous layer plays a major role in regulating tree recruitment. Many studies have found that grasses and forbs compete heavily with tree seedlings for water, light and nutrients, and that their presence is a major cause of high tree seedling mortality (Fensham & Kirkpatrick 1992, Davis et al. 1999, Smit & Olff 1998, Gordon & Rice 2000, Rey Benayas et al. 2003, Davis et al. 2005).

In Australian grassy woodlands, competitive interactions have changed markedly since European settlement, and the persistence of trees is threatened by a lack of tree recruitment (Hobbs & Atkins 1991, Yates et al. 2000a, Dorrough & Moxham 2005). There has been a shift towards dominance by exotic annuals – which generally have faster growth rates than native species (Leishman et al. 2007) – and increased herbaceous biomass as a result of clearing, livestock grazing and fertiliser use (Whalley et al. 1978, Kirkpatrick 1994, Pettit et al. 1995, Dorrough et al. 2006). Soil enrichment promotes exotic plant dominance (e.g. McIntyre & Lavorel 1994, Prober et al. 2002, Chalmers et al. 2005), and nitrate and phosphorus are key determinants of exotic grass and forb biomass in degraded grassy woodlands (Prober et al. 2002, Prober et al. 2005, Dorrough et al. 2006).

Nutrient enrichment has been demonstrated to have a negative impact on tree recruitment where tree seedlings are competing with the herbaceous layer for limited resources (e.g. Allcock 2002, Rey Benayas et al. 2003, Chalmers et al. 2005, Davis et al. 2005). However, in the absence of competition from understorey species, seedlings of a range of trees benefit from soil nitrogen (N) and phosphorus (P) enrichment. These include conifers (DeLucia et al. 1989, Myrold et al. 1989), alpine willow (Bowman & Conant 1994), mixed temperate hardwood (Magill et al. 1997), tropical montane rainforest (Tanner et al. 1998) and boreal forest trees (Högberg et al. 2006).
Soil nutrient enrichment has been shown to increase growth of forest eucalypts in a range of soil types in Australia and elsewhere. Growth responses are primarily to nitrogen (Moore & Keraitis 1971, Cromer & Jarvis 1990, Kirschbaum et al. 1992), phosphorus (Kirschbaum et al. 1992), or both in interaction (Halsall et al. 1983, Fabião et al. 1995); the main effects being increased shoot to root ratio, specific leaf area and photosynthetic rate (Stoneman 1994). Improved soil nutrition was also shown to reduce seedling mortality of *Eucalyptus marginata* seedlings by about 30% (Stoneman et al. 1993). The preferred form of nitrogen supply is also important and differs between species, with nitrate-nitrogen producing a stronger response than ammonium-nitrogen in the woodland eucalypt *Eucalyptus albens* (Moore & Keraitis 1971), but ammonium-nitrogen the preferred source of N uptake by the Tasmanian forest eucalypt *Eucalyptus nitens* (Garnett et al. 2003).

As herbaceous annual competitors also respond strongly to elevated soil nitrogen, carbon additions to soil in the form of sucrose (sugar), sawdust or activated-carbon have been used to reduce soil nitrate in order to restrict the growth of nitrophilic annuals in Europe (Eschen et al. 2007, Szili-Kovács et al. 2007); America (Morghan & Seastedt 1999, Blumenthal et al. 2003, Averett et al. 2004, Kulmatiski & Beard 2006); and Australia (Prober et al. 2005). Carbon addition enhances microbial processes that can reduce the autumn peak in soil nitrate (Cookson et al. 2006). The indirect effects of carbon addition on the establishment and growth of tree seedlings within annual pastures have not previously been investigated.

As growth of both herbaceous (Wilson & Simpson 1993) and woody species (Stoneman 1994) are positively correlated with soil nutrient enrichment, my working hypothesis is that in grassy woodlands, the interaction of these responses results in an ecological tradeoff: the existence of a point along a gradient of soil fertility at which the benefit to eucalypts of improved nutrition is balanced by the cost of increased competition from herbaceous species. Embedded in this resource-competition tradeoff hypothesis are several questions which I wished to test for woodland eucalypts in field conditions. At the broadest level, I wished to investigate whether increased soil fertility due to fertiliser addition poses a threat to *Eucalyptus* spp. recruitment and early growth in Australian temperate grassy woodlands. More specifically I asked:
(1) Do *Eucalyptus* spp. and exotic pasture grasses both show a positive growth response to soil nutrient additions when competitors are excluded?

(2) Does an increase in growth of exotic grasses lead to a negative growth response on *Eucalyptus* spp. seedlings?

### 4.2 Methods

#### 4.2.1 Experimental design

A factorial plot experiment was established at Albury NSW (160 a.s.l., 36°05', 146° 55', ~800mm mean annual rainfall) on a yellow kandosol soil. The experimental site has a long history of grazing by sheep and cattle and is typical of many areas of moderate soil fertility in the region where grazing and fertiliser application have elevated levels of soil extractable P (Colwell) from pre-European levels of <5 mg kg\(^{-1}\) to >20 mg kg\(^{-1}\).

The site once supported open grassy woodland, comprising *Eucalyptus albicans* (White Box), *Eucalyptus polyanthemos* (Red Box), *Eucalyptus mellidora* (Yellow Box) and *Eucalyptus blakelyi* (Blakely’s Red Gum). The site had been cleared of trees and was dominated by the exotic annual grasses *Bromus mollis, Hordeum leporinum* and *Lolium rigidum*. This grassland was representative of the ‘Fertilised Pasture’ state identified in the State and Transition Model (McIntyre and Lavorel 2007) outlined in Chapter 3, characterised by high grazing pressure and high soil fertility.

Combinations of four nutrient treatments (two rates of carbon addition, control and fertiliser) and two herbicide treatments (hereafter referred to as ‘pasture’ or ‘bare’) were applied to 1.5 m\(^2\) plots. Each plot was separated by a 1.5 m buffer and treatments were randomised within five blocks (giving 40 plots in total) which were fenced to exclude livestock and marsupials. The broad spectrum herbicide Round-Up\(^{®}\) (glyphosate, 7.2 g L\(^{-1}\) as isopropylamine salt) was applied at the recommended rate to half of the plots in late April 2006, which was effective in removing pasture competition. Dead above-ground biomass was removed when dry and these bare plots were maintained by regular manual weeding of small seedlings with minimal disturbance to the soil surface.

Nutrient treatments were designed to increase and decrease the availability of the most commonly limiting macronutrients, nitrogen and phosphorus, which are
widely considered to influence pasture growth and have been shown to increase *Eucalyptus* establishment in Australian forests (Stoneman *et al.* 1993).

I used Di-Ammonium Phosphate (DAP) as fertiliser to enhance nitrogen and phosphorus levels and sugar to reduce soil nitrate. The key transformation processes are shown in Fig. 4.1. Gaseous loss of nitrogen as ammonia is expected to be small in soils of neutral to acid pH (Morel 1983) and de-nitrification should be driven by sucrose addition, through microbial activity at anaerobic microsites.

![Diagram of nitrogen transformation processes](image)

**Fig. 4.1:** Key nitrogen transformation processes within the soil. DAP fertiliser addition provides a source of ammonium ions, whose conversion to NO3- (nitrification) is strongly influenced by soil moisture, temperature and abundance of nitrifying organisms (fungi and bacteria).

Studies on ammonia loss from rice floodwater show that volatilisation of ammonia to the atmosphere is primarily controlled by the concentration of ammonia gas in the air close to the surface and the rate of transfer from the air close to the interface into the atmosphere (Leuning *et al.* 1984). This process is regulated by the concentration of ammoniacal nitrogen, pH and temperature. In my experimental soils (pH ~ 5.9), volatilisation losses of ammonia are expected to be very small.

Treatments were as follows: high sugar (HS) application of 1.0 kg m⁻² of granulated white sugar, shown to substantially reduce soil nitrate available for plant uptake (Prober *et al.* 2005); low sugar (LS) application of 0.33 kg m⁻²; control (no additions); and Di-Ammonium Phosphate (DAP; (NH₄)₂HPO₄) fertiliser addition of 15 g m⁻² (35.2 kg ha⁻¹ of P; 31.8 kg ha⁻¹ of N). The first round of nutrient addition/depletion began in autumn (early May 2007, almost five months before planting, Fig. 4.2), and nutrient treatments were repeated quarterly until February 2008.
Eight seedlings each of 6 month old nursery-grown *E. albens* (White Box) and *E. microcarpa* (Grey Box) of similar size (approximately 25cm in height and already exceeding the height of the pasture) were planted in each plot within an area of 1 m² in mid spring (mid October). This left 0.5 m² of the treated area within each plot for soil sampling with minimal disturbance to adjacent seedlings. Three seedlings which died within a week of planting were replaced, but subsequent deaths were not. The relatively short duration of the experimental trial allowed for a close planting distance in comparison with commercial forestry operations and trials.

From the time of planting, plots were hand-watered from one to three times weekly (10-20 mm per application) to supplement lower than average rainfall, and bring total monthly rainfall closer to long term averages. Watering ceased at the start of summer (early December, 75 days after planting, Fig. 4.2).

Seedling survival was monitored weekly over the spring and summer (from late November until February), and leaf area of surviving seedlings was assessed at the end of the experiment (start of February; 130 days after planting, Fig. 4.2) as a surrogate for seedling growth. Leaf areas were estimated non-destructively using a graphical scale and leaf-count tally, converted to actual areas with the image analysis software ImageJ® (NIH Image; Rasband 2007).

Above-ground herbaceous biomass of each plot (hereafter referred to as grass biomass) was sampled by cutting all shoot material within a 900 cm² quadrat placed to be representative of average plot coverage. Herbaceous biomass was sampled in spring, at the time of its peak annual biomass. Samples were dried at 75 °C for 3 days prior to weighing.

The effects of sugar and fertiliser treatments on soil fertility were assessed using three indicators; soil nitrate-nitrogen (NO₃⁻-N), extractable phosphorus (extractable P) and total nitrogen (Total N). Three soil cores (2 cm diameter, 10 cm depth) were collected from each plot (15 sub-samples per treatment) in summer after senescence of the annual pasture species. These were pooled and mixed before being sent for laboratory analysis (Methods were: NO₃⁻-N extracted using 1:10 ratio of soil:2M KCl, mixed for 1 hour, filtered/centrifuged and then analysed on a segmented flow auto-analysers; extractable P by the Colwell method, using a 1:100 ratio of soil: 0.5M NaHCO₃
mixed for 16 hours, filtered/centrifuged, with colourimetric finish; Total N by digestion
in sulphuric acid and catalyst, mixed with NaOH, distilled with collection of ammonia
gas in boric acid and back-titration using standardised sulphuric acid).

Fig. 4.2: Experimental schedule showing seasonal timing of plot treatments, seedling
transplantation and period of supplementary hand-watering.

4.2.2 Analysis

Seedling survival and final leaf area were assessed by ANOVA to test for experimental
treatment effects, species differences and possible interactions. For seedling survival,
separate ANOVAs were performed for the end of the period of hand-watering (late
spring) and at the end of summer (two months after watering ceased) and these were
tested for species effects.

The seedling growth ANOVA used untransformed leaf area data, pooled and
averaged over the remaining number of seedlings in each plot for each species. Due to
the large difference in initial seedling leaf area between species, seedling growth was
analysed separately for *E. albens* and *E. microcarpa*.

Treatment effects on soil nitrate-N, soil extractable P, soil total N and grass
biomass were also tested by ANOVA on untransformed data. Post-hoc pair-wise
comparisons of means (Bonferroni method) were used to test for significant
differences between treatments. A linear regression model of grass biomass was
produced using the three soil fertility indicators as predictor variables, and correlations
between these variables were tested (Pearson method).
4.3 Results

4.3.1 Manipulation of soil fertility using sugar and fertiliser

Two outlier plots with very high nitrate-N found in the pasture control treatment (see Fig. 4.5) were removed prior to the soil nutrient analyses (and were excluded from presentation in Fig. 4.3). An analysis of the data with outliers retained did not show any significant difference in nitrate-N between fertilised plots and control plots. However, the increased average nitrate-N in the control resulted in a significant difference in nitrate-N between the low sugar treatment and the control.

In bare plots, soil nitrate was significantly higher in the Di-Ammonium Phosphate (DAP) fertiliser treatment than the high sugar treatment at the end of summer (late January). However, neither rate of sugar application nor fertiliser treatment significantly altered soil nitrate-N from the levels found in control plots (Fig. 4.3a). In contrast, levels of soil extractable P were more than doubled by the addition of DAP fertiliser compared with control plots, while sugar application did not affect extractable P (Fig. 4.3b). Nutrient treatments had no significant effect on Total N (Fig. 4.3c).

With one exception, soil fertility of pasture plots at the end of the growth season did not differ significantly from bare plots (Fig. 4.3). Extractable P was lower in pasture than bare plots within the fertiliser treatment ($p < 0.05$) but did not differ between pasture and bare plots within the other nutrient treatments (Fig. 4.3).
4.3.2 Grass biomass: treatment effects and soil fertility

An ANOVA of above-ground grass biomass in pasture plots (assessed at its maximum in November 2006) showed that fertilised plots had significantly higher biomass than the control and high sugar treatments (Fig. 4.4). However, the low sugar treatment was not significantly different from the other treatments (Fig. 4.4), and the high sugar treatment was not significantly different from the control. Thus, as hypothesised, nutrient addition using fertiliser was effective in stimulating a 76% increase in grass biomass above control plots, but unexpectedly sugar application did not reduce biomass compared to the control.
Mean grass biomass (t/ha)

Fig. 4.4: Mean grass biomass of plots with pasture for each nutrient treatment. Treatments which share the same letter are not significantly different at $P = 0.05$. Error bars show ± 1 S.E.

Further analysis using linear regression to investigate the influence of soil nitrate-N, extractable P and Total N on grass biomass produced a significant relationship ($P < 0.001$, $R^2 = 0.829$) when two outlying data points were excluded from the analysis (Table 4.1). These may be explained by ‘hotspots’ of livestock manure residues, noting that soil and grass samples were separated by necessity. Removal of outliers greatly strengthened the relationship between soil nitrate-N and grass biomass (Fig. 4.5) but had negligible effect on the relationships between extractable P, total N and grass biomass. Individually, grass biomass was strongly correlated to soil nitrate-N ($R = 0.77$, $P < 0.001$), and extractable P was weakly correlated with grass biomass ($R = 0.48$, $P < 0.05$). There was no significant correlation between soil nitrate-N and extractable P ($R = 0.45$, $P > 0.05$), or between grass biomass and Total N ($R = -0.22$, $P >0.05$) or other soil fertility indicators (Fig. 4.5, Table 4.1).
Table 4.1: Linear regression model of grass biomass including soil fertility predictors nitrate-N, Extractable P and Total N, showing (a) significance and (b) coefficients.

(a)  

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
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<td>3</td>
<td>9.448</td>
<td>22.571</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>5.860</td>
<td>14</td>
<td>0.419</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.206</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( R^2 = 0.829 \) (Adjusted \( R^2 = 0.792 \))

(b)  

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardised Coefficients</th>
<th>Standardised Coefficients</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>3.626</td>
<td>1.159</td>
<td>3.129</td>
<td>0.007</td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>0.132</td>
<td>0.021</td>
<td>0.787</td>
<td>6.302</td>
</tr>
<tr>
<td>Extractable P</td>
<td>0.032</td>
<td>0.023</td>
<td>0.180</td>
<td>1.413</td>
</tr>
<tr>
<td>Total N</td>
<td>-13.107</td>
<td>6.344</td>
<td>-0.236</td>
<td>-2.066</td>
</tr>
</tbody>
</table>

Fig. 4.5: Scatterplots of grass biomass against soil nitrate-N. The two outlying data points (filled symbols) were excluded from the GLM in Table 4.1.
4.3.3 Eucalypt seedling survival

Results did not support the hypothesis that eucalypts respond positively to increasing soil nutrient in the absence of herbaceous competitors. Tree survival was uniformly high during the hand-watering phase (prior to enforced drought in December). The effects of pasture and nutrient treatments on eucalypt survival were negligible during the hand-watering phase.

From the onset of the droughting period (after hand-watering ceased in early December), seedling survival dropped rapidly in pasture plots compared to bare plots. Nutrient treatments had no significant effect on seedling survival rate for either tree species, regardless of the watering regime, and no interactions between nutrient and pasture treatments were found (Table 4.2). However, seedling survival stabilised in January, two months after droughting was imposed (Fig. 4.6). Final *E. albens* seedling survival at the end of summer (late January) was 8% (S.E. = 3%) for pasture plots and 48% (S.E. = 6%) for bare plots; for *E. microcarpa*, seedling survival was 4% (S.E. = 2%) in pasture plots and 66% (S.E. = 4%) in bare plots. The ANOVA showed the pasture treatment to be the main factor influencing final seedling survival; survival was significantly lower in pasture plots than bare plots (*P* < 0.001, Fig. 4.6, Table 4.2). A species by pasture interaction indicated that in bare plots, survival was higher for *E. microcarpa* than *E. albens*.

![Survival of (a) Eucalyptus albens and (b) Eucalyptus microcarpa seedlings during the droughting phase after hand-watering ceased (see Fig. 4.2). Means are averaged across nutrient treatments. Bars show ± 1 S.E.](image)
Table 4.2: ANOVA of seedling survival to the end of summer (late January) including fixed design effects and significant interactions.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>13</td>
<td>4299.429</td>
<td>12.765</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>78908.203</td>
<td>234.270</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>1033.203</td>
<td>3.067</td>
<td>0.085</td>
</tr>
<tr>
<td>Pasture</td>
<td>1</td>
<td>50626.953</td>
<td>150.306</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nutrient</td>
<td>3</td>
<td>215.495</td>
<td>0.640</td>
<td>0.592</td>
</tr>
<tr>
<td>Block</td>
<td>4</td>
<td>285.156</td>
<td>0.847</td>
<td>0.501</td>
</tr>
<tr>
<td>Pasture * Nutrient</td>
<td>3</td>
<td>17.578</td>
<td>0.052</td>
<td>0.984</td>
</tr>
<tr>
<td>Species * Pasture</td>
<td>1</td>
<td>2392.578</td>
<td>7.103</td>
<td>0.010</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.715$ (Adjusted $R^2 = 0.659$)

4.3.4 Eucalypt seedling growth: treatment effects

Seedling leaf area in late summer (130 days after planting) was significantly higher in bare plots than pasture plots for both species (*E. albens* = 4.7 fold increase, $P < 0.001$; *E. microcarpa* = 7.6 fold, $P < 0.001$; Fig. 4.7). Seedling leaf area showed no significant response to nutrient treatments in either bare or pasture plots for either species, and there was no interaction between pasture and nutrient treatments (Table 4.3). The size increase of seedlings in bare plots compared to pasture plots was large (almost x10), minimising the effect of variation at planting. There was a significant species by pasture interaction representing the large difference in leaf areas between the species (Fig. 4.6). Exclusion of the two previously identified ‘high nitrate – low biomass’ outlier plots had no effect on models of eucalypt seedling survival or growth, so these were retained in the analyses presented in Tables 4.2 and 4.3.
Table 4.3: ANOVA of seedling leaf area at the end of summer (late January) including fixed design factors and significant interactions.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>32</td>
<td>8438.233</td>
<td>22.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>373348.392</td>
<td>980.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>60637.530</td>
<td>159.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pasture</td>
<td>1</td>
<td>174155.942</td>
<td>457.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Block</td>
<td>4</td>
<td>1259.906</td>
<td>3.31</td>
<td>0.018</td>
</tr>
<tr>
<td>Nutrient</td>
<td>3</td>
<td>809.141</td>
<td>2.13</td>
<td>0.110</td>
</tr>
<tr>
<td>Species * Pasture</td>
<td>1</td>
<td>18821.939</td>
<td>49.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species * Nutrient</td>
<td>3</td>
<td>335.074</td>
<td>0.88</td>
<td>0.458</td>
</tr>
<tr>
<td>Pasture * Nutrient</td>
<td>3</td>
<td>609.148</td>
<td>1.60</td>
<td>0.202</td>
</tr>
<tr>
<td>Pasture * Block</td>
<td>4</td>
<td>704.334</td>
<td>1.85</td>
<td>0.135</td>
</tr>
<tr>
<td>Nutrient * Block</td>
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<td>274.251</td>
<td>0.72</td>
<td>0.724</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.938$ (Adjusted $R^2 = 0.896$)

Fig. 4.7: Average above-ground growth per seedling of each species in bare and pasture plots. Error bars show ± 1 S.E.
4.3.5 Eucalypt seedling growth: influence of grass biomass

Overall, elimination of pasture competition using herbicide had the greatest effect on increasing eucalypt seedling establishment and growth. As hypothesised, tree seedling growth was severely stunted in pasture plots compared with bare plots, even in the lowest pasture biomass plots of less than 2 t ha$^{-1}$ (Fig. 4.8), which was well below the average grass biomass of 3.4 t ha$^{-1}$.

Contrary to my hypothesis, applications of sugar and fertiliser had little effect on the success of tree seedlings in terms of survival or growth rate, even though these nutrient treatments were successful in manipulating soil fertility and the biomass production of the pasture. I found that tree seedlings responded strongly to the presence or absence of pasture, but differences in pasture biomass had no significant effect.
Fig. 4.8: Scatterplots of plot-averaged seedling above-ground growth against grass biomass for (a) *Eucalyptus albens* and (b) *Eucalyptus microcarpa* in bare plots (open symbols) and pasture plots (closed symbols).
4.4 Discussion

4.4.1 Manipulation of soil fertility

Neither of the two sugar treatments was effective in reducing soil nitrate-N concentration below that in the control plots, though there was a non-significant trend towards decreasing soil nitrate-N with increasing sugar addition. Although the sugar dosage of 1 kg m\(^{-2}\) was twice that used in a similar study conducted in the central-western slopes of NSW (Prober et al. 2005), the base level of nitrate-N at 20 mg kg\(^{-1}\) in my control plots was almost twice as high as nitrate-N at their study site, which was considered a high nutrient environment. Soil nitrate is reduced by microbial processes that are enhanced by carbon addition (Cookson et al. 2006), but the effectiveness of carbon treatments in nutrient-enriched sites, such as my study site, may require more frequent additions rather than higher quantities of carbon.

Addition of DAP fertiliser resulted in large, significant increases in soil extractable P, but not in soil nitrate-N, as shown by changes within bare plots. Consequently the intentions of the fertiliser treatment (to boost available nitrogen and phosphorus) were only achieved for phosphorus. Fertiliser addition still resulted in significant increases in grass biomass compared to the control and sugar treatments, suggesting that grass growth was limited by available phosphorus rather than available nitrogen. However, nitrate-N was a better predictor of grass biomass than phosphorus in a linear regression of grass biomass against soil nitrate-N for individual plot data. This may be explained by the lag between grass and soil sampling and potential immobilisation of nitrogen during the lag period.

There was a surprising similarity in soil nitrate-N for all plots at the end of the growing season. Explanations are that growth of pasture grasses has little impact on soil nitrate in this relatively nutrient-enriched environment, or senescence of grass roots in herbicide-treated plots may increase mineralisation.

While substantial, phosphorus uptake by the pasture in fertilised plots (as shown by the difference in soil extractable P between pasture and bare plots) was not large enough to reduce extractable P to levels found in the corresponding control plots. This suggests that residual available phosphorus was present at the end of the season, and
therefore pasture growth was not likely to have been limited by phosphorus in the fertilised plots. Soil extractable P and nitrate-N were correlated, as they were applied in uniform ratio using one fertiliser source, and soil nitrate-N and available P appear to co-limit grass biomass production in this system.

4.4.2 Effects of soil fertility on pasture growth

The response of grass biomass to the established nutrient treatments mirrored the soil nitrate patterns between nutrient treatments; neither sugar treatment nor fertiliser treatment differed significantly from the control in the ANOVA, but the most extreme treatments (high sugar and fertiliser) produced significantly different biomass levels.

The regression model, on the other hand, showed a highly significant positive response of pasture growth to increasing soil nitrate-N. The weak response of pasture to experimental nutrient treatments appears to be at odds with this finding; however these results are not contradictory when one considers the high level of variability in soil nitrate-N that we found within nutrient treatments. Surprisingly, the most variable of treatments in terms of soil nitrate-N was the control. We suspect that outlying data points (removed from the analysis) were the result of residues from livestock manure in some soil samples.

That nitrate-N was the best predictor of herbaceous biomass is not a novel finding in an agronomic context (though soil phosphorus is usually assumed to be more limiting to pasture growth on the geologically old soils of Australia); this finding accords with previous work by Prober et al. (2002), who showed that (non-native) annual grasses and forbs are better able to use the autumn peak in soil nitrate-N that occurs in systems where annuals are present, than Australian native perennial species that are adapted to low nutrient soils.

4.4.3 Eucalypt survival and growth

I found that soil nutrient additions increased grass biomass and influenced the amount of unused soil nitrate-N and extractable P, but did not affect leaf area of either *E. albens* or *E. microcarpa*. The presence of pasture grasses was important, as survival and leaf area of both tree species was greatly reduced in pasture plots compared with bare plots, regardless of pasture biomass.
I hypothesised that changes in soil fertility and its influence on grass biomass (and therefore competition) would be the main mechanism driving the eucalypt seedling survival response. Contrary to this hypothesis, neither eucalypt survival nor leaf area was affected by increasing soil nitrate-N in either pasture or bare plots, despite numerous prior studies demonstrating a positive eucalypt growth response to increased nitrogen supply (Moore & Keraitis 1971, Cromer & Jarvis 1990, Wong et al. 1992).

In my experiment, either growth responses to nutrients were not expressed in terms of changes to seedling leaf area, or were not detectable within the duration of the experiment. Although woody species typically have lower potential growth rates than herbaceous annuals, reflecting their respective strategies as stress-tolerant or ruderal species (Grime 2001, Reich et al. 2003), the large increase in leaf area of *E. albens* and *E. microcarpa* measured in bare plots over the course of the experiment shows that treatment effects on eucalypts were detectable, but my hypothesis relating to nutrient response was not upheld. The lack of response by tree seedlings to increases in soil nitrate-N, even in bare plots, suggests that pre-existing nitrate levels were more than adequate for tree seedling growth and that other resources (e.g. water) were limiting (Davis et al. 1999). The high level of available P (~ 20 mg kg\(^{-1}\)) at my study site is typical of fertilised pastures in south-east Australia, whose available P content far exceeds typical values found in reference (pre-European) grassy woodlands (i.e. < 10 mg kg\(^{-1}\) P; McIntyre 2008) to which eucalypts are adapted.

My results confirm the strong negative influence of the herbaceous understorey on tree seedling survival and growth. The sudden increase in mortality in pasture plots when droughting was imposed (from uniformly high survival rates across all treatments during the period of hand watering) suggests competition for moisture was driving survival and growth responses. Davis *et al.* (1999) reached a similar conclusion in their study of *Quercus* growth and survival along a water-light-nutrient gradient, highlighting the indirect nature of resource use mechanisms driving species responses (Tilman 1988).

The high survival during the watering phase also suggests that long-term monthly rainfall averages were sufficient to support seedlings through spring. This cannot be
assumed based on the presence of adult trees in the same rainfall zone, nor from the presence of seedlings in unmodified systems that may exhibit a different pattern of soil drying (Clarke et al. 2005). Several laboratory-based studies have assessed the effects of simulated soil matric potentials on Eucalyptus germination (reviewed by Stoneman 1994), however translation of these results to seedling survival and growth in the field is problematic, since matric potentials fluctuate greatly over time and space. The predicted inverse relationship between grass biomass and tree seedling growth was supported by my results, as much higher tree growth occurred in bare plots than pasture plots. However, for seedling growth in pasture plots only, my hypothesis was that tree growth would increase along the gradient of decreasing grass biomass, gradually approaching the levels of growth observed in bare plots. Growth was negligible in all pasture plots irrespective of grass biomass, and as biomass levels as low as 1.5 t ha\(^{-1}\) (ungrazed pasture) suppressed seedlings, this prediction was not supported. (A biomass of 1.5 t ha\(^{-1}\) reflects a short (< 6cm height) or sparse pasture, and is around the minimum spring benchmark for annual pastures in this region, as targets are typically around 3-4 t ha\(^{-1}\) where legumes are present (Bell 2006)).

Results in Figure 4.7 show a negative exponential relationship between grass biomass and seedling growth, and a gap in the biomass gradient (between 0 and 1.5 t ha\(^{-1}\) dry matter) which was not achieved by applying sugar at my site. The largest rate of increase in seedling growth would be expected to occur between these values, assuming that a continuous relationship exists between seedling growth and grass biomass.

Davis et al. (1999) considered the impact of reduced pasture competition on tree seedling growth response - as mediated by increased available N - to be of far greater ecological significance than the direct response of seedlings to nitrogen input per se. They did, however, find that one of their two deciduous woody study species, Pin Oak (Quercus ellipsoidalis) grew faster with increased nitrogen, while Bur Oak (Quercus macrocarpa) did not. Similarly, I found pasture competition to be the strongest predictor of tree seedling establishment and growth, but neither of my study species responded to soil fertility changes.
The crux of nutrient-competition interaction experiments is that the level of nutrient inputs to a system does not necessarily equate to increased nutrient availability to all species within that system, particularly if nutrients are consistently and more quickly used by faster growing herbaceous species (Davis et al. 1998, 2000, Tilman 1988). For example, in grassy ecosystems in Australia, annual exotic herbs generally possess higher potential growth rates (Leishman et al. 2007) and more pronounced growth responses to nitrate (Prober et al. 2005) than native perennial herbs that are adapted to low nutrient soils. As slower-growing, nutrient efficient species, newly recruited eucalypt seedlings are unlikely to benefit from soil enrichment where a significant proportion of the herbaceous understorey is exotic.

4.4.4 Consequences for tree recruitment

This study confirmed that competition from herbaceous species impacts strongly on young eucalypt seedlings during their establishment. Under natural conditions, young eucalypt seedlings may survive the negative impacts of herbaceous species long enough to outgrow their influence if competition is temporarily reduced or removed, as may occur following physical disturbance to the grass layer or if additional resources (such as unusually high rainfall) are available.

As there were no native perennial grasses at our site – a common situation in the more intensively grazed and cultivated areas of south eastern Australia – I could only seek to manipulate the abundance or growth of the exotic annual grasses dominating the site in order to benefit tree growth. At my study site, grass biomass would need to be reduced to well below 1.5 t ha\(^{-1}\) for tree seedling growth to occur during spring; a level which would be considered very low for any pasture in temperature areas of south-eastern Australia. This can be achieved by use of herbicides. The addition of sugar to reduce nitrate levels, as trialed by Prober et al. (2005), was shown in this experiment to be insufficient.

It is notable that seedling mortality was not 100% in pasture plots (though near to it), and therefore rare recruitment events into an herbaceous understorey are still possible. However, these results do not provide good estimates of seedling mortality rates from natural seeding into pastures, since I planted seedlings rather than seed, and watered our plots. Watering would have enhanced survival overall, and naturally
established seedlings would have had deeper tap roots than planted seedlings. With planted seedlings, the impacts of competition for light between pasture species and tree seedlings that occurs when seedlings are very small are also avoided. Light competition is commonly a key determinant of seedling establishment in herbaceous vegetation but responses are complex. Shading can also increase seedling survival in arid environments due to improved soil-water relations (Davis et al. 1999).

Restoration measures which can greatly reduce competition from the herbaceous understorey and increase survival and growth rates of tree seedlings (e.g. Wittwer et al. 1986) include the use of broad-spectrum and residual herbicides, scalping (removal of nutrient rich topsoil) and cultivation, though they are likely to be most useful at sites of high soil nutrient enrichment, where the understorey is dominated by exotic annual species, and where the understorey is of little conservation value itself. Alternative measures to reduce soil nitrate such as carbon additions have been shown to be effective in reducing growth of exotic annual species and shifting understorey composition in favour of native species (Prober et al. 2005, Kulmatiski & Beard 2006). However, as shown by our results, the effectiveness of this treatment appears to be reduced at very high nitrate levels.

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- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia

5.1 Introduction

Bottlenecks in tree population dynamics typically occur at the emergence and early seedling growth life stages of recruitment, when individuals are highly sensitive to environmental stresses and mortality is high (Harper 1977). The key mechanisms limiting tree recruitment differ between biomes and across the environmental gradients they contain. For example, in intact tropical forests of central America (Hubbell et al. 1999, Hooper et al. 2005, Uriarte et al. 2005) and temperate coniferous and mixed hardwood forests of north America (Finzi & Canham 2000), recruitment is dependent on gap formation, and is usually attributed to light availability and variation in species’ shade tolerance. However, water stress (and climate) is the dominant ecological filter in many lower rainfall environments, for example in oak forests in North America (Gordon & Rice 2000, de Steven 1991b, Jurena & Archer 2003), savannas in southern Africa (Knoop & Walker 1985, Sankaran et al. 2008) and Australia (Fensham et al. 2005), and deserts in Egypt (Andersen & Krzywinski 2007).

Seed dispersal and seedling predation are also important regulators of tree population dynamics in some systems (e.g. Zimmerman et al. 2000, Clarke & Davison 2001, Castro et al. 2002). While nutrient availability seldom limits tree recruitment directly, indirect impacts of nutrient on tree recruitment and growth arise from changes to the productivity and composition of the herbaceous layer, and associated changes in resource availability (Davis et al. 1998, 1999, Allcock 2002, Chapter 4).

Very low levels of recruitment threaten the persistence of woodland trees in the wheat-sheep farming zone of south-east Australia, which formerly supported grassy woodlands. Prior to widespread clearing and the introduction of European farming systems, these were characterised by a sparse overstorey of Eucalyptus species, and an understorey dominated by perennial tussock grasses, interspersed with perennial forbs (Prober & Brown 1994).

In woody-herbaceous (‘tree-grass’) ecosystems such as savannas and grassy woodlands – where the canopy is open and tree seedling recruitment tends to be highly episodic – water availability (and therefore climate), seed supply, seed
predation, and fire are the most commonly cited drivers of tree recruitment (Clarke 2001, 2002, van Wijk 2002, Sankaran et al. 2004, 2008). Tree-grass interactions are also the focus of applied studies aiming to restore the woody component to abandoned pastures ('old-fields') or former woodlands, and competition from the herbaceous layer is a major barrier to tree recruitment in these systems (Berkowitz et al. 1995, Davis et al. 1999, Rey Benayas et al. 2003). The impact of the herbaceous layer is generally attributed to its association with soil moisture availability, but mechanisms are often unable to be identified (e.g. de Steven 1991b, Smit & Olff 1998, Gordon & Rice 2000, Davis 2005).

Potential barriers to tree recruitment can be numerous and may act in succession (e.g. Dovčiak et al. 2005, Mills et al. 2006). Consequently, their relative influence on tree population dynamics may vary over time due to shifts in resource availability or removal of other barriers (e.g. de Steven 1991b). This is consistent with two favoured theories of tree-grass coexistence (House et al. 2003) being: 'competitive exclusion' (or 'disclimax community'), whereby there is a tendency for grasses or trees to dominate the other life form unless disturbance prevents it, resulting in alternative stable equilibrium states; and 'multiple stable states', where spatial or temporal variability in resources and disturbances allow different tree-grass ratios to exist at various times as a result of non-equilibrium dynamics, where changes in resources or disturbance can shift the equilibrium point and trigger a transition to a new state of tree-grass coexistence. Mills et al. (2006), support the concept of multiple possible stable states by suggesting that possible routes to tree-grass coexistence will include interactions of abiotic and biotic factors, operating at a different strength at different scales.

As the mechanisms which limit tree recruitment are expected to change along abiotic and biotic gradients (Mills et al. 2006), I propose that the mechanisms limiting tree recruitment are likely to reflect both climate variability, and different landuses. The temperate grassy woodlands of south-eastern Australia experience a mediterranean climate, where summers are typically hot and dry. Above-average rainfall is expected to increase the likelihood of tree recruitment by reducing the seedling mortality caused by drought stress during the first spring and summer after germination (Curtis 1990, Clarke 2002). However, increased competition for resources due to changes in composition of the herbaceous layer following 'pasture
improvement’ (introduction of palatable exotic grasses and forbs) and invasion by 
exotic annual weeds in agricultural areas once dominated by grassy woodlands may 
counteract increased water supply (Berkowitz et al. 1995). Both human-induced 
changes to the composition and competitive ability of the herbaceous layer need to be 
considered.

Changes to understorey composition in south-east Australian woodlands since 
European arrival due to grazing, fertilisation and cultivation history have been 
described by McIntyre and Lavorel (2007). Their state and transition model of plant 
community dynamics categorises grasslands (and derived pastures) into five broad 
states of increasing modification and soil enrichment: (i) reference grassland (now rare; 
Prober et al. 2002a), (ii) native pasture, (iii) fertilised pasture, (iv) sown pasture, and (v) 
enriched pasture (Chapter 3, Fig. 1). In this model, states are characterised by both the 
dominant life form and soil fertility associated with altered management. Transitions 
between states need not be linear, and while they are in theory reversible, in practice, 
the return from nutrient-enriched states dominated by exotic species to native 
vegetation dominance is improbable without intensive management inputs, even 
without consideration of native species’ dispersal limitation due to fragmentation 
(Cramer et al. 2007).

This study assesses the potential for tree recruitment within three grassland 
states found in modified grassy woodlands of south-eastern Australia: native pasture, 
fertilised pasture and enriched grassland.

The aim of this experiment was to determine the key mechanisms limiting tree 
regeneration in low and high rainfall years in three grassland states typical of 
aricultural land uses in south-eastern Australia. More specifically, I investigated how 
the manipulation of light, water and competition from herbaceous species affected 
the germination, growth and survival of seedlings of the woodland tree Eucalyptus 
microcarpa.

My hypotheses are:

(1) that recruitment of E. microcarpa will differ between grassland states in both 
average and high rainfall years (simulated by irrigation) reflecting changes in water 
limitation;
(2) that native perennial pasture will support more tree recruitment than the exotic annual pasture (‘fertilised pasture’), and the exotic annual pasture more than the exotic perennial pasture (‘enriched grassland’), in response to improved water availability and greater light interception;

(3) that grassland state, light, and water supply will have an interactive effect on tree seedlings, with light having a greater influence than water in the high-biomass exotic perennial pasture, and water availability having a greater influence in the lower biomass exotic annual and native perennial pasture (Fig. 5.1).

**Fig. 5.1:** Visual representation of hypothetical recruitment responses to water and light manipulations within pasture plots at the three experimental sites (representing native perennial-, exotic annual-, and exotic perennial-dominated grassland states). Predictions are that water availability will be the greatest limitation to recruitment in Native perennial and Exotic annual grassland states, and that light would limit recruitment in the Exotic perennial grassland state, independent of water availability.

### 5.2 Methods

A field-based factorial experiment was established in autumn 2007 to test the effects of pasture competition, soil moisture and light on both the emergence and seedling establishment life stages of the woodland tree *E. microcarpa* (Grey Box) within a grassy understorey. The experiment was carried out in three grassland states of distinctly different plant community composition, reflecting differing land use histories of grassy woodlands, now mostly cleared of trees for agricultural use. The sites were located within 3km of each other, near Albury NSW (160 a.s.l., 36°05, 146° 55’, ~800mm mean annual rainfall) and subject to similar climate and rainfall (verified using rain gauges).

All three sites shared the same landscape position (valley floor), had negligible slope
and (therefore) an open aspect. Soil surveys of the area (NSW Soil and Land Information System 2010) indicate that all three sites are characterised by yellow podzolic soils (clay-loams). The differences between the study sites are attributed to change in their management since the development of European farming systems, not any inherent difference between the sites prior to their development.

While experimental treatments were replicated within each grassland state, the grassland states themselves were not replicated due to the number of treatments in the experiment and the time required for application of the irrigation treatment. Thus, the experiment is pseudo-replicated. This restricts the statistical analyses that can be applied to the design, but does not prevent ecological interpretation of the results, as long as conditions in the chosen experimental sites are known and considered representative of many parts of the landscape (Morgan 1997). The grassland states I investigated (described below) have been well documented in numerous studies (reviewed by McIntyre & Lavorel 2007) and chosen sites were considered to be typical of these states.

5.2.1 Site selection

Sites were selected based on a visual assessment of dominant species. Further characterisation of each site included assessments of soil chemical and physical properties, and descriptors of sward composition and physical structure. Soil and above-ground biomass sampling and chemical analysis methods follow those described in Chapter 4.

The site chosen as representative of the native perennial state (hereafter the ‘Native’ state) was located in a travelling stock reserve that had been subject to periodic light grazing, and to our knowledge, had not been fertilised or cultivated or sown with exotic pasture species. The retention of native vegetation in the ‘native state’ represented an administrative boundary associated with the Travelling Stock Reserve system (Lunt & Spooner 2005), not a bias towards particular landform. Few exotic species were present at this site, which contained large areas of bare ground. The most abundant species in the herbaceous layer were the native perennial $C_3$ grasses Austrodanthonia sp. and Austrostipa sp.
The site representative of the ‘fertilised pasture’ (hereafter the ‘Annual’ state) was immediately adjacent to that used in chapter 4. This site had a long history of grazing and fertiliser use, and was being grazed by cattle prior to fencing of the experimental site in autumn. This site was dominated by the exotic annual grasses *Bromus mollis*, *Lolium rigidum* and *Hordeum leporinum* in winter/spring, and later by the nitrogen-fixing exotic legume *Trifolium subterraneum* in late spring/summer, when winter-growing grasses had senesced.

The site representative of the ‘enriched grassland’ dominated by exotic perennial grasses (hereafter referred to as the ‘Phalaris’ site) was dominated exclusively by the exotic perennial *Phalaris aquatica* L., which is considered an important grass in ‘improved’ pastures in south-eastern Australia (Auld & Medd 1987). It forms dense clumps up to 1.5m high when left ungrazed, especially in wetter areas, and once established, competes heavily with other species (Semple & Koen 2003). It is listed as one of 49 exotic species impacting rare or threatened native flora in Australia and occurs in all states (Groves *et al.* 2003).

### 5.2.2 Experimental treatments

At each site, eight treatments representing all combinations of ± herbicide (hereafter referred to as bare or pasture plots), ± irrigation (irrigated, rainfed) and ± light were assigned to separate plots of 1.2 m by 1.8 m separated by 1.5 m buffers within six randomised replicate blocks (48 plots in total). The sites were fenced to protect them against grazing by livestock, rabbits and hares during April and May.

The herbicide glyphosate (‘Round-Up®’ (N-phosphenomethylglycine), Monsanto Corp., St. Louis) was applied in autumn (April) 2007 and achieved broad spectrum plant death within a few weeks. All standing biomass and litter was removed by hand several months later. Hand-weeding continued during spring and summer where needed, taking care to minimise soil disturbance. The control (pasture) plots consisted of an ungrazed herbaceous sward which differed significantly between the three grassland states, as outlined later in the site characterisation.

Irrigation of selected plots began at the time of seed sowing (September 2007) and ceased in early January 2008. Plots were irrigated as often as was feasible, between one and four times weekly, delivering between 1.75 to 4.4 mm (or 17.5 to 44
L m$^{-2}$) per irrigation event. Plots were irrigated less frequently following rainfall (> 20 mm) but the quantity of water supplied was always consistent across irrigated plots. My aim was to reduce water stress of plants in the irrigation treatment to the greatest degree possible. At no stage during the experiment was ‘pooling’ of water visible, or water-logging evident. Dam water was delivered to each plot from a car-mounted 1200L capacity tank, through a fixed network of PVC piping, fitted with 90°-angle ‘micro-spray’ nozzles mounted on risers of 40 cm height. Because plots were rectangular (not square), to achieve a full overlap of the 90° arcs required watering slightly into the plot buffers. This did not appear to affect non-irrigated plots except on windy days, when watering was avoided. Rate of water application (and coverage) was manually adjusted through pressure supplied by the petrol-driven water pump.

The light treatment was designed to open the pasture sward to natural sunlight without significantly changing below-ground competition for resources. The treatment consisted of two parallel retaining wires which were cut from rigid fencing wire, and bent to 90° at each end, effectively creating a wire staple of 40 cm length. These were pressed into the ground together, then carefully levered outwards to either side whilst pinning the sward down under them to create a linear (North-South) opening to natural light. These were exactly aligned over the strip-shaped quadrats to which tree seed was sown. Although sideways compression of the sward foliage in this way would be expected to slightly reduce evapotranspiration (and therefore water use) of the pasture alongside these quadrats, the change to below-ground interactions would be minimal in comparison to the compensatory changes that would occur if shoots were cut. Wallace & Macko (1993) have demonstrated such compensatory changes. Wire restrainers constituting the ‘+ light’ treatment were also applied in the ‘+ herbicide’ (bare) plots where the sward had already been removed to maintain a fully crossed experimental design and test for the possible influence of wire on seedling emergence.

In late September, each plot was sown with three 2.25 g of *E. microcarpa* (Grey Box) seed, equally divided between three linear strip quadrats (25 cm by 2 cm; 0.75g seed/quadrat). These were aligned on a north-south axis, and spaced 30 cm apart within the northern half of each plot.

One week later (late September), seven *E. microcarpa* (Grey Box) seedlings were planted in the southern half of each plot (an area of 1m$^2$). Seedlings were nursery-
grown and approximately 6 months old. At the time of planting, seedlings were trimmed to a uniform height of 20 cm, and had similar initial leaf area (range of 0.12 to \(0.16 \text{ m}^2 \times 10^{-2}\) for sample of 20 seedlings). No seedlings died within the first fortnight after planting, so subsequent deaths were attributed to treatment effects, rather than transplant shock.

Plots were observed for signs of germination, and the first full assessment of germination across all three grassland states took place in late October. Germinants were individually ‘tagged’ using toothpicks in order to differentiate between new and old seedlings and determine the maximum cumulative germination for each plot, since some early mortality occurred while new seedlings were emerging. Subsequent monitoring of seed-sown (germinant) survival as well as survival counts of planted (tubestock seedlings) were carried out every two to three weeks from spring to the conclusion of the experiment in late summer (February).

Leaf areas of all planted seedlings were assessed once in mid-summer (January) as a substitute for average seedling growth, since initial leaf area was negligible. Leaf areas were estimated non-destructively using a graphical scale and leaf-count tally, converted to actual areas with the image analysis software ImageJ\(^\circ\) (NIH Image; Rasband 2007). Seedling height data were also assessed and showed trends correlating with leaf area. Leaf area was a more sensitive predictor of higher ecological relevance, so only leaf area data is presented in this paper.

### 5.2.3 Site characterisation

Above-ground herbaceous biomass (hereafter referred to as grass biomass) of each of the 24 herbicide-free plots at each grassland state was sampled by cutting all shoot material within a 30 cm square quadrat placed to be representative of average plot coverage. Samples were dried at 75 \(^\circ\)C for 3 days prior to weighing. Ground cover (% live cover) was assessed in spring using a 1m square quadrat with 100 equally spaced point markers. Soil moisture at field capacity was assessed at 10 cm depth using a soil moisture probe meter (MPM160, ICT International Pty Ltd), 24 hours after high rainfall in October 2007. Soil water infiltration rates were assessed using a 20 cm diameter cylinder internally marked with a 1mm graduation ruler. The cylinder was buried to 3 cm below the soil surface at its base, filled with water to 40 mm height and allowed to
drain by gravity. The rate of drop in water level was recorded every 30 seconds for a maximum of 15 minutes to obtain the post-wetting rate of water infiltration, which stabilised after several minutes.

Three soil bulk density samples were collected from each site using a metal core of 50mm diameter and depth. The samples were pooled for each site prior to oven drying at 80°C for three days, and weighing. Six separate soil samples were collected to 10cm depth from each site and combined prior to being analysed at the Environmental and Analytical Laboratories (Charles Sturt University, Wagga Wagga) for ammonium-N, nitrate-N, total N, extractable P, pH, and total organic C. Methods followed those described in Chapter 3.

5.2.4 Analysis

‘Grassland states’ (Native, Annual, Phalaris) were included as a block effect in the analysis of germination, seedling leaf area and planted-seedling survival data, since each state was represented by only one site. Interactions between states and treatments were tested within each block. Analysis of seed-sown seedling survival was undertaken only for the Phalaris state, as mortality was close to 100% in the Native and Annual states.

Germination was analysed using poisson regression due to a large number of zero counts in two of the three states. Over-dispersion required the use of F-values to test for factor significance rather than the less conservative chi-square test.

An alternative model of germination that excludes zero counts is also presented. This applied an ANOVA to log-transformed germination data in order to compare treatment means, which is not achieved by analysis of deviance in poisson regression. Post-hoc pairwise comparisons (Tukey) of germination were used for the eight combinations of herbicide, irrigation and light treatments. Although opening of the sward with wire restrainers in the ‘+ light’ treatment was superfluous in the ‘+ herbicide’ (bare) plots where the sward had already been removed, the fully crossed design was retained to be sure the wire was not influencing seedling emergence in any way. As the light treatment had no effect on emergence in either bare plots or pasture plots, data from the ± light treatments was pooled in subsequent analyses.
Repeated measures analysis could have been used if I were interested in finding the date or period of time when treatment differences were visible. As the key result was in differences between treatments at the end of the first summer – a bottleneck in the eucalypt life cycle – an ANOVA was used to assess February survival data.

The leaf area data was pooled and averaged according to the remaining number of seedlings in each plot for each species, such that values represent average leaf area of an individual seedling.

Survival and log-transformed final leaf area of planted seedlings were assessed by ANOVA to test for herbicide, irrigation or possible interactions, but did not include a light treatment which was redundant because tubestock seedlings were taller than the grass sward at the start of the experiment.

5.3 Results

5.3.1 Site characteristics

The Native state was the least fertile of the three states, and the Phalaris state the most fertile. The Phalaris state also had the highest soil moisture content at field capacity, highest water infiltration rate, and highest grass biomass. With the exception of soil ammonium-nitrogen (NH4+ as N), the native annual state displayed soil fertility values that were lower than the values for the annual state and the Phalaris state (Table 5.1).
Table 5.1: Selected soil chemical and physical characteristics of the three study sites (mean, standard deviation (σ), and number of samples (n)).

| Experimental Site | Phalaris | | | Annual | | | | Native | | |
|-------------------|----------|---|---|--------|---|---|--------|---|---|
|                   | Mean     | σ | n  | Mean   | σ | n  | Mean   | σ | n  |
| **Soil chemical fertility indicators** | | | | | | | | | |
| NH$_4^+$ as N (mg kg$^{-1}$) | 49.8     | - | 1  | 1.0     | - | 1  | 2.9     | - | 1  |
| NO$_3^-$ as N (mg kg$^{-1}$) | 87.3     | - | 1  | 28.3    | - | 1  | 1.6     | - | 1  |
| Total N (mg kg$^{-1}$) | 2110     | - | 1  | 2190    | - | 1  | 993     | - | 1  |
| Extractable P (Colwell; mg kg$^{-1}$) | 22.8     | - | 1  | 33.9    | - | 1  | 12.9    | - | 1  |
| pH                | 5.0      | - | 1  | 5.9     | - | 1  | 5.3     | - | 1  |
| Total organic C (mg kg$^{-1}$) | 34000    | - | 1  | 28000   | - | 1  | 19000   | - | 1  |
| **Soil physical properties** | | | | | | | | | |
| Soil bulk density (Mg m$^{-3}$) | 0.800    | 0.03 | 3  | 0.903   | 0.01 | 3  | 0.951   | 0.03 | 3  |
| Soil moisture at field capacity (% vol/vol) | 19.2 | 2.5 | 6  | 16.5 | 1.4 | 6  | 9.2 | 7.0 | 6  |
| Infiltration rate (mm hr$^{-1}$) | 150 | 94 | 3  | 90 | 15 | 3  | 23 | 3 | 3  |
| **Sward characteristics** | | | | | | | | | |
| Grass biomass (t ha$^{-1}$) | 9.19 | 2.33 | 6  | 3.28 | 1.16 | 6  | 1.19 | 0.32 | 6  |
| Ground cover (%) | 98.0 | 3.8 | 12 | 92.0 | 7.0 | 12 | 23.2 | 6.3 | 12 |

5.3.2 *Eucalyptus microcarpa* germination

Grassland state (block factor) had a bigger impact on *E. microcarpa* germination than within-block treatments, explaining 39% of total model deviance in the poisson regression model, compared to 35% explained by herbicide and 4% by irrigation (Table 5.2). The impacts of soil properties on germination were demonstrated by differences between the bare (control) treatments at each site, which were expected to show similar responses (see treatments 5 and 6 in Fig. 5.2).

Contrary to my expectations, germination of *E. microcarpa* was highest in the Phalaris state, followed by the Annual state and the Native Perennial state; the latter two states having very few germinants (Fig. 5.2).

The relative importance of within-site plot treatments to germination was similar in the three states; the herbicide treatment had the biggest impact on germination, followed by the irrigation treatment, with the highest number of germinants found in the bare-irrigated plots, followed by the bare-rainfed plots. The light treatment had no significant effect in any of the grassland states, and no interactions between light and other treatments were found (Table 5.2). The 3-way interaction of irrigation, light and block was almost significant (p = 0.06), reflecting the trend toward higher germination.
within pasture than bare ground for rainfed plots in the Annual state (see treatment 7, Fig. 5.2).

When zero counts were excluded from the analysis, irrigation was only significant in interaction with herbicide, reflecting increases in the number of germinants in bare plots. Irrigation effects were insignificant in pasture plots (Table 5.3). This response did not interact with block effects. An herbicide by block interaction indicated that herbicide did not significantly increase the quantity of germination in the Native state when plots without germination were excluded.

Table 5.2: Poisson regression model of maximum cumulative *E. microcarpa* germination per plot, including all main effects, two-way and three-way interactions. Grassland state is included as a block effect.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Deviance</th>
<th>Residual d.f.</th>
<th>Residual Deviance</th>
<th>F value</th>
<th>P (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>143</td>
<td>5293.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>2062.595</td>
<td>141</td>
<td>3230.6</td>
<td>127.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herbicide</td>
<td>1</td>
<td>1855.452</td>
<td>140</td>
<td>1375.1</td>
<td>229.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irrigation</td>
<td>1</td>
<td>226.468</td>
<td>139</td>
<td>1148.6</td>
<td>28.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Light</td>
<td>1</td>
<td>0.093</td>
<td>138</td>
<td>1148.5</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Herbicide*Block</td>
<td>2</td>
<td>30.952</td>
<td>136</td>
<td>1117.6</td>
<td>1.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Herbicide*Irrigation</td>
<td>1</td>
<td>0.004</td>
<td>135</td>
<td>1117.5</td>
<td>0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Irrigation*Block</td>
<td>2</td>
<td>32.386</td>
<td>133</td>
<td>1085.1</td>
<td>2.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Light*Block</td>
<td>2</td>
<td>10.46</td>
<td>131</td>
<td>1074.7</td>
<td>0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Light*Irrigation</td>
<td>1</td>
<td>1.026</td>
<td>130</td>
<td>1073.7</td>
<td>0.12</td>
<td>0.72</td>
</tr>
<tr>
<td>Light*Herbicide</td>
<td>1</td>
<td>23.557</td>
<td>129</td>
<td>1050.1</td>
<td>2.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Herbicide<em>Irrigation</em>Block</td>
<td>2</td>
<td>35.731</td>
<td>127</td>
<td>1014.4</td>
<td>2.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Light<em>Irrigation</em>Block</td>
<td>2</td>
<td>46.771</td>
<td>125</td>
<td>967.6</td>
<td>2.9</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Number of Fisher Scoring Iterations: 13
**Table 5.3:** ANOVA of log-transformed *E. microcarpa* germination data with zero values excluded. Only significant interaction terms have been retained in the model. Grassland state is included as a block effect.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>19.7</td>
<td>8</td>
<td>2.5</td>
<td>17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>28.2</td>
<td>1</td>
<td>28.2</td>
<td>203.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Block</td>
<td>5.1</td>
<td>2</td>
<td>2.6</td>
<td>18.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herbicide</td>
<td>3.3</td>
<td>1</td>
<td>3.3</td>
<td>23.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irrigation</td>
<td>0.16</td>
<td>1</td>
<td>0.16</td>
<td>1.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Light</td>
<td>0.19</td>
<td>1</td>
<td>0.19</td>
<td>1.4</td>
<td>0.24</td>
</tr>
<tr>
<td>Herbicide * Irrigation</td>
<td>0.89</td>
<td>1</td>
<td>0.89</td>
<td>6.4</td>
<td>0.014</td>
</tr>
<tr>
<td>Herbicide * Block</td>
<td>2.2</td>
<td>2</td>
<td>1.1</td>
<td>7.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>9.0</td>
<td>65</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>118.2</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>28.7</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.685$ (Adjusted $R^2 = 0.647$)

**Fig. 5.2:** Mean number of *Eucalyptus microcarpa* germinants per plot for herbicide and irrigation treatments for three grassland states, dominated the exotic perennial grass *Phalaris aquatica* (‘Phalaris’), exotic annual grasses (‘Annual’) or native perennials (‘Native’). Means are based on all plots, including zero counts.
5.3.3 Survival of seed-sown seedlings

Seedling survival to the end of the experiment in late summer was influenced by state and within-state treatments in a similar manner to germination, with state (block) being the most important predictor of final survival.

For the Phalaris state, which had the most *E. microcarpa* germinants of the three states, there were significant herbicide, irrigation and interaction effects on the number of surviving seedlings at each monitoring stage over the course of the experiment (Fig. 5.3). Mortality of seedlings in rainfed (non-irrigated) – bare plots was highest in the first month (a 62 % drop from 47 to 18 seedlings per plot from November to December) and slowed over subsequent months until the number of surviving seedlings stabilised in January 2008 (Fig. 5.3). In the irrigated – bare plots, seedling mortality was less severe and more consistent over the three months from November (with 16 % mortality from November to December), but seedling numbers did not appear to have fully stabilised by the conclusion of monitoring in February 2008.

In the Annual state, herbicide and irrigation effects were initially present, but all seedlings in the bare plots in early November had died by the start of December (in rainfed plots) or by January (in irrigated plots), so that no seedling survived in any treatments past January. Seedling germination in the Native state was too low for statistical analysis of survival.

The Phalaris state was the only state in which a large number of seed-germinated seedlings survived through to the end of summer, and these occurred exclusively in bare plots. An investigation of the effects of spring grass biomass on seedling survival showed a significant but weak negative relationship when bare plots were included ($R^2 = 0.30, F = 19.4, P < 0.001$), but no relationship between survival and biomass across pasture plots (Fig. 5.4). Hence grass biomass was not a useful predictor of seedling survival beyond the presence or absence of pasture (Table 5.4).
Fig. 5.3: Survival of seed-sown *E. microcarpa* seedlings across the three grassland states (dominated by the exotic perennial grass Phalaris, exotic annuals or native perennials), and across herbicide treatments (bare o vs. pasture •) and irrigation treatments (solid line) vs. rainfed (broken line). Letters indicate the presence of significant herbicide (h), irrigation (i), or herbicide-irrigation interaction (x) effects (*P* < 0.05) determined by ANOVA at each monitoring date. Bars show ± 1 S.E.

Fig. 5.4: Seed-sown seedlings surviving to late summer (February) plotted against herbaceous biomass and labelled by irrigation treatment (rainfed x, and irrigated •). Data points running parallel with the x axis are from pasture plots, while those running parallel with the y axis are from bare plots.
Table 5.4: ANOVA table of seed-sown seedling survival to the end of summer at the Phalaris state, including main treatments, significant interactions and the covariate grass biomass. ANOVAs were not carried out on final survival data at the Annual and Native states, as mortality in February was close to 100% in all treatments.

<table>
<thead>
<tr>
<th>Phalaris site Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>11841.9</td>
<td>7</td>
<td>1691.7</td>
<td>9.24</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>698.8</td>
<td>1</td>
<td>698.8</td>
<td>3.81</td>
<td>0.058</td>
</tr>
<tr>
<td>Herbicide</td>
<td>872.2</td>
<td>1</td>
<td>872.2</td>
<td>4.76</td>
<td>0.035</td>
</tr>
<tr>
<td>Irrigation</td>
<td>1968.2</td>
<td>1</td>
<td>1968.2</td>
<td>10.75</td>
<td>0.002</td>
</tr>
<tr>
<td>Light</td>
<td>286.5</td>
<td>1</td>
<td>286.5</td>
<td>1.56</td>
<td>0.218</td>
</tr>
<tr>
<td>Herbicide * Irrigation</td>
<td>2057.4</td>
<td>1</td>
<td>2057.4</td>
<td>11.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Irrigation * Light</td>
<td>186.8</td>
<td>1</td>
<td>186.8</td>
<td>1.02</td>
<td>0.318</td>
</tr>
<tr>
<td>Herbicide * Light</td>
<td>302.1</td>
<td>1</td>
<td>302.1</td>
<td>1.65</td>
<td>0.206</td>
</tr>
<tr>
<td>Grass biomass</td>
<td>2.7</td>
<td>1</td>
<td>2.7</td>
<td>0.01</td>
<td>0.904</td>
</tr>
<tr>
<td>Error</td>
<td>7321.0</td>
<td>40</td>
<td>183.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25790.0</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>19163.0</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.618$ (Adjusted $R^2 = 0.551$)

5.3.4 Survival of planted seedlings (tubestock)

Results for tubestock seedlings paralleled those for seedling germination and survival in that pasture state (block effect) had a bigger impact on survival of transplanted *E. microcarpa* seedlings (tubestock) to the end of summer than experimental treatments (Table 5.4) though both herbicide and irrigation significantly increased seedling survival. The highest number of remaining seedlings occurred within the Phalaris state (average 45.8% survival), followed by the Annual state (20.2%) and the Native state (12.5%).

Unlike the germination and germinant survival responses, the influence of the irrigation treatment on planted-seedling survival differed between states. There was no significant herbicide by block interaction, indicating similar effects of herbicide on survival across the three states. However, there was a significant irrigation by block interaction, reflecting the larger effect of irrigation in the Native state than the Phalaris state and the Annual state.

In the Phalaris state, effects of both herbicide and irrigation treatments were significant ($P < 0.001$, $P < 0.01$ respectively). Survival was highest in the bare (+ herbicide) plots, with irrigation resulting in further small increases within both bare and pasture plots in comparison to the rainfed treatment (Fig. 5.5a). There was no
herbicide by irrigation interaction effect on final seedling survival in the Phalaris state (Table 5.4).

In the Annual state, herbicide and irrigation treatments had a significant effect on seedling survival for the most part of the spring-summer monitoring period, with the bare plots again having higher survival than pasture plots, and irrigated plots higher survival than rainfed plots (Fig. 5.5b). However, a rapid increase in mortality in the bare-irrigated plots towards the end of the experiment meant that all treatments had relatively low survival to the end of summer. Herbicide application (bare plots) led to a significant (p < 0.001) though only moderate increase in final seedling survival, whereas the irrigation treatment had no measurable impact.

The Native state produced quite a different seedling survival response from the other experimental sites. There was no significant difference in survival between bare and pasture plots, but irrigation led to a small but significant increase (p < 0.01). Although the irrigation effect was significant, average survival to February in irrigated plots in the Native state was low (25.0%) and no seedlings survived in the rainfed plots.

Table 5.4: ANOVA table of treatment effects on planted (tubestock) seedling survival to the end of Summer (February 8th), based on seedling counts. Grassland state is included as block effect.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>243.2</td>
<td>11</td>
<td>22.1</td>
<td>5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>206.6</td>
<td>1</td>
<td>206.6</td>
<td>53.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Block</td>
<td>119.6</td>
<td>2</td>
<td>59.8</td>
<td>15.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herbicide</td>
<td>19.9</td>
<td>1</td>
<td>19.9</td>
<td>5.2</td>
<td>0.025</td>
</tr>
<tr>
<td>Irrigation</td>
<td>49.6</td>
<td>1</td>
<td>49.6</td>
<td>12.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herbicide*Block</td>
<td>19.6</td>
<td>2</td>
<td>9.8</td>
<td>2.6</td>
<td>0.081</td>
</tr>
<tr>
<td>Irrigation*Block</td>
<td>29.2</td>
<td>2</td>
<td>14.6</td>
<td>3.8</td>
<td>0.025</td>
</tr>
<tr>
<td>Herbicide * Irrigation</td>
<td>1.46</td>
<td>1</td>
<td>1.46</td>
<td>0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Herbicide<em>Irrigation</em>Block</td>
<td>3.9</td>
<td>2</td>
<td>1.96</td>
<td>0.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Error</td>
<td>506.9</td>
<td>132</td>
<td>3.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>956.7</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>750.1</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.324$ (Adjusted $R^2 = 0.268$)
Fig. 5.5: Survival of planted (tubestock) seedlings from the mid-spring transplant until late summer, displayed by pasture competition (bare plots ○; pasture plots •) and irrigation treatment (irrigated – solid lines; rainfed – broken lines) combinations, in the (a) Phalaris (b) Annual and (c) Native states.

5.3.5 Growth of planted seedlings (tubestock)

Growth of planted *E. microcarpa* seedlings, assessed as leaf area in late summer, closely matched the seedlings’ survival response. Again, block had a significant impact on seedling responses. Substantial seedling growth occurred in the Phalaris and Annual states, but was much higher in the former, while seedling growth in the Native state was negligible. As well as having far more surviving seedlings, seedling growth was higher in bare plots than pasture plots, and higher in irrigated plots than rain-fed plots, though the relative influence of herbicide differed between states, as shown by the herbicide by block interaction (Table 5.5). Herbicide had little effect in the Native state.

Herbicide application increased seedling growth (*F* = 27.8, *P* <0.001) to a greater degree than irrigation (*F* = 5.1, *P* < 0.05) but herbicide had greater effect in the higher biomass Phalaris and Annual states than the Native state (Fig. 5.6).

Grass biomass of plots in spring differed greatly between the three grassland states (Fig. 5.7), which was predicted based on a visual assessment of their biomass in autumn, and was one of the primary reasons they were selected for the study. However, within each site, irrigation had relatively little impact on grass biomass and while showing a trend towards increased growth, grass biomass in irrigated plots...
Table 5.5: ANOVA of log-seedling leaf area ($m^2 \times 10^{-2}$) showing main effects and interaction terms.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>50.3</td>
<td>10</td>
<td>5.0</td>
<td>29.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>50.5</td>
<td>1</td>
<td>50.5</td>
<td>294.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Block</td>
<td>1.8</td>
<td>2</td>
<td>0.9</td>
<td>5.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Herbicide</td>
<td>4.8</td>
<td>1</td>
<td>4.8</td>
<td>27.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irrigation</td>
<td>0.9</td>
<td>1</td>
<td>0.9</td>
<td>5.1</td>
<td>0.025</td>
</tr>
<tr>
<td>Herbicide * Block</td>
<td>2.1</td>
<td>2</td>
<td>1.1</td>
<td>6.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Irrigation*Block</td>
<td>0.019</td>
<td>2</td>
<td>0.009</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Herbicide*Irrigation</td>
<td>0.19</td>
<td>1</td>
<td>0.19</td>
<td>1.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Herbicide<em>Irrigation</em>Block</td>
<td>0.026</td>
<td>1</td>
<td>0.026</td>
<td>0.15</td>
<td>0.70</td>
</tr>
<tr>
<td>Error</td>
<td>35.7</td>
<td>208</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>633.2</td>
<td>219</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>86.1</td>
<td>218</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.585$ (Adjusted $R^2 = 0.565$)

Fig. 5.6: Back-transformed estimated marginal means for surviving seedlings leaf area ($m^2 \times 10^{-3}$) for pasture and irrigation treatment combinations. Bars show ± 1 S.E.

was not statistically different from that in rain-fed plots ($p > 0.05$). The impact of changes in grass biomass on *E. microcarpa* growth in the pasture plots was negligible at all sites. Though, as for survival of seed-sown and planted seedlings, regression of seedling growth against grass biomass (separated by site) revealed a significant relationship for the Phalaris state when both bare plots and pasture plots were included in the analysis ($F = 12.4, P < 0.01, R^2 = 0.42$), confirming the significant impact of grass competition on tree seedling growth.
Fig. 5.7: Grass biomass of rainfed (non-irrigated) and irrigated pasture plots in the three grassland states, which were dominated by the exotic perennial grass *Phalaris aquatica*, a mix of exotic annual grasses (Annual) and native perennial grasses and forbs (Native), respectively.

5.4 Discussion

Grassland state had the biggest impact on seedling emergence and survival of planted seedlings, while the presence of herbaceous vegetation had a large negative impact on all response variables and was a stronger determinant of seedling growth than water supply. The response of seedlings to the presence of pasture was independent of its abundance (measured as above-ground biomass) – a result also found by Peltzer & Köchy (2001). The vast majority of seedling recruits occurred in plots where pasture had been removed. For the few seedlings that did emerge amongst existing vegetation, the majority occurred in the Phalaris state, and the least in the Native state, which was the opposite response to that hypothesised.

Irrigation of plots was of secondary importance, and was only effective in increasing eucalypt emergence and survival in plots where pasture had been removed. Through a process of elimination, these results identify water availability as the primary mechanism limiting recruitment at all three experimental sites. The light treatment had no effect on eucalypt germination in irrigated or non-irrigated plots at any of the experimental sites.

It is unlikely that nutrient availability had a direct influence on eucalypt emergence or growth, since earlier experimental work (Chapter 4) found nutrient to
have no effect on eucalypt seedling growth in abandoned annual grass-dominated pastures in the region.

Herbaceous vegetation or dense litter can act as a physical barrier to seedling emergence and root/shoot growth (Facelli et al. 1999), but separating this mechanism from resource limitations is difficult, particularly for below-ground competition. I did not attempt to differentiate between different impacts of below-ground plant-plant interactions, beyond measuring the effects of the herbaceous community on soil moisture. As there was minimal litter at our experimental sites, and the nature of the light treatment was to open the sward to allow light penetration to soil level, it is unlikely that the sward created a physical barrier to above-ground growth within the light treatment. Roots of pasture species may have been a physical barrier to tree root penetration, which Fensham and Kirkpatrick (1992) suggest limited forest eucalypt recruitment in southern Australia. It is possible that the response they observed was the result of moisture stress, though it is difficult to differentiate between below-ground mechanisms acting upon seedling recruits. I observed a slight increase in seedling emergence, survival and growth with irrigation, which suggests that if seedling emergence and/or root penetration was physically restrained by grassland species, the effect was reduced by increased soil moisture.

I had expected to observe effects of light limitation in the dense irrigated Phalaris sward, thereby investigating whether water availability was the only barrier to recruitment in this enriched grassland state. The irrigation treatment did increase water availability, which was shown by direct soil moisture readings and by significant pasture biomass and eucalypt seedling emergence and survival responses. However, irrigation did not remove water limitation altogether because the maximum practical application of surface irrigation could not overcome the high evaporation rates associated with hot, dry summer days. Consequently, the irrigation treatment in this study simulated a summer of above average rainfall, but not an environment free of water stress. Gordon & Rice (2000) also found that water availability was the key driver of tree seedling recruitment and growth in Quercus douglasii woodlands in California. The relationship was independent of whether soil moisture increases were due to the direct effects of increased watering, or increased availability of water due to reduced inter-specific competition.
In this experiment, seedling survival mirrored emergence responses, such that treatment effects on seedling establishment success were compounded over time. This consistency in emergence and survival response to competition from neighbours and manipulation of resource levels is not a feature of all communities. Smit et al. (2006) note that conditions which are safe for seeds are not necessarily safe for seedlings; interactions between herbaceous vegetation and newly recruited tree seedlings may change from facilitative to competitive (or vice-versa) at different life stages (Callaway & Walker 1997).

Facilitation of tree seedling emergence by the herbaceous layer is possible when the improvement in soil water relations due to shading exceeds the cost of increased competition from the sward for light and water (Holmgren et al. 1997). Examples of facilitation include the increased emergence of maple (de Steven 1991a, Berkowitz et al. 1995) ash, pine (de Steven 1991a) and tropical rainforest tree seedlings (Zimmerman et al. 2000) amongst grasses in old-fields when compared with germination in areas with all vegetation removed. Also, the association of tree saplings with ‘nurse shrubs’ reduced seedling predation (Smit et al. 2006). In most of these cases, the net positive effect of neighbours on seedling recruitment was short-lived, but the influence of emergence patterns on tree distribution can sometimes persist into later life stages (Garcia & Houle 2005). As potential facilitation and competition effects on tree seedling recruitment occur simultaneously (Holmgren et al. 1997), the net negative impact of the pasture sward on tree seedlings in our study indicate that any potential facilitative effects on recruitment of shading by the pasture were far outweighed by competition for soil moisture.

The potential for abiotic factors to moderate the influence of biotic factors on tree population dynamics (or vice versa; Mills et al. 2006) means that there may be multiple pathways to successful tree establishment within a given ecosystem (Dovčiak et al. 2005). For example, if soil water availability is generally limiting to eucalypt recruitment in south-eastern Australia, above-average rainfall at the time of seedfall may temporarily alleviate moisture stress, stimulating seedling emergence at a level high enough to compensate for high mortality at the seedling stage. This would result in a discrete climate-driven recruitment event. Similarly, removal or modification of the herbaceous layer by natural or man-made disturbances may create a pulse in
availability of resources (or release from resource limitation), leading to a recruitment pulse. Conversely, very low levels of recruitment may still be possible in average rainfall years if a small number of individuals are able to survive despite intense intra- and inter-specific competition for resources. This would result in slow but regular recruitment, rather than discrete, less predictable recruitment events. Watson et al. (1997) note that ‘background’ recruitment is quite common but generally overlooked by the literature in systems where large magnitude episodic recruitment contributes to population dynamics. In their study of recruitment in two arid-zone shrubs in southern Australia, ‘event-driven’ recruitment accounted for only 50-70% of total recruitment, so that both continuous processes and episodic events need to be considered.

In this experiment, removal of competition from the grass layer greatly increased germination compared to pasture plots under the same water regime, resulting in a ‘pulse’ effect. Irrigation reduced the negative impact of pasture competition on recruitment compared with unwatered plots, allowing seedlings to survive for longer in pasture plots, even though all ultimately perished. This indicates a trend towards the potential for ‘continuous’ recruitment. Whether or not disturbance to the grass layer historically resulted in ‘pulse’ or ‘continuous’ recruitment would have depended on the scale of the disturbance; for example fire, compared to isolated, small soil excavations by animals.

Between-state differences in the strength of treatment effects on survival of planted seedlings in my study is an example of change in influence of abiotic and biotic factors on recruitment across a productivity gradient (as discussed by Mills et al. 2006). Although the irrigated/herbicide-treated plots had the highest survival in all three states, irrigation had the greatest impact on seedling survival in the Native state, while the herbicide treatment was more important in the Phalaris and Annual states. Opposite results would be expected from infiltration rates and moisture holding capacity, since both these measures were lowest in the Native state. However, it is not surprising that the influence of the herbaceous layer on seedling survival is relatively less in the Native state, than in the Phalaris and Annual states, since the pasture biomass and therefore water requirements of the herbaceous layer in the Native state were very low. Removal of competition from herbaceous species in the Native site would result in only a minor increase in soil water relations relative to direct inputs.
from irrigation, and this was shown by direct measures of soil moisture in control (pasture) and herbicide-treated (bare) plots. In this low productivity environment, abiotic stresses (notably rainfall limitation) are the most important driver of recruitment success. The greater influence on recruitment of competition from herbaceous species compared with the simulated increase in rainfall (irrigation) in the Phalaris and Annual states reflects their influence on soil water availability as the key limiting mechanism in this system. This supports my original hypothesis, at least in part; though I could not supply enough water to demonstrate any potential restrictions on recruitment due to light availability (Fig. 5.9).

**Fig. 5.8:** (a) Hypothesised responses of tree recruitment to manipulation of water and light resources when competing with herbaceous species, and (b) responses observed.
By far the most important finding of this study was the impact of site location on recruitment success, relative to the impacts of within-site experimental treatments (Fig. 5.2, Fig. 5.3). I had expected that differences in tree recruitment success between the experimental sites would be primarily due to direct effects of the competition from the different types of pasture they supported, and that differences in recruitment in the herbicide (bare) plots, if found, would be minimal and reflect differences in soil properties.

The difference in soil properties between the three grassland states were substantial, and had as much, if not greater impact on seedling emergence, survival and growth than within-site experimental manipulation of plots. The far higher water holding capacity and lower compaction of the Phalaris state (reflected by bulk density) was expected from its higher soil organic matter content. Whether or not soil compaction has physical effects on eucalypt germination additional to the effects of compaction on soil moisture relations is investigated in Chapter 6. Although variability of soil fertility within the site cannot be known from one sample, there was almost two orders of magnitude difference in the mean levels of both nitrate-N and ammonium-N between the ‘native’ grassland state and the ‘phalaris’ grassland state. By definition (see McIntyre & Lavorel 2007), grassland states embody both characteristics of the sward and soil fertility, which are generally confounded at medium to long time frames because of the dynamic nature of community composition (Quétier et al. 2007). Just as soil fertility plays an important role in determining pasture composition, pasture composition can influence soil chemical and physical properties through organic matter buildup and negative or positive feedback loops in nutrient cycling (Ehrenfeld 2003). Consequently, the selection of the experimental sites based on differences in above-ground sward characteristics pre-empted that differences in soil properties would be found.

Because of the dynamic and confounded nature of soil-plant interactions within grassland states, it is likely that soil property differences observed between the sites reflected both human-induced (management) changes compounded over many decades, and pre-existing differences in soil resources due to the influence of parent materials or topography, though differences due to the latter were predicted to be minor.
The surprising aspect of these findings was not the degree of variation in fertility between the states per se, but that the impact of site fertility on tree recruitment would be so much greater than the biotic influences within each site. In a system where soil moisture is limiting, rainfall is seasonal and inter-annual rainfall variability is high, the moisture holding capacity of the soil at the time of seedling emergence is critical. The finding that highly enriched grasslands such as Phalaris-dominated communities are capable of supporting more tree recruitment than nominally less ‘degraded’, native-dominated systems when herbaceous competition is removed presents a philosophical difficulty for restoration ecologists.

Ungrazed swards of exotic perennial grasses such as *Phalaris aquatica* quickly displace and exclude native herbaceous species, but their high litter production and deep roots may increase soil organic matter and aid soil water infiltration, creating a positive feedback loop. Laungani & Knops (2009) similarly found that annual communities benefit from the recycling of soil nitrogen (Laungani & Knops 2009). Generally, exotic species deposit large amounts of litter in comparison with native species whose longer leaf lifespan corresponds to their strategy for survival in highly infertile environments (Wright et al. 2002). The potential for exotic species to increase soil organic matter, water infiltration and moisture holding capacity in the short term, and therefore benefit recruitment of native tree species (in association with intensive control measures such as herbicide use) come at a great cost to native species diversity of the understorey. Soil fertility exposes these native grassland states to increased risk of (re-) colonisation by exotic species (Ehrenfeld 2003), thereby reinforcing the existing impacts on understorey composition. Once the herbaceous layer is removed, soil nitrogen would be expected to decline due to exposure of soil to the elements (Prober et al. 2008), meaning that benefits to tree recruitment created by enriched grassland states would not be sustained, though soil phosphorus would be expected to persist (Standish et al. 2006). Short-term management interventions aimed at restoring the overstorey may be at odds with restoration goals for the grassy understorey.

The consequences of these findings for recruitment of woodland eucalypts in south-eastern Australia are in some respects positive. I have shown that significant disturbance or removal of the herbaceous layer is needed for high levels of recruitment to occur, regardless of its composition. However, very low seedling emergence was
possible within exotic species-dominated pastures, and where competition from herbaceous species was removed altogether, recruitment levels were (at least initially) higher in the enriched, exotic-species dominated grassland states, due to improved soil water relations at these more fertile sites.

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**Chapter 1: Introduction**
- Why is natural regeneration of woodland eucalypts important?
- Key research question: How does modification of the grass layer affect eucalypt recruitment?

**Chapter 2: Literature review**
- How have grassy woodlands been changed by land management?
- How do State and Transition models describe vegetation change?
- What are the main barriers to tree recruitment?
- How are vegetation changes caused by land management practices predicted to affect tree recruitment?
- How does seed supply vary in space and time due to natural processes and management influences?

**Chapter 3: Assessing the potential for natural tree regeneration between grassland states - a landscape-scale germination trial**
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- Does the duration of livestock exclusion affect recruitment potential?

**Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility**
- How does soil nutrient enrichment influence tree recruitment in an exotic pasture?
  - Do eucalypts and exotic pasture species both respond positively to nutrient additions when competitors are excluded?
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**Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia**
- What are the mechanisms limiting tree recruitment in high and low rainfall years?
- How do light, water and herbaceous biomass manipulations affect tree seedling germination, growth and survival?

**Chapter 6: The effects of soil compaction on germination and early growth of *Eucalyptus albens* and an exotic annual grass**
- Is eucalypt germination and growth inhibited in highly compacted soils?
- Is *Eucalyptus albens* more sensitive to soil compaction than a common grass weed, *Vulpia myuros*?

**Chapter 7: Summary and synthesis**
- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 6: The effect of soil compaction on germination and early growth of *Eucalyptus albens* and an exotic annual grass

6.1 Introduction

Soil compaction is prevalent in agricultural areas worldwide (Drewry et al. 2008). The impacts of soil compaction on crop and pasture growth are mostly adverse and are the subject of many reviews (Unger & Kaspar 1994, Greenwood & McKenzie 2001, Lipiec & Hatano 2003, Drewry et al. 2008). Remnant native vegetation and woody species used for reafforestation can also be adversely affected by soil compaction. This has been demonstrated in laboratory studies of seedling growth for two New Zealand bushland species (Bassett et al. 2005) and the Northern American Douglas-fir (Heilman 1981), in forest stands of *Pinus* spp. subject to heavy machinery loads during harvesting (Kozlowski 1999), and in heavily grazed *Eucalyptus salmonophloia* woodlands in Western Australia (Yates et al. 2000a).

Soil compaction in agricultural areas can occur through use of heavy machinery, trampling by livestock, slumping/settling of soil, or exposure of surface soil to the elements following fire or heavy grazing (Håkansson & Voorhees 1998, Kozlowski 1999, Drewry et al. 2008, Prober et al. 2008). Compaction caused by livestock trampling is especially severe in stock camps, which are distinct areas within a field where animals are inclined to gather. Stock camps are typically near the shelter of remaining paddock trees; the zone to which tree recruitment from natural seedfall is mostly restricted.

Low levels or absence of tree recruitment is cited as a major threat to the persistence of remnant woodlands in many agricultural areas in Australia (Yates & Hobbs 1997). Climatic factors, competition from exotic species, grazing/trampling of seedlings by livestock, predation of seed, and poor seed quality due to inbreeding of isolated trees are frequently cited as barriers to woodland regeneration (Nadolny 1995, Yates et al. 2000b, Semple & Koen 2001, 2003, Dorrough & Moxham 2005). The effects of soil compaction on tree recruitment in Australia are likely to be widespread, as most agricultural land has been affected by compaction to some degree (Greenwood & McKenzie 2001).
Compaction is characterised by increased soil bulk density, increased soil strength (mechanical resistance to penetration), increased soil water tension, and decreased air-filled porosity and infiltration. These effects may persist for years to decades after removal of the causal process (Braunack & Walker 1985, Greenwood & McKenzie 2001). The consequences of compaction for plant growth are reduced water, nutrient and oxygen availability (Kozlowski 1999), especially in early life stages (Smith et al. 2001, Bassett et al. 2005).

Plants differ in their tolerance of soil strength (Ferrero 1991, Smith et al. 2001, Williamson & Neilsen 2003, Bassett et al. 2005), so compaction levels commonly recorded in agricultural areas may limit germination and growth of some species but not others. Cass (1999) and Marerechera et al. (1991) suggest a soil penetration resistance of 3 MPa as a possible maximum threshold for survival of many crop species. Root growth is likely to be restricted by soil strengths greater than 1 MPa, and seedling emergence may be unsuccessful in soils between 1 MPa and 2 MPa at field capacity, as resistance is likely to exceed 3 MPa before wilting point. However, few experimental studies have tested the response of Australian native plant species to increases in soil compaction.

Smith et al. (2001) found that at 7% moisture, two Corymbia species were able to penetrate a sandy loam soil of bulk density 1.6 Mg m$^{-3}$ but not 1.8 Mg m$^{-3}$; while at 10 % soil moisture, both species could penetrate at the higher bulk density, though root length was reduced by around 60%. Misra & Gibbons (1996) found that root length of Eucalyptus nitens also decreased with increasing bulk density, from 0.7 to 1.0 Mg m$^{-3}$, corresponding to penetration resistances of 0.4 to 4.2 MPa.

Eucalyptus albens Benth. (White Box) is the dominant overstorey species in many grassy woodlands on the Western Slopes of New South Wales. These ecosystems have been highly modified by agriculture, and like other woodland eucalypts, the long-term persistence of E. albens is threatened in many cases by poor regeneration (DECC 2008). This species is often included in species lists for restoration activities (eg. Berwick 2007, Earl et al. 2001, Stelling 1998).

This study aimed to investigate the effects of soil compaction on emergence and early root/shoot growth of E. albens germinants and compare their response to that
of an exotic annual grass species *Vulpia myuros* (L.) J.Gmelin (Rat’s Tail Fescue), which is abundant in many degraded woodlands and a common competitor of small eucalypt seedlings (Prober *et al.* 2002). These two species are hereafter referred to as *Eucalyptus* and *Vulpia*. I predicted that *Vulpia* would be more tolerant of increases to soil bulk density than *Eucalyptus*, suggested by its very high abundance in heavily grazed woodlands (Prober *et al.* 2002). The results of this study are discussed in the context of current attempts to improve rates of *Eucalyptus* recruitment from natural seedfall in the agricultural areas where it occurs.

### 6.2 Methods

#### 6.2.1 Preparation of soil cores

The effect of soil compaction on the germination, survival and early growth of *Eucalyptus* and the exotic annual grass *Vulpia* was compared in a fully randomised laboratory experiment using reconstructed soil cores of varying bulk density.

Soil for the experiment was collected near Benalla in North Central Victoria (36°31'S 145°43'E) from the upper 25 cm of the soil profile within a paddock subject to more than a century of livestock grazing but which had been rested in the previous 5 years. The site had yellow duplex soils characteristic of former woodlands on mid slopes in the region, and an A horizon bulk density of around 1.3 Mg m⁻³. This represented the upper end of the bulk density range found for long-grazed paddocks in the region (Skinner, unpublished data, 2006). Soil was passed through a 2 mm sieve, air-dried, and stored in the laboratory until use. A soil mix of 20% water content by mass (within the plastic limit of the soil) was prepared in a single batch by adding deionised water to sieved soil of known water content. The soil was mixed, left to equilibrate overnight in a sealed tub and mixed again before use the following day.

Five replicate cores of each of five soil bulk density levels (1.0, 1.1, 1.2, 1.3 and 1.4 Mg m⁻³) were created by packing pre-determined weights of the soil mix into cylindrical poly-vinyl (PVC) piping of 71 mm internal diameter and 100 mm length using a hydraulic press and custom-made piston with 10 mm gradations. These bulk densities are not as high as can be found in agricultural areas and some heavily grazed remnant woodlands (Ferrero 1991, Yates *et al.* 2000b), but preliminary compression trials established 1.4 Mg m⁻³ as the maximum practical bulk density for this soil, as
higher compression levels resulted in deformation of the PVC tubing. A void of 10 mm was left at the top of each core, giving a final soil depth of 90 mm and volume of 356.3 cm². To attain relative uniformity of surface compaction, soil was compressed from the top in one event as one layer. After soil compression, the bottom of each core was sealed with tightly fastened clear plastic ‘cling-film’ and aluminium foil.

6.2.2 Seed addition and laboratory conditions

_Eucalyptus albens_ seed used in the experiment was collected near Tarawingee, Victoria (36°21’S 146°19’E) in April 2005 (sourced from the North East Community Seedbank), and _Vulpia myuros_ seed near Young, NSW (34°15’5 148°14’E) in 2003 (supplied by S. Prober). In March 2007, either 20 _Eucalyptus_ seeds or 10 _Vulpia_ seeds were placed on the surface of each core, based on species differences in seed size. Prior to this, laboratory germination tests identified seed viabilities of over 98% for both _Eucalyptus_ and _Vulpia_ seeds. Seeds were not pre-treated. Germination was initiated by hand-spraying with water (1.5 ml/core), then cores were placed in a humidified crib (as per Misra & Gibbons, 1996) under fluorescent lights (PAR = 95 μmol m⁻² s⁻¹) set to a 12hr/12hr light/dark cycle in a 23°C constant temperature room. Germination occurred in all cores within four days. The fungicide furalaxyl was applied as a light mist over all cores three times weekly to protect seedlings from fungal attack.

Three weeks after the start of the experiment, seedlings were thinned to a maximum of 12 per core for _Eucalyptus_ and eight per core for _Vulpia_, to minimise intraspecific competition effects on seedling shoot and root growth while providing insurance against early _Eucalyptus_ mortality. Seedlings were harvested 6 weeks after sowing by splitting the PVC piping, soaking soil cores in water overnight and extracting seedlings and root-washing by hand. Seedlings were photographed prior to the separation of roots for automated analysis of total root length (primary + laterals) using the software WinRhizo® (Regent Instruments Inc., 2007). Maximum shoot length, maximum root length and maximum vertical depth of root penetration (root depth) of all surviving seedlings was measured manually from photographs of extracted seedlings (Fig. 6.1) with the aid of the image analysis software ImageJ® (NIH Image 2007).
Assessments of shoot length (A to B), primary root length (B to C) and root depth (indicated by double-ended arrow), were carried out manually from photographs with the aid of the image analysis software ImageJ® (NIH image 2007), which used pixel units to obtain length estimates for curved shoots and roots. Total root length (including secondary roots) and other root variables were obtained from automated root scans using the software WinRhizo® (Regent Instruments Inc., 2007).

6.2.3 Analysis

All statistical analyses were carried out on core-level averages of surviving seedlings using SPSS® (Release 14.0.2, 2006). Two Eucalyptus cores and one Vulpia core were excluded from the analysis due to fungal attack on seedlings. Data were checked for normality and transformed where required to achieve homogeneity of variance. Analysis of variance was carried out on average shoot length, average maximum root length and the transformed ln (average root depth) of seedlings, with species and bulk density as fixed factors within a General Linear Model. The model was investigated for possible species*bulk density interaction effects, and ANOVAs were then run independently for each species. Post-hoc Tukey multiple comparisons were used to test for differences significant at $P < 0.05$.

6.3 Results

Bulk density treatments (1.0, 1.1, 1.2, 1.3 and 1.4 Mg m$^{-3}$) had no effect on germination, shoot height, primary root length, root to shoot ratio, total root length (including primary and lateral roots), root surface area, root volume or number of root tips for either species. However, species differences were large. Vulpia had significantly higher values than Eucalyptus for all the attributes above with the exception of root volume (Table 6.1).
In contrast to the above variables, greater soil bulk density led to significantly less root soil penetration for both species (\( P \leq 0.001 \); Table 6.2; Fig. 6.2). The increase in bulk density from 1.0 Mg m\(^{-3} \) to 1.4 Mg m\(^{-3} \) led to a 77% and 75% reduction in root depth of *Eucalyptus* and *Vulpia* respectively (Fig. 6.2).

**Table 6.1:** Species means for germination, shoot and root response variables which were not influenced by soil bulk density. Means represent averages of all surviving seedlings for each core, pooled across all bulk density treatments (N = number of cores). \( P \)-values show significance of differences between species.

<table>
<thead>
<tr>
<th>Response variable</th>
<th><em>Eucalyptus</em> (n=23)</th>
<th><em>Vulpia</em> (n=24)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>Shoot length (mm)</td>
<td>70.0 ± 2.5</td>
<td>92.5 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum root length (mm)</td>
<td>16.6 ± 5.1</td>
<td>97.9 ± 4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total root length; primary + laterals (mm)</td>
<td>45.4 ± 5.9</td>
<td>122.0 ± 5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>1.1 ± 0.05</td>
<td>3.5 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Root surface area (mm(^2) x 10(^{-3} ))</td>
<td>5.1 ± 0.40</td>
<td>9.5 ± 0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Root volume (mm(^3) x 10(^{-6} ))</td>
<td>5.0 ± 2.1</td>
<td>5.9 ± 1.6</td>
<td>0.059</td>
</tr>
<tr>
<td>Number of root tips</td>
<td>17.4 ± 0.8</td>
<td>39.1 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 6.2:** ANOVA of ln(average root depth) showing species and bulk density effects, and the lack of interaction between species and bulk density.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>15.712</td>
<td>9</td>
<td>1.746</td>
<td>10.550</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>239.209</td>
<td>1</td>
<td>239.209</td>
<td>1445.561</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species</td>
<td>2.089</td>
<td>1</td>
<td>2.089</td>
<td>12.626</td>
<td>0.001</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>13.103</td>
<td>4</td>
<td>3.276</td>
<td>19.796</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species * Bulk Density</td>
<td>0.043</td>
<td>4</td>
<td>0.011</td>
<td>0.065</td>
<td>0.992</td>
</tr>
<tr>
<td>Error</td>
<td>5.957</td>
<td>36</td>
<td>0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>259.219</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>21.669</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( R^2 = 0.725 \) (Adjusted \( R^2 = 0.656 \))

At the lowest soil bulk density (1.0 Mg m\(^{-3} \), which is the least compacted soil), the root depth of *Eucalyptus* was equivalent to its average root length, indicating vertical root growth. However, at higher compaction levels, root depth fell short of average root length, due to oblique (non-vertical) growth. By contrast, *Vulpia* root depth was less than its average root length even at the lowest bulk density. *Vulpia* had oblique root growth in all of the experimental treatments.
Fig. 6.2: Mean root depth of 6 week old (a) *Eucalyptus albens* and (b) *Vulpia myuros* seedlings across five bulk density treatments. Bars show ± 1 SE. Treatments with the same letter(s) above error bars are not significantly different ($P > 0.05$). The dotted lines indicate maximum root length (in comparison to depth) of seedlings for each species across all bulk density treatments. Data were back-transformed for presentation.
6.4 Discussion

6.4.1 Effects of bulk density on germination, shoot and root growth

In this study, increasing soil bulk density reduced root depth to a similar degree for *Eucalyptus* and *Vulpia*. Misra & Gibbons (1996) also observed a large (71%) reduction in primary root depth of *Eucalyptus nitens* with increase in bulk density from 0.7 Mg m$^{-3}$ to 1.0 Mg m$^{-3}$. I considered root depth (maximum vertical root penetration) to be the most relevant indicator of compaction effects, especially for young seedlings during the critical phase immediately following germination. Under field conditions, the large (~75%) reduction in root depth at the highest soil bulk density for both *Eucalyptus* and *Vulpia* would translate to a reduction in water availability due to surface drying. This would reduce the fitness of individual seedlings in times of moisture stress.

Soil physical characteristics and climatic conditions combine to determine the depth to which wicking of soil moisture by surface evaporation occurs and the degree to which root limitations will affect seedling success (Unger & Kaspar 1994). Desiccation due to high temperatures and soil surface drying is likely to be the greatest risk to seedling survival immediately after germination, but the importance of maximising root depth remains in later phases of establishment as seedlings compete for soil moisture with surrounding vegetation.

Under laboratory conditions I found that germination was unaffected by bulk density. Adequate moisture was provided for seed imbibition, and emergence of radicle and cotyledons relied only on seed reserves. Similarly, mortality of newly emerged seedlings was not seen, even in cases where radicles failed to immediately penetrate the soil surface, since the humid conditions provided protection against desiccation. Although this humid environment is not typical of field conditions, my aim was to test the effects of increasing penetration resistance on seedlings, rather than the correlated effects of moisture stress.

In contrast to root depth, I found that most root and shoot growth attributes of *Eucalyptus* and *Vulpia* did not differ across bulk density levels. This result was unexpected, but is explained by the oblique growth of both primary and secondary roots observed in soil cores for both species. This oblique growth was associated with a significant reduction in root depth with increasing bulk density. The maintenance of
root length through oblique growth appears to have prevented stunting of shoot growth associated with nutrient deficiency or moisture stress due to restricted soil exploration by roots (Kozlowski 1999). I found no evidence of shoot growth being affected by root growth changes in this study, since the volume of surface soil accessed by oblique-growing roots can be assumed to equal roots of the same length growing to depth.

Some surface root growth was seen for the New Zealand natives *Cordyline australis* and *Leptospermum scoparium* in a study comparing bulk densities of 0.7, 0.84 and 0.91 Mg m\(^{-3}\) for silty clay, and silty clay loam soil types (Bassett *et al.* 2005). In their study, increased compaction led to a substantial reduction in the number of seedlings penetrating the soil surface and longer time to penetration where it occurred. I was unable to determine the time to root penetration in our cores, as some seedlings which appeared to have only surface root growth were found when harvested to have roots which had penetrated the soil surface, though these remained shallow.

### 6.4.2 Differences in response of Eucalyptus and Vulpia

The exotic annual grass *Vulpia* had longer roots and shoots than the woody perennial *Eucalyptus* for each individual bulk density treatment in our experiment. This corresponds to *Vulpia*’s higher initial growth rate and larger seed size, and is consistent with the accepted life history strategies of *Vulpia* and *Eucalyptus* as ruderal and competitor species respectively (Grime 1977). Contrary to our hypothesis, *Eucalyptus* and *Vulpia* showed similar relative declines in root depth with increasing soil bulk density, showing that both species are sensitive to high levels of soil compaction despite innate differences in root thickness and physiology. This similarity was surprising, because *Vulpia* was predicted to be less sensitive to compaction, based on the observation that it, like other annual exotic weeds, is highly abundant and resilient in degraded areas where other species are not. Unlike *Eucalyptus*, the failure of *Vulpia* roots to achieve their maximum potential depth in the least compacted soil (as indicated by root depth less than average root length) suggests that *Vulpia* is as sensitive to soil compaction as *Eucalyptus* (independent of species differences in root length). Therefore the success of *Vulpia* in Australian woodlands is in spite of its sensitivity to compaction, and is most likely due to its fast growth and high fecundity,
enabling it to overcome limitations of other stresses. The tendency of *Vulpia* toward oblique root growth even at low soil bulk density could be an exploratory characteristic of fibrous root systems in general, which at maturity are shallower than the tap-root systems of woody species (Schenk & Jackson 2002). This may provide an advantage for *Vulpia* in compacted soil areas or a strategy to deplete soil water in the topsoil and so outcompete other shallow-rooted species, though this was not specifically tested in this study.

The sensitivity of *Eucalyptus* and *Vulpia* to compaction, while not significantly different in our experimental conditions, might differ between species at lower soil moisture contents. The slower average root extension of *Eucalyptus* than *Vulpia* (reflected by difference in root length at the end of the experiment) could convey an increased drought tolerance. However, the merits of such a strategy would to a large degree be undermined by the expected reduction in fitness of shallow-rooter individuals in exposed environments. *Eucalyptus*, as a smaller seeded, slower growing species, has a greater reliance on successful root penetration for survival.

### 6.4.3 Application to restoration activities

As soil bulk density levels in agricultural areas frequently exceed the maximum bulk density used in our experiment (Ferrero 1991, Yates *et al.* 2000b, Greenwood & McKenzie 2001), impacts of compaction on seedlings may be even more severe in the field. However, extrapolation of laboratory studies on plant responses to compaction to field situations should be made with caution. Soil bulk density measurements recorded in this study provide an indication of relative compaction within a given soil type and context but in most situations, absolute values cannot be simply compared between two soil types. Whilst the response of roots to compaction is a response to soil strength or penetration resistance rather than bulk density *per se*, the uniformity of bulk density allows me to more accurately test the relative effects of compaction on plant growth over a period of several weeks. For this reason, I chose to vary bulk density at constant high moisture content.

The re-created soil cores used in my study lacked biopores which may be present in field soil samples of equivalent bulk density. Biopores can reduce the impact of compaction by improving oxygen diffusion and providing ‘pathways’ for roots to
bypass zones of high soil strength (Lipiec & Hatano 2003). Nevertheless, the advantage of our chosen method was the ability to accurately manipulate soil bulk density, which would not have been possible in the field. My results show that *Eucalyptus* and *Vulpia* are significantly disadvantaged by high levels of soil compaction. In particular, *Eucalyptus* seedlings are more susceptible to surface drying in compacted than uncompacted soils due to reduced root depth. Therefore *Eucalyptus* seedlings would face a greater risk of desiccation during the critical months after a regeneration event.

Nothwithstanding previous cautionary comments, these results have important implications for restoration of woodland remnants. The degree to which compacted soils can be ameliorated through natural biological processes and rest from grazing is site-specific, and varies due to differing soil types, past land use history, prevailing climate (wetting and drying cycles), plant composition and soil biota (Greenwood & McKenzie 2001; Yates *et al.* 2000ab). Natural recovery of degraded soils is a long-term process that may take centuries (Webb *et al.* 1983; Lovich & Bainbridge 1999). For example, in grazing exclosure sites in the South West Slopes of NSW, gradual but significant reductions in soil compaction were found seven to nine years after the removal of grazing (Spooner & Briggs 2008).

Amelioration of soil compaction by ripping is often used to prepare soils for replanting or direct seeding. Although mechanical disturbance can greatly assist natural regeneration, it may be undesirable or impractical in many areas. Consequently, closer attention to degree of compaction at potential regeneration sites is important to managers’ expectations for tree seedling establishment success. This may aid the prioritisation of resources towards sites of higher regeneration potential.

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Chapter 1: Introduction
- Why is natural regeneration of woodland eucalypts important?
- Key research question: How does modification of the grass layer affect eucalypt recruitment?

Chapter 2: Literature review
- How have grassy woodlands been changed by land management?
- How do State and Transition models describe vegetation change?
- What are the main barriers to tree recruitment?
- How are vegetation changes caused by land management practices predicted to affect tree recruitment?
- How does seed supply vary in space and time due to natural processes and management influences?

Chapter 3: Assessing the potential for natural tree regeneration between grassland states - a landscape-scale germination trial
- How does eucalypt germination and survival differ between pasture states?
- Does the duration of livestock exclusion affect recruitment potential?

Chapter 4: Eucalyptus recruitment in degraded woodlands: no benefit from elevated soil fertility
- How does soil nutrient enrichment influence tree recruitment in an exotic pasture?
  - Do eucalypts and exotic pasture species both respond positively to nutrient additions when competitors are excluded?
  - Does increased pasture growth reduce tree growth?

Chapter 5: Mechanisms regulating tree recruitment in three woodland understorey states in south-eastern Australia
- What are the mechanisms limiting tree recruitment in high and low rainfall years?
- How do light, water and herbaceous biomass manipulations affect tree seedling germination, growth and survival?

Chapter 6: The effects of soil compaction on germination and early growth of *Eucalyptus albens* and an exotic annual grass
- Is eucalypt germination and growth inhibited in highly compacted soils?
- Is *Eucalyptus albens* more sensitive to soil compaction than a common grass weed, *Vulpia myuros*?

Chapter 7: Summary and synthesis
- What are the key findings from my experimental work on the effects of understorey modification on woodland eucalypt recruitment?
- How might the effects of land management practices interact to limit recruitment?
Chapter 7: Summary and synthesis

In this chapter I first summarise the findings of previous chapters in this thesis. I then integrate the results, consider the limitation of my experiments, and describe improvements that could be made to aid interpretation. Next I develop a new conceptual model that describes the effects of grassland states and episodic events on tree recruitment, and I discuss the implications of my findings for management and future research.

Natural regeneration of woodland eucalypts from seed has the potential to contribute to catchment-scale revegetation targets in areas set aside from agricultural production. The legacy of agricultural land use is extensive modification of the understory, yet the impact of understorey modification on tree recruitment is not known. Consequently, I investigated how woodland eucalypt recruitment differs between grassland states, and the mechanisms by which grassland states influence tree recruitment.

Livestock grazing was removed in my experiments, since grazing is widely recognised as restricting tree recruitment and fencing to exclude livestock is typically the first step taken in restoration programs aimed at promoting the natural regeneration of trees.

Grassy woodlands in south-eastern Australia have changed greatly owing to agricultural development since European settlement. In pastoral areas the overstorey has been cleared to a large extent and the understorey has been modified by cultivation, grazing and fertiliser application, resulting in dominance of exotic species across most of the landscape. Woodland eucalypts remain only within small remnant areas or as isolated paddock trees, and since recruitment of eucalypt seedlings is restricted, the survival of grassy woodlands is threatened.

The literature from Australian woodlands shows that recruitment of woodland eucalypts is naturally episodic and infrequent, and is limited by bottlenecks at the germination and seedling life stages. Various studies have shown that eucalypt germination relies on the coincidence of mass seedfall and high rainfall to overcome competition from the herbaceous layer. High levels of seed predation by ants and intense competition from the grass layer are also key natural barriers to recruitment.
Historically, fire has played a key role in promoting eucalypt recruitment by stimulating mass seedfall and satiating seed predators, temporarily removing competition from the grass layer and providing a fertile ash seedbed suitable for germination. Recruitment can still occur in the absence of fire, albeit less often and with lower seedling densities.

Agricultural development has reduced the potential for tree recruitment in several ways including fire suppression and less seed production. Seed deposition per unit area has been reduced by both clearing of adult trees and lower seed production per tree caused by isolation of trees and inbreeding. Competition from grasses is known to be a major barrier to recruitment, but the effects of understorey modification on eucalypt recruitment have not been well studied; this has been identified as a major gap in tree recruitment models in Australia. In light of the large extent of understorey modification and increasing interest in using natural regeneration to restore grassy woodlands, a better understanding of the effects of understorey modification on recruitment is needed.

In this chapter I assessed germination of two common grassy woodland trees, *Eucalyptus albens* and *Eucalyptus microcarpa* in five different grassland states that were categorised by management history and dominant species. Sites that had been recently grazed were compared to those that had been fenced to exclude livestock for more than three years. Germination occurred in all pasture types, including dense annual and perennial exotic pastures and was significantly higher in the annual exotic grass (*Bromus/Hordeum*) dominated grassland state than other states. However, the overall germination percentage (0.67%) across all states was very low. The duration of fencing did not affect germination significantly, and the difference in germination between grassland states was not explained by pasture biomass.

Severe spring drought caused all seedlings to die during summer showing climate has an over-riding effect on tree recruitment in grassy woodlands providing seedfall is adequate. Recruitment is unlikely to occur in years of low rainfall due to seedling desiccation soon after emergence. In years of high rainfall, it is expected that pasture composition and other understorey characteristics will have a stronger influence on seedling survival and tree recruitment.
My results showed that the *Bromus/Hordeum* exotic annual grass-dominated grassland state provided more favourable conditions for woodland eucalypt germination than the other grassland states. Due to the high risk of observing 100% mortality in low rainfall years, successive experiments were subsequently undertaken locally where irrigation could be used to supplement rainfall.

Poor tree recruitment is commonly attributed to increased competition from dominant exotic annual grasses and forbs in the understorey – a situation that often occurs due to soil nutrient enrichment. In Chapter 4 I investigated the effects of soil nutrient manipulations on the competitive interaction between eucalypt seedlings and pasture species, in order to test whether the benefits to trees conferred by increased soil nutrients were outweighed by increased competition from a pasture of exotic annual grasses. In this experiment, survival and growth of six-month old seedlings were assessed in irrigated plots with and without competition from different pasture species. Secondly, this experiment trialed the use of refined sugar and fertiliser to decrease and increase soil nitrate respectively.

Soil nutrient manipulations did not affect seedling survival or growth, whether or not in competition with pasture. In contrast with observations of other researchers, I found that the sugar treatment did not significantly reduce soil nitrate or pasture biomass.

Pasture biomass from the lowest (~1.5 t ha\(^{-1}\)) to the highest (~7 t ha\(^{-1}\)) level suppressed eucalypt seedling growth similarly, but where competition from pasture species had been completely removed using herbicide, seedling survival and leaf area more than doubled in comparison to pasture plots. The negative impact on eucalypt recruitment of competition from the herbaceous layer was consistent across all experimental work undertaken. Differences in eucalypt growth between pasture plots and bare plots were attributed to the effects of pasture on competition for soil moisture rather than light availability, since seedlings were too large to be shaded by the pasture.

These results suggest that soil nutrient enrichment to levels typical of grazing land in south-east Australia has no benefit to eucalypt recruitment, either with or without competition from grasses. The expected cost to eucalypts of increased competition from pasture species with increasing soil nutrient was not realised due to
the ability of pasture to suppress eucalypt seedling growth, even when biomass was very low.

I found that seedling mortality stabilised by mid-summer. Although significantly lower than in competition-free plots, survival of seedlings in the pasture was as high as 8% for *E. albens* and 4% for *E. microcarpa*. Thus on average under these experimental conditions one seedling would survive to the end of summer for every 13 *E. albens* seedlings and 25 *E. microcarpa* seedlings present in spring.

There are many limitations on the extrapolation of experimental results to natural situations and landscapes. The use of irrigation to establish seedlings in this study means that estimates of seedling survival may be conservative overestimates. Conversely, the use of nursery grown seedlings (as a way of investigating seedling growth and survival without the inherent risk of relying on sufficient germination *in situ*) is likely to underestimate survival due to larger shoot-root ratios (and shallower roots) compared with seedlings of the same age that have germinated in the field. The mortality rates observed for seedlings during their first summer were specific to the prevailing climatic conditions and to that life stage of seedling growth. Overall, mortality at the germination stage would be much higher under natural conditions than in my experiments, as indicated by the landscape scale experiment in chapter 3.

In chapter 5, a further field experiment was designed to examine interactions between eucalypt germination, survival and growth in competition with pasture species when light and water status were manipulated.

Results showed firstly that competition for soil water was the primary mechanism limiting tree recruitment even when water supply was maximised, and secondly, that differences in soil physical properties between sites affected eucalypt seedling growth and survival as much as within-site manipulations of pasture biomass, light availability and soil water. Between-site soil effects reflected the influence of differences in soil water availability, highlighting the importance of landscape features and underlying soil properties (particularly soil organic carbon content and moisture holding capacity) for successful recruitment.

Watering generally increased germination, growth and survival of seedlings greatly, but not more than removal of grasses using herbicide. However in the native
grassland state investigated in my experiment, herbaceous biomass was very low, so removal of the sward had minimal effect.

Light limitation was not observed in any of the pastures, which is not surprising in view of the extremely high levels of photosynthetically active radiation (PAR) in spring and summer in southern Australia. I conclude that competition for soil water is the primary mechanism that limits eucalypt recruitment and drives differences between grassland states. Removing competition from the grass layer resulted in substantial increases in germination and seedling survival at the three experimental sites, but the size of the effect was far greater in the exotic-dominant pastures than the native-dominant pasture.

Land use impacts on recruitment are not restricted to the effects of competition from grasses. Compaction of surface soil by livestock or heavy machinery can substantially reduce the rate of root soil penetration and put young seedlings at a greater risk of desiccation due to more rapid surface soil drying. In a laboratory experiment (Chapter 6) I found that an increase in soil bulk density from 1.0 Mg m\(^{-3}\) to 1.4 Mg m\(^{-3}\) (at 20% soil moisture) resulted in a 75% decrease in root depth for six week-old *E. albens* seedlings.

Comparison of root depth of *E. albens* seedlings with an annual exotic grass competitor (a common weed), *Vulpia myuros*, under laboratory conditions of imposed compaction showed a similar root depth response for both species. I concluded that any competitive advantage to *V. myuros* present in field conditions is not due to a greater tolerance of soil compaction.

Competition from the herbaceous layer had a consistently large, negative impact on eucalypt germination in all experimental work undertaken. The major obvious inconsistencies in my findings were the very low level of germination in Phalaris-dominated pastures relative to other grassland states in Chapter 3, compared to relatively higher germination in the Phalaris pasture in Chapter 5, which was attributed to the extremely low water availability due to drought in the Chapter 3 experiment as described (Sect. 3.4).
7.1 Synthesis: Soil water limitation

In accord with many reports in the literature, I found that competition for soil water was the key limitation to seedling emergence, survival and growth (Chapters 3, 4 and 5). This has interesting consequences for the interpretation of the impacts of understorey modification on recruitment in southern Australia. At continental scales, numerous models have identified mean annual precipitation as the most important predictor of woody species cover (Fensham et al. 2005, Sankaran 2008). At smaller scales, investigations of the mechanisms of recruitment limitation in grassy woodland systems have also found that competition for soil moisture rather than competition for light is the driver of seedling success (Davis et al. 1999, Sánchez-Gómez et al. 2006). In a minority of cases herbaceous vegetation increases seedling emergence, most commonly through improved soil moisture as an indirect effect of shading (Holmgren et al. 1997, Davis et al. 1999, Suding & Goldberg 1999).

I confirmed that the grass layer competes strongly with tree seedlings in grassy woodlands and savannas and ‘wooded pastures’. This is identified in most experimental studies of tree-grass interactions, and has been demonstrated experimentally for several eucalypt species within pastoral areas of Australia (e.g. Cluff & Semple 1994, Lawrence et al. 1998, Semple & Koen 2003, Moxham & Dorrough 2008). However, few studies have experimentally tested the effects of alternative grassland states on recruitment (Semple & Koen 2003).

It is commonly hypothesised that competition from exotic species inhibits eucalypt regeneration (Curtis 1990, Windsor 2000, Yates Hobbs & Atkins 2000, Cramer et al. 2007). This hypothesis is supported by correlations between exotic annual grass cover and lower probability of tree recruitment found in two landscape-scale surveys of agricultural areas in south-east (Dorrough & Moxham 2005, Spooner & Briggs 2008). However, the mechanisms behind these responses are not elucidated. Inhibition of eucalypt recruitment by exotic annual weeds has been attributed to increased intensity of competition for soil water (Cramer et al. 2007), associated with homogenisation of soil surface microtopography and loss of ‘microcatchments’. However, in the experiment in chapter 5, I found higher soil water availability and higher tree recruitment in the exotic annual state than the native perennial state. My
experimental findings did not support the hypothesis of reduced recruitment potential in exotic annual pastures compared to predominantly native pastures. No evidence was found for occurrence of a recruitment threshold in the State and Transition model of Fig. 3.1, which was hypothesised to occur between the ‘Sown pasture’ and ‘Enriched grassland’ state. My findings suggest that a recruitment threshold associated with water use of herbaceous competitors is likely to exist within all grassland states irrespective of species identity; with the location of this threshold tempered by the soil properties that affect moisture availability.

In order to integrate these apparently conflicting observations, I developed a conceptual model of the benefits and costs to tree recruitment of soil water availability as influenced by land management practices. Figures 7.2 ands 7.3 represent alternative models of landuse impacts on tree recruitment to the State and Transition models presented in Chapters 2 and 3.

7.2 Conceptual framework for effects of rainfall variability on recruitment

Patterns of recruitment vary across a range of spatial and temporal scales. While mass tree recruitment events across years are typically the result of release from interspecific competition following landscape-scale disturbances such as fire or flooding (Gill 1997, Windsor 2000), more regular recruitment of fewer seedlings at small scales (‘background recruitment’) can still occur given sufficient seed supply and above-average rainfall (Curtis 1990, Watson 1997, Clarke 2001).

A hypothetical example of how the probability of recruitment responds to variation in rainfall between years is provided in Fig. 7.1, adapted from Lopez et al. (2008). This curve is based on recruitment of *Prosopis* spp. obtained using tree-ring and historical climate data along a rainfall gradient in Peru and Chile, but for the purposes of this discussion, the values of annual precipitation are arbitrary, since the response is specific to the species and the environment.

The solid line in Fig. 7.1 indicates the probability that a given value of mean annual rainfall exceeds a threshold for recruitment for a specified species; in this example, a probability of 0.05 is reached at just under 100mm annual rainfall. Since rainfall is only one of many factors identified as important for successful recruitment
(chapter 2), recruitment may still be low in many years where the annual rainfall is above the purported threshold. The threshold probability curve does not inform the recruitment response to increasing rainfall above the threshold value itself, but numerous experimental studies and landscape-scale models support an increased likelihood of recruitment with increasing rainfall. Here, eucalypt recruitment response to rainfall is presented in terms of its relative probability under a particular set of conditions.

The middle of the three broken lines in Fig. 7.1 represents the probability of recruitment occurring across a gradient of increasing rainfall in an undisturbed understorey in ‘reference condition’. The lower (left-most) inflexion in the curve indicates the presence of recruitment threshold associated with rainfall limitation, below which recruitment is unlikely to occur; while the upper (right-most) inflexion indicates an upper rainfall threshold, above which recruitment is limited by other factors. Management changes vegetation composition and soil properties, and these in turn control the quantity and seasonality of water uptake, so management practices may increase or decrease the water available for tree seedlings, and thus shift the probability of recruitment to the left or right along the rainfall axis. Since the various influences on eucalypt emergence and survival - including rainfall, seed production, seed predation, competition from the herbaceous layer, soil nutrient cycling – can interact (chapter 2), changes in the recruitment probability response owing to land management are likely to be more complex than a horizontal shift in the curve, and could result in an overall increase or decrease in the probability of recruitment at the highest rainfall values.
**Fig. 7.1:** Conceptual model of precipitation thresholds to recruitment, and the response of recruitment to increasing precipitation. The left-hand axis and solid line indicate the probability that a given value of mean annual rainfall exceeds a threshold for recruitment for a specified species (adapted from Lopez et al. 2008). The horizontal dotted line indicates the statistical probability value of interest; in this case, a p-value of 0.05. The broken line (right-hand axis) depicts the probability of recruitment occurring along a gradient of increasing annual rainfall, which may be shifted to the left or right by a relative decrease or increase in water uptake associated with changes to pasture composition.

The threshold responses described above also operate at monthly, weekly or even daily scales (Lopez et al. 2008), such that recruitment potential also varies greatly within years. Lack of soil moisture in the hot, dry summer months is an effective barrier to seedling emergence, and a major cause of seedling mortality (Stoneman 1994). Consequently, the probability of recruitment over summer is very low, and the windows of maximum opportunity for recruitment occur outside this period. Like the recruitment response to annual rainfall, management-driven changes to pasture composition and soil properties can influence water deficits over spring, summer and autumn.

A conceptual model of how understorey modification can alter the windows of opportunity for eucalypt recruitment is presented in Figures 7.2 and 7.3.

Figure 7.2 gives an example of how soil moisture content changes throughout the year, and how differences in soil water use by alternative pasture states can alter
recruitment potential within years. This shape of this curve is typical of most southern Australian systems (Dunin et al. 1999, BOM 2009), and reflects the pattern of rainfall minus evaporation, with a slight temporal delay (i.e. shifted slightly to the right) because of soil storage and because plant uptake is gradual. In this model of two hypothetical pastures, Pasture ‘B’ dries the soil more rapidly than Pasture ‘A’. This results in an extension to the period during which soil moisture limits recruitment, as indicated by the shaded bar above the graph. While not directly comparable, pasture states ‘A’ and ‘B’ could be interpreted as being analogous to grassland states ‘Fertilised pasture’ (dominated by exotic annuals) and ‘Native pasture’ (dominated by native perennials) respectively, based on their predicted water use.

The same generic seasonal soil moisture curve (solid line) and the predicted temporal barrier to recruitment (shaded bar) are presented in Fig. 7.3. A further limitation to recruitment is low seed supply outside the main summer period of seed release, due to predation and the relatively short period of seed viability after release from the capsule. This is represented by the dotted line in Fig. 7.3, whereby the probability of high seed supply corresponds to the occurrence of hot summer winds that stimulate seed release. The period of predicted seed limitation to recruitment (shaded bar at top of Fig. 7.3) is superimposed on the period of moisture limitation, leaving two ‘windows of maximum opportunity’ for recruitment (shown by the gaps between the shaded bars).

These windows of opportunity are a function of the natural processes of rainfall, seed fall, seed predation and seed deterioration and water uptake by competitor species. An example of a shift the timing and duration of windows of opportunity for recruitment in different pasture states is given in Fig. 7.4. In this example, the constraints to recruitment over summer due to soil water limitations in pasture state ‘B’ begin earlier in the season, and last longer than in pasture state ‘A’. This leads to a narrowing of the windows of opportunity for recruitment in pasture state ‘B’, and an overall lower probability of recruitment throughout the year.

Thus, if the hypothesised impacts of compositional change on soil water presented in Fig. 7.2 are sufficiently large, the seasonal windows of opportunity for tree recruitment described in Figs. 7.3 and 7.4 may be significantly shortened or lengthened as an indirect result of land management actions.
Fig. 7.2: A model of soil water availability under alternative pasture states. Higher soil water uptake by pasture ‘B’ than pasture ‘A’ results in more rapid drying of the soil profile, leading to an increase in the length of the summer period unsuitable for germination and less likely to support young seedlings, thereby decreasing the potential number of recruits and the probability of successful recruitment in that year. The same result may be observed as a result of the confounding of grassland states and soil properties that influence water content.

Fig. 7.3: A model of recruitment ‘windows of opportunity’ for an unspecified pasture state. The maximum probability of recruitment occurs in windows either side of the summer period of critical soil water deficit, when seed supply is sufficiently large to offset background levels of germination failure and seedling mortality. The windows of opportunity are represented by the gaps in the shaded bar at the top of the figure.
Fig. 7.4: A model of recruitment ‘windows of opportunity’ in two different pasture states. The recruitment probability curve is a function of biotic and abiotic interactions, as shown in Fig. 7.3. The windows of opportunity (shown by gaps in the shaded bars at top) reflect the periods when recruitment probability is at a maximum. The shift in these windows is brought about by altered soil water conditions in alternative pasture states.

7.3 Benefits and costs of soil water availability

Given the importance of soil water to recruitment, further interrogation of the literature was required to tease out the influence of pasture species on soil water availability, namely seasonality, rate of water use and capacity to dry topsoil.

Differential rates of soil drying between pasture types are evident between spring and autumn (Ridley et al. 2001), corresponding with the critical period for eucalypt seedling survival (Fig. 7.2). Ridley et al. (2001) found that soil water deficits were greater (i.e. soil was drier) under the perennial legume Lucerne (*Medicago sativa*) than under an annual pasture, and Lucerne was not only able to dry the soil profile to greater depth, but it was better at drying the soil within the root zone of the annual species. These effects were at their greatest between late spring and mid-summer (Ridley et al. 2001). Superior soil water extraction from topsoil and at depth by perennial grasses compared with annual grasses has also been shown by Dear & Cocks (1997), Bell et al. (2006) and Southwell et al. (2008).

Southwell et al. (2006) compared soil water beneath a native perennial C₄ grass (*Themeda australis* and *Bothriochloa macra*) pasture, and a native perennial mixed
C₃/C₄ grass (*Austrodanthonia* sp. and *B. macra*) pasture with a sown annual ryegrass (*Lolium multiflorum*) ‘control’ pasture. In their study, soil water deficits were greatest under the mixed C₃/C₄ native perennial pasture, followed by the C₄ native perennial pasture. Both perennial pastures produced a greater water deficit than the exotic annual pasture. Differences in soil water deficit between the pastures were were apparent from spring onwards, but were far more pronounced in late summer through autumn (Southwell *et al.* 2008). Figure 7.2 illustrates the general responses observed by Southwell *et al.* (2006), where the soil water content under the perennial and annual pastures is shown by the broken and solid lines respectively.

Virgona & Southwell (2006) note an important grazing interaction in their comparison of soil drying under the native perennial grass *Austrodanthonia* sp. and the exotic perennial grass *Phalaris aquatica*. The native grass was better at drying the soil profile under short grazing rotations, while *Phalaris* was more effective under longer (8-week) rotations.

From these studies it can be concluded that soil drying is influenced by species composition as well as immediate management actions, and generally, perennial pastures are better at drying the soil profile than annual pastures over the spring to autumn period. In light of the importance of soil water for seedling emergence and survival, these findings imply that the potential for tree recruitment in spring or autumn should be greater under annual-dominated pastures than perennial pastures, due to the lower soil water deficits (and greater soil water availability) under annual pastures.

There are three possible explanations for an inverse relationship between the cover of exotic annuals and probability of tree recruitment.

Firstly, annuals extract water more quickly than slower-growing perennial herbs (Gordon & Rice 2000), resulting in increased competition for surface soil moisture following small pulses of rain.

Secondly, greater structural homogeneity of the understorey in exotic annual-dominated pastures may be associated with a reduction in microsites or ‘microcatchments’ (Yates Norton & Hobbs 2000). Homogenisation of pasture structure and microtopography can reduce effective plant available water at micro-scales (Yates Norton & Hobbs 2000, Cramer *et al.* 2007), and smaller plant basal areas in annual
pastures would be expected to leave less gaps or ‘inter-tussock spaces’ in the sward. The potential importance to recruitment of heterogeneity in pasture structure is supported by highly heterogeneous spatial structure of seedling emergence at fine spatial scales (Garcia & Houle 2005). The effects of homogenisation are also likely to be the result of the management practices associated with exotic annual dominance (i.e. clearing, removal of woody debris, grazing, cultivation).

Thirdly, exotic annual grasses may inhibit tree recruitment by litter acting as a physical barrier that prevents seed-soil contact, thus reducing seed ‘available’ for germination, though this is rarely quantified (Peterson & Facelli 1992, Castro et al. 2002). Consequently, potential benefits to tree recruitment of increased soil water availability under annual pastures compared with perennial pastures might be countered by several associated adverse effects.

7.4 Soil properties determine the influence of grassland state

In contrast to the ecological restoration literature (e.g. Yates Norton & Hobbs 2000, Spooner et al, 2002, Semple & Koen 2003, Dorrough & Moxham 2005, Cramer et al. 2007), my experimental findings did not support the hypothesis of reduced recruitment potential in exotic annual pastures compared to predominantly native pastures. Nor was the hypothesised recruitment threshold associated with the shift from ‘Sown pasture’ to ‘Enriched grassland’ state in the State and Transition model of Fig. 3.1 observed. Where the herbaceous layer is undisturbed, the native pasture state proved to be the least favourable environment for recruitment; both emergence (while still very low) and seedling survival were significantly higher in the annual state (Chapters 3 and 5). I attributed this response to differences in plant available soil water between the annual and native states, in accord with the literature regarding water uptake by annual and perennial pastures. An alternative model

I found that recruitment was greater in the phalaris state than the annual or native states (Chapter 5), even though soil water extraction by phalaris is expected to be greater than annual-dominated pastures (Virgona & Southwell 2006). Thus neither origin nor lifeform of the dominant species within each state was sufficient to predict recruitment potential on their own.
I attributed these results to more favourable soil properties – especially better soil water retention – in the phalaris state than the annual state and native state. More soil water was available for extraction by the herbaceous layer and eucalypt recruits in the phalaris state, and differences in the net water supply available to tree recruits between the states were independent of absolute water uptake by the herbaceous layer. Soil water at field capacity was highest in the phalaris state, followed by the annual state and the native state, and this explained the large differences in recruitment between states even when competition from herbaceous species was removed (Chapter 5).

As might be expected, soil moisture holding capacity within the three grassland states corresponded well to soil organic carbon, which is an important indicator of soil health because of its influence on soil structure, soil water infiltration and storage (Sanjari et al. 2008).

My study suggests that differences in recruitment between grassland states reflect soil physical properties, principally moisture holding capacity, to a greater degree than competition from pasture species. The three states I investigated shared lithology and climate, so differences between the states’ composition and soil properties were attributed to land use impacts.

7.5 Are the effects of soil properties and species composition inseparable?

There are several reasons why particular soil properties may be a feature of grassland states at a landscape scale and therefore inseparable from the suites of species they support. Firstly, at large spatial scales it is highly likely that soil fertility and pasture composition are confounded as a result of preferential tree clearing and pasture improvement in the most fertile, rich soils which were recognised as the most productive areas for cropping and livestock grazing (Prober & Thiele 1995). A related outcome of bias in the pattern of agricultural development is that native grassland states, like remnant woodlands, are mostly restricted to non-arable areas with low nutrient, shallow soils of low water holding capacity (Dorrough & Moxham 2005).

Secondly, soil properties may again be confounded with pasture composition if past and continuing management actions that created and maintained alternative
states also directly altered soil properties. Examples include the lasting effects of fertiliser application and disturbance on soil available phosphorus and nitrate (Dorrough et al. 2006, Prober et al. 2002) and the effects of cultivation and heavy livestock grazing on soil compaction (Greenwood & McKenzie 2001, Wilson et al. 2008). For example, in North America, loss of soil organic carbon associated with decades of cultivation and intensive agriculture was shown to be reversible following restoration of areas to prairie, but full replacement was estimated to take more than a century (Potter et al. 1999).

Thirdly, positive feedback loops may exist between species composition and soil properties, such that changes to soil properties that may have been initiated by management actions are perpetuated by the influence of plants on those properties. Exotic annual grasses have been shown to alter soil nitrogen cycling which favours their own re-establishment (Prober et al. 2002). Production of plant litter and soil organic matter – which have a large influence on soil water infiltration rates, water holding capacity, soil structure – can increase pasture growth and thus favour the persistence of high biomass pastures (Lodge et al. 2006). High biomass exotic-dominant and nutrient enriched states produce nutrient-enriched organic matter that increases the speed of litter decomposition and rate of change to soil structure (Lodge et al. 2006).

7.6 Management implications

Despite the large impact of competition with herbaceous layer on tree recruitment, there has been little prior research on the influence of grassland states on tree recruitment. Notwithstanding the limitations of experimental work in identifying universal climatic and biotic thresholds to recruitment, my findings greatly improve our understanding of the mechanisms limiting tree recruitment in the Grassy Woodlands of south-eastern Australia.

While the relative impact of grassland states upon recruitment is small in comparison to the effects of presence or absence of the herbaceous layer and of water supply (Chapters 4 and 5), their relevance may be greater in the context of anthropogenic ‘novel ecosystems’ where disturbance regimes have been vastly altered. In agricultural areas of southern Australia, disturbances to the herbaceous
layer from fire and native mammal soil ‘diggings’, both considered drivers of recruitment, are now rare (Yates et al. 1994b, Noble 1997, Martin 2003). While soil disturbance by rabbits may still occur in areas fenced to exclude livestock, herbivory offsets any potential benefit of this disturbance. Consequently, low levels of ‘background’ recruitment into undisturbed areas (Watson et al. 1997) – and the effects of grassland states upon it – are likely to be of greater relevance to the maintenance of tree populations than in the past, when ‘event-driven’ recruitment was more reliable.

Estimates have been made of the frequency of recruitment needed to maintain tree populations based on current rates of tree decline (Dorrough & Moxham 2005, Gibbons et al. 2008). These suggest that recruitment needs to occur only several times per century, but future decline in the number of hollow-bearing trees is already inevitable, even if widespread recruitment were to occur now.

The occurrence of large recruitment events still relies on the coincidence of high seed fall and high spring and summer rainfall to overcome bottlenecks in seeding survival, particularly in the absence of disturbances which remove competition from the herbaceous layer. Management cannot change these factors. However effective rainfall and therefore plant available soil moisture is strongly influenced by soil properties. I have shown that the soil properties associated with grassland states are able to influence seedling emergence and survival; more fertile states with greater soil water infiltration and storage are more likely to support recruitment, irrespective of differences in water uptake by pasture species.

Changes in the likelihood of recruitment success in any given year are likely to translate to an increased frequency of recruitment over sufficiently long periods, since small changes in seedling mortality can determine the difference between presence or absence of recruitment in that year.

My research has shown that abundance of exotic annual grasses alone is not a good indicator of the likelihood of tree recruitment, since highly modified grassland states can result in recruitment given adequate seed supply and soil moisture. Attempts to restore areas by promoting natural regeneration of trees from seed must first consider the availability of seed (Vesk et al. in press), and then soil physical condition as an important influence on seedling survival.
Determination of the tolerance of woodland eucalypt seedlings to varying periods of exposure to extreme soil water deficits requires controlled soil water conditions that are difficult to achieve in the field, but Vallejo et al. (2005) were able to create a survival probability curve for oak and pine seedlings in a Spanish summer relating to duration between large rain events. Obtaining critical period thresholds for eucalypts relating to duration they can withstand extreme soil water deficits would allow soil moisture and recruitment probability under different climate scenarios to be modeled for a range of grassland states.

My research has shown that modification of the understorey as the result of management practices affects woodland eucalypt recruitment, and that the responses of seedling emergence, growth and survival to different grassland states is driven by soil characteristics associated with the states. Recruitment is greatly restricted by competition from the herbaceous layer in all states, native and exotic, perennial and annual. Recruitment can occur in undisturbed pastures albeit at very low levels. I found that recruitment was higher in an intact exotic perennial pasture state than intact an exotic annual pasture state, and was lowest in a native perennial pasture state. Following removal of the herbaceous layer, the highest potential for recruitment again occurred in an ‘enriched’ grassland state previously dominated by exotic perennial species. This suggests that improved soil water relations due to increased soil carbon and improved soil water infiltration and storage such as in the exotic perennial pasture state can override the predicted cost to recruitment of higher soil water uptake by the pasture.

This research has improved our understanding of the influence of understorey modification on woodland eucalypt recruitment in agricultural landscapes by highlighting the important role soil properties associated with states play in altering soil water available to eucalypt recruits. Both potential seed supply and soil condition are important factors determining tree recruitment potential in areas flagged for restoration using resting alone.

Further research is needed to quantify the relationships between grassland states and a wider range of soil properties (extending the work of Prober et al. 2005 and McIntyre & Lavorel 2007) at the landscape scale. This will aid in the refinement of existing models of recruitment potential under different management regimes.
References


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