Initiating Temperate Grassland Restoration by Controlling the Dominant Weed Species; a case study with *Nassella trichotoma*

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Abstract

Temperate grasslands are globally important biomes, in that they (i) provide habitat for a wide diversity of species, (ii) sequester large stocks of carbon, and (iii) provide forage for important pollinators (Chapter 1). These ecosystems often fall within highly fertile areas, and consequently humans have come to depend on them to provide high quality forage for grazing livestock and land for agricultural development. Temperate grasslands are considered to be critically endangered on a global scale. The grazing industry relies upon healthy and productive grasslands for the production of a substantial proportion of human food products, however, when these systems incorporate unsustainable land-management practises, such as over-grazing and continual fertilisation with inorganic matter, has resulted in a significant decline in important native grass species. This has resulted in encroachment of unpalatable, noxious plants, which decrease the quality of available forage. One such noxious weed species, *Nassella trichotoma*, known commonly as serrated tussock, is having a significance impact on the constitution of temperate grasslands and grazing systems, globally, due to its unpalatability and competitive growth form. In order to return temperate grasslands to a fully-functional and a high-quality forage state, human intervention in terms of ecosystem restoration is required. The control of noxious species, together with the reintroduction and establishment of native species, is a critical step for restoration efforts with the return of native plant diversity, and the re-establishment of ecosystem services, such as habitat for higher trophic levels.

This thesis reviews and overlaps the scientific disciplines of ecosystem restoration (Chapter 2), weed science relating to *N. trichotoma* (Chapter 3), and environmental management in order to provide solutions for controlling *N. trichotoma* in non-native grassland communities (Chapter 4). The effect of direct herbicide application, soil tillage, grazing exclusion, fire, and broadcasting native seeds for the control of this dominant weeds in a total of 13 different combinations is investigated. The experimental plots were surveyed over a four-year period and soil cores were collected over a three-year period to survey the seedbank density. It was found that the inclusion of fire significantly increased the establishment of the native broadcast species. Also, without the integration of fire or tillage, *N. trichotoma* recovered, and consequently was observed to be the dominant species in the final sampling period.

To support the findings of Chapter 4, research into the seed longevity and seedbank persistence of *N. trichotoma* was undertaken in Chapter 5. It was found that less than 10% of the seeds were observed to be viable after 12 months of burial in field. In addition to this, the longevity of the seeds was determined by rapidly ageing the seeds through exposure to high relative humidity and

temperature. This process determined that *N. trichotoma* produces transient seedbanks, referring to those that persist for 12 months or less, and therefore the seedbank would be reliant on new seed input annually to remain a competitive threat. This implies that management control of new seed fall is essential to prevent the reestablishment of the seed bank.

The seedbank persistence for *N. trichotoma* is complicated by disturbance events such as fire. To investigate this impact, four different collection years; 2016, 2017, 2018, and 2019 were subjected to increasing heat (80, 100, 120, or 140^{O}C) and time of exposure (1, 3, 6 or 9 minutes) by placing them into a temperature-controlled oven for the given treatment. It was found that only the 140^{O}C treatment was significant for killing *N. trichotoma*, as detailed in Chapter 6. High moisture content (95%) increased the seeds sensitivity to radiant heat, with all tested temperature effective for killing this species. The seedlings were not killed by the tested treatments.

Management implications and recommendations for the control of *N. trichotoma* in temperate grasslands (Chapter 7) include; (i) the use of herbicide in Autumn to prevent seed set in the following summer, and (ii) in addition to initial herbicide, use, subsequent fire treatment and broadcasting native seeds appear to provide ongoing competition against *N. trichotoma* reestablishment in treated areas. Further, high fire intensities, where the soil is heated to 140^{O}C or more, can kill *N. trichotoma's* seedbank and prevent its recruitment. In all cases of treatment, monitoring recruitment from the seedbank is recommended for up to one year after treating a site. This thesis suggests that localised eradication of *N. trichotoma* is achievable in as little as three years if (i) above-ground plants are treated, (ii) seedling recruitment from the seedbank is managed intensely within the first year, (iii) high densities of native grass is established to provide competition, and (iv) the addition of new seed is prevented.

Declaration

This is to certify that:

i) The thesis compromises only my original work towards the Ph.D. except where indicated in the preface,

ii) Due acknowledgement has been made in the text to all other material used,

iii) The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: [redacted]

Date: 16th of September 2022

Preface

Chapters of thesis published in peer-reviewed journals:

Chapter 2. Humphries T, Florentine SK, Dowling K, Turville C, Sinclair S (2021) Weed management for landscape scale restoration of global temperate grasslands. *Land Degradation and Development* 32(3): 1090–1102

Chapter 3. Humphries T, Dowling K, Turville C, Sinclair S, Florentine SK (2020) Ecology, distribution and control of the invasive weed *Nassella trichotoma* (Nees) Hack. ex Arechav.: A global review of current and future challenges. *Weed Research* 60: 392–405.

Chapter 5. Humphries T, Florentine SK. (2022) Assessing seedbank longevity of the invasive tussock grass; *Nassella trichotoma*, through in-field burial and laboratory-controlled ageing. *Plants* 11: 2377. https://doi.org/10.3390/ plants11182377

*Contribution (%) by T. Humphries to each published paper.

Chapter 2: Research design 90%; Literature review and write up 90%; Data collection 100%; Research Analysis 80%.

Chapter 3: Research design 90%; Literature review and write up 90%; Data collection 100%; Research Analysis 100%.

Chapter 5: Research design 90%; Literature review and write up 90%; Data collection 100%; Research Analysis 100%.

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Thank-you to Christopher Turville for your patience and assistance with the statistical analysis. The analysis for several chapters in this thesis seemed quit daunting at first, however with your guidance, these concepts became simplistic and well understood.

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Thank-you to Steve Sinclair for providing an industry perspective to this project. It was very important to me that my research was applicable to land management, and with your support, we were able to make more impactful conclusions.

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I also acknowledge the Wadawurrung Peoples as the Traditional Owners of the land of which was used for this experimental research, and we pay our respects to Elders past, present and emerging. I'd like to thank James Neil from Parks Victoria for his support and assistance during my field work and for providing access to the Little Raven North site, and for all the work that went into the prescribed burn. I also would like to thank the Country Fire Authority (CFA) for conducting the prescribed fire treatment., and AusEco Solutions for implementing the herbicide application.

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Chapter 1. Thesis Introduction

Thesis Introduction

Introduction

Temperate grasslands are one of the most threatened biomes on the planet (IUCN, 2013). Temperate grasslands fall within high fertility zones, and thus, a significant proportion of these lands have been converted for agriculture and livestock grazing activities (Tikka et al. 2001; Gerla et al. 2012; Gaworecki 2016). Agricultural practices use mechanical (such as tillage) and chemical (including fertilizer addition or liming) processes to modify soil conditions for crops and pastures, which subsequently favours the establishment of invasive plants (Prober et al. 2004). Weeds are the most important contributor to the loss of biodiversity and reduction in ecosystem services, as they displace native species and can actively alter the disturbance regimes (Prober et al. 2005). As a result, passive restoration in weed dominated systems is ineffective, making research into practical and effective landscape scale active restoration methods vital (Stromberg et al. 2007).

Active restoration involves the integration of different management techniques to return a degraded ecosystem to one that resembles its historical origin, is self-sustaining, and resilient to disturbance (Higgs et al. 2014). It commonly includes one or more components of; revegetation (Page and Bork 2005; Torok et al. 2012), weed density reduction (Johnson et al. 2018), soil alteration (Sandel et al. 2011; Brown et al. 2017), landscape contouring (Keesstra et al. 2018), and grazing management (Lengyal et al. 2012; Torok et al. 2018). Revegetation is an important element to consider in isolated and highly fragmented landscapes where migration of native propagules from a remanent site is not feasible (Tognetti and Chaneton 2012). Reducing the weed density can be achieved by removing entire plants or leaf litter (Musil et al. 2005; Johnson et al. 2018; Bourdot and Saville 2019) or by implementing fire (Zaloumis and Bond 2011; Waller et al. 2016), and this is critical for generating sites for establishing competitive native species (Wilson and Partel 2003; Waller et al. 2016). Soil alteration and disturbance is important for flushing out weed seedbanks (Stromberg et al. 2007), and can assist in altering the soil to resemble historical conditions (Prober et al. 2005). This can be achieved through mechanical disturbances such as tillage (Waller et al. 2016) and soil scalping (Gibson-Roy et al. 2010), or chemical alterations such as carbon (Huddleson and Young 2005) or fertilizer addition (Eze et al. 2018). Implementing practices that exclude, include or mimic grazing may be considered as this can promote the long-term success of the restoration project (Klaus et al. 2018). Even with the abundance of intervention options, the lack of ongoing management and funding limits the extent and success of many grassland restoration projects (Freudenberger and Gibson-Roy 2011).

For restoration projects to be successful over long-term time scales, several ecological and evolutionary factors for invasive plants must be addressed. These include the seedbank persistence (Gardener et al. 2003), and the ability of the plant and its seeds to respond to various disturbance events, such as fire (Tangney et al. 2018). For long-term management, it is critical to understand how long a dominant weeds seedbank can persist, as transient seedbanks are usually less problematic than those that are persistent (Burnside et al. 1996). Further, understanding how the seeds responds to climatic events can assist in the development of control tactics that flush or kill the seedbank. Herbicides are one of the most economical and effective methods for weed management, however, their over-reliance has resulted in increased cases of herbicide resistant weeds (Heap 2019). Further, ongoing reliance on chemical control can pose serious health hazards on living organisms either as a direct or indirect result of these pollutants entering the air, water and soil (Sharma et al. 2019).

Temperate grasslands throughout Australia, New Zealand and South Africa have been prolifically invaded by the South American grass, *Nassella trichotoma* (Nees) Hack. (Serrated Tussock) (EPPO 2012). This course, tufted-grass is having a devastating effect on agricultural and natural systems as this weed displaces native and palatable grasses, reduces soil nutrients, and can quickly forms dense monocultures, making it one of the biggest threats to livestock carrying capacity (Osmond et al. 2008; DPI 2016). *Nassella trichotoma* can also be problematic within its native range and populations have been observed to be emerging in France (EPPO 2012) and the USA (USDA 2020). *Nassella trichotoma* is a long-lived perennial grass with high fecundity, it is drought, fire and frost tolerant, and due to its low crude protein, grazing avoidant (CRC 2013). This weed self-facilitates by reducing soil nutrients to unproductive levels, allowing it to maintain its dominance. Management strategies for this species have been developing since the 1930s in Australia (Campbell and Vere 1995), yet it is still widespread and one of the leading causes for landscape-scale degradation of temperate grasslands.

In Victoria, Australia, the Greater Western Plains grasslands located to the west of Melbourne are considered one of the most degraded ecosystems in the country, with less than 1% remaining intact

(Garrard and Bekessy 2016; Abrahams 2020). This area has been subjected to rapid urban development, over grazing, unnatural disturbance regimes, and is overrun with several aggressive grassland weeds. Of these introduced plant species, *N. trichotoma,* is amongst the most wide-spread and difficult to manage (Badgery et al. 2003; Goldson et al. 2015). To date, *N. trichotoma* has invaded over two million hectares in Australia's south-east, and a further estimated 32 million hectares are considered climatically suitable for its establishment (Hamilton 2012; DPI 2016). This is a weed of both environmental and agricultural significance and it is therefore critical that new research focusing on reducing this weed at a landscape scale is developed in order to protect economic and environmental assets for the long-term future.

Theoretical principals for ecological restoration

Ecological restoration is a relatively new scientific discipline and theoretical principals and standards are continuously being developed and defined (Suding and Hobbs 2009; Gann et al. 2019). Before restoration activities can be implemented, it is essential to understand how a system moves from its historical state to a degraded state. The simplest model to illustrate this concept is the State and Transition model (Figure 1.1). This model simplistically shows a disturbance event moving an intact ecosystem to a degraded state (Westerby et al. 1989). For clarity, an intact system refers to an ecosystem in a state that is providing high ecosystem functionality and is able to support high biodiversity, while the inverse describes a degraded system. Disturbance can be gradual and continuous or a sudden regime shift (Scheffer and Carpenter 2003). Once a site is in a degraded state, the ecosystem can develop new feedback loops that hold the system in an Alternative Stable State (Suding and Hobbs 2009; Schroder 2009). Therefore, active restoration is required to disrupt these new feedback loops in order to restore the ecosystems functionality.

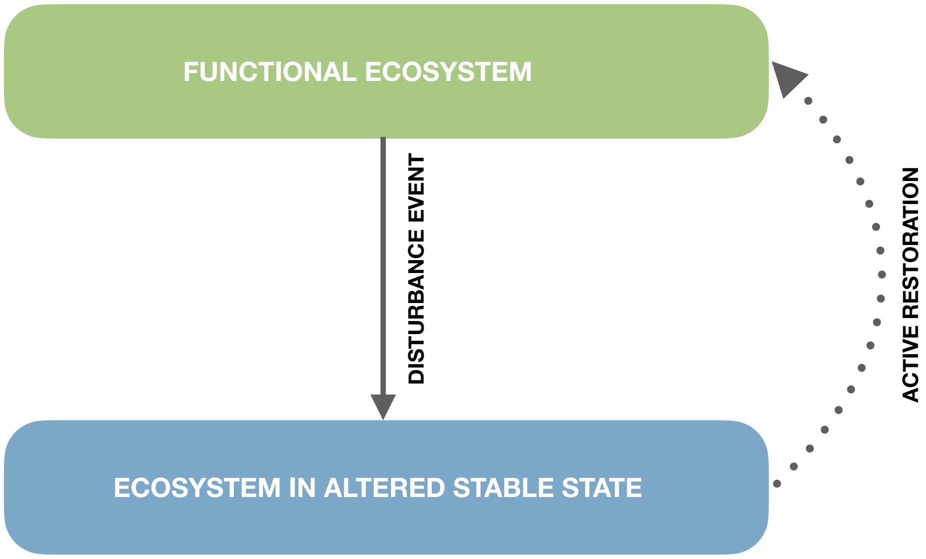

Figure 1. An example of a simple State and Transition model. This shows a disturbance event moving a functional system into an altered state. In this state, the ecosystem processes have changed and the ecosystem is no longer functioning as normal. New feedback loops keep this system from naturally returning to its former functionality. The dotted arrow shows active recovery is required to return ecosystem processes and functions. This model is based on Suding and Hobbs (2009).

It is rare that a fully functioning ecosystem will move into an altered state after a single disturbance event, as these systems are usually resilient to "normal" disturbance patterns (Walker et al. 2006). When the frequency, intensity or disturbance type is changed, loss of ecosystem function may then occur. The Threshold model (Figure 1.2) explains how a fully-functional, intact ecosystem may move into a degraded state by passing two thresholds; biotic and abiotic (Suding and Hobbs 2009). When an ecosystem passes the first threshold, biotic interactions are lost, resulting in moderate loss in ecosystem functionality. Implementing active restoration efforts at this point are usually very successful (Suding and Hobbs 2009). However, when the system pushes beyond the abiotic threshold, the system loses its core functionality, and even with active restoration efforts, a system in this state may never fully recover to the historical condition, and thus, restoration efforts should aim to restore ecosystem processes (Suding and Hobbs 2009).

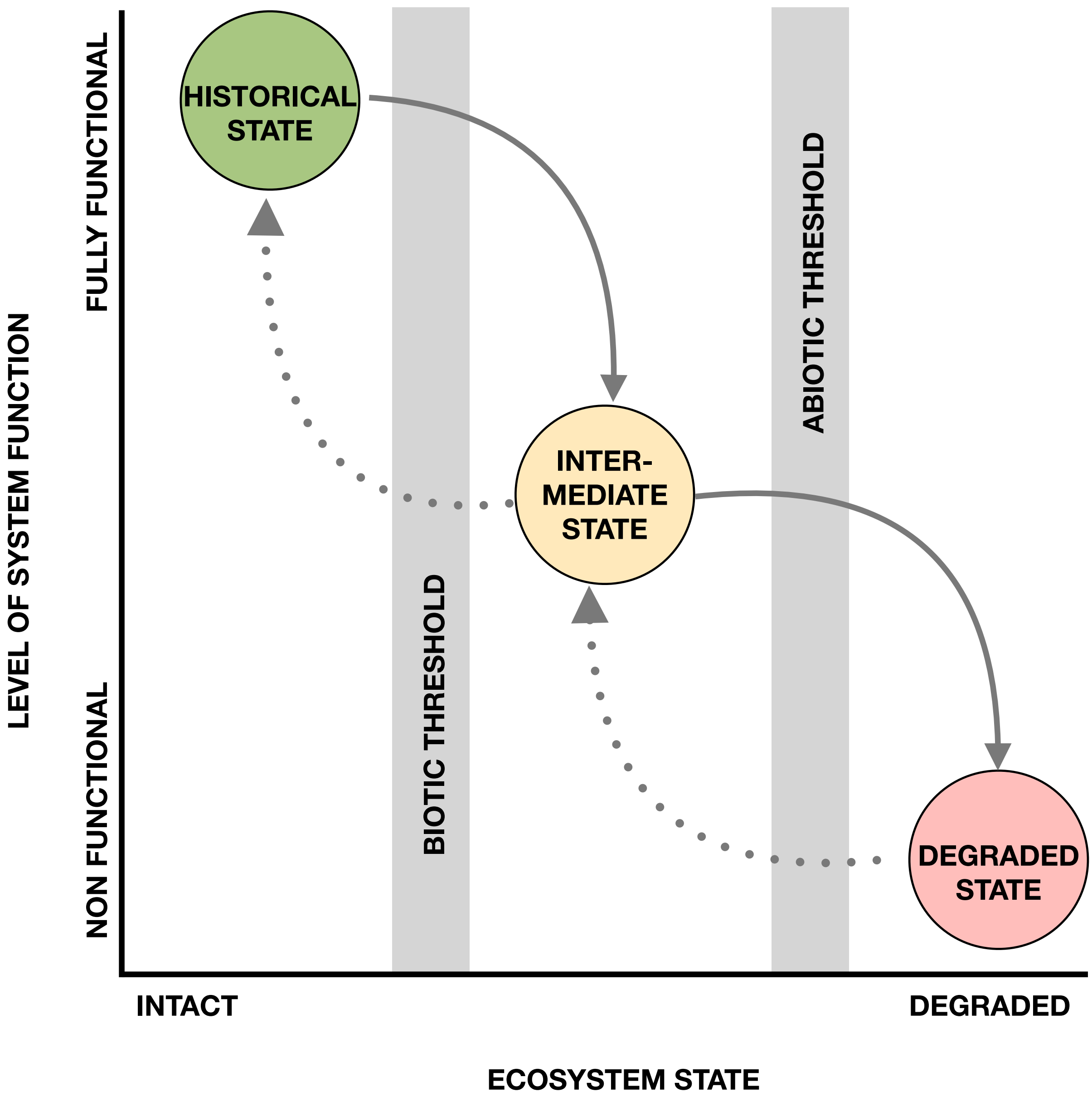

Figure 2. An example of a simple Threshold model. The green circle represents an ecosystem that is intact and highly functional. If a disturbance event occurs that results in the loss of biotic interactions, for example the loss of a pollinator, the system will be pushed past the first threshold. The yellow circle represents an ecosystem is somewhat functional and somewhat intact. If another disturbance event occurs that results in the loss of abiotic processes, such as soil salinity, it will be pushed past the second threshold. The orange circle represents an ecosystem that is non-functional and degraded. Once a threshold is passed, it requires active restoration (represented by the dotted arrow) to move it back to its previous state. This model is based on Suding and Hobbs (2009).

Proposed area of research inquiry

This study lies at the intersection of three interconnected scientific disciplines; land-scape restoration ecology, weed science and land management. The restoration ecology component will be focused on the promotion of native species through integrated control efforts. The weeds science component will be addresses ways to diminish the density of dominant weeds above and below ground density for long-term control. The land management component addresses design methods that have relevance to *N. trichotoma* dominated grasslands across the world. It is therefore critical that this proposed project develops practical management implications that can be easily communicated with land managers and subsequently implemented with ease.

Contribution to existing knowledge

Many ecological restoration projects are small scale, low-budget, and fail to address multiple degrading factors leading to inconclusive results (Suding and Hobbs, 2009). This research specifically aims to provide practical solutions for landscape-scale management of *N. trichotoma*. Techniques selected for this study are already being widely applied for the management of this weed, but it is known that they are often ineffective. Therefore, this study aims to improve the effect of current management practices that are widely applied by implementing these techniques in an innovative sequence to achieve synergistic effects. The proposed study will practically address the long-term management of *N. trichotoma* by attacking it below (seedbank) and above (canopy) the ground. This detailed knowledge will facilitate management recommendations with greater confidence.

Research questions

This thesis attempts to demonstrates how targeting *N. trichotoma* can initiate the restoration of a degraded grassland currently dominated by this species. The overarching question is:

Can long-term restoration of a degraded temperate grassland be achieved by targeting the above and below ground density of the dominant weed?

In addition to this, we also aim to gain a better understanding of the seed ecology of *N. trichotoma* will facilitate future management strategies. Additional and supporting research questions are:

I. Can integrating herbicide, fire, grazing exclusion, tillage and competition effectively reduce the above and below ground cover of *N. trichotoma*?
II. What is the seedbank longevity for *N. trichotoma*?
III. How does *N. trichotoma* seeds and seedlings respond to fire?

Study design and thesis layout

This thesis includes two review chapters and three experimental chapters. This study was designed with the anticipation that the methods could be adaptable to other temperate grassland systems dominated by *N. trichotoma* and potentially other invasive *Nassella* species.

Chapter 2 presents a metanalysis review of the literature regarding previous restoration attempts on different grasslands across the world. The effect of both passive and active restorations is explored and how various biotic and abiotic factors can impede their success. This review describes the various restoration techniques used and their success at reversing the site from a degraded state. This chapter highlights the most widely used control methods and how they can be applied with greatest effect. The limitations of ecological restoration are also explored with factors including budget, time, resources, and degree of degradation all being important elements to factor into the restoration design.

Chapter 3 reviews the literature regarding the biology, ecology, history and management of *N. trichotoma*. It is known that invasive plants establish negative-feedback loops that can alter ecosystem processes, such as fire regimes or seedbank communities, to facilitate their own dominance (Briske et al. 2006). Understanding the biological and ecological traits of an invasive species highlights suitable control methods that break these self-facilitating feedback loops and improve the restoration outcomes.

Chapter 4 fuses the key findings of the two review chapters to conduct a landscape-scale grassland restoration. The study is conducted within the Greater Western Plains grasslands, located in Victoria, Australia. This grassland community is highly altered and degraded, with as little as 1% of its original extent remaining intact (Garrard and Bekessy 2016; DELWP 2018; Abrahams 2020). From the early 1900’s, these grasslands were modified for grazing and cropping, and are now left abandoned and dominated by weeds (Garrard and Bekessy 2016; Abrahams 2020). In more recent times, these areas have been subjected to urban expansion, resulting in a highly fragmented landscape. *Nassella trichotoma* is a species of particular concern within this vegetation community due to its; extensive spread, aggressive and competitive nature, and difficulty to control (Osmond et al. 2008; DELWP 2018). The management strategies adopted in this chapter are specifically designed to target of the above- and below-ground density of this species, and the subsequent increase in density of two broadcast native grasses. This study combines vegetation surveys and soil sampling to determine vegetation shifts as a result of the integrated treatments implemented.

Chapter 5 explores the seed longevity and seedbank persistence of *N. trichotoma* through the burial of viable seeds at different; times, depths, and durations to determine seedbank persistence. Predicting seed longevity can be achieved through the rapid ageing technique where the seeds are exposed to high humidity and temperature. These experiments provide clarity for making management recommendations regarding ongoing management and monitoring of this weed's seedbank recruitment. As persistent seedbanks can thwart ecological restoration efforts, understanding the seed longevity for problematic weeds is essential for defining a time scale to manage seedling recruitment.

Chapter 6 investigates *N. trichotoma's* response to temperatures induced by fire. One important way *N. trichotoma* alters its environment is by increasing the intensity of fire, which often results in native grass species being killed and displaced. Fire regimes can be strategically manipulated in weed management programs to break these established negative feedback loops, and therefore, breaking the dominance cycle of the invasive plant. Fire can also be used to flush or devitalize the seedbank (Franzese and Ghermandi 2020), and the intensity of the burn is affected by several environmental factors such as fuel load, seasonality, topography and plant moisture content (Bradstock et al. 2010; Kreye et al. 2013). Understanding temperature thresholds of seeds, and how these are altered with various seed moisture contents can assist land manages in planning fire treatments in the appropriate season for the highest impact on targeting this species seedbank. Chapter 6 explores the effect of radiant heat on the germination capacity of different seed ages and hydration levels.

Chapter 7 synthesises the research findings and proposes management strategies and future research.

Chapter 2. Weed management for landscape scale restoration of global temperate grasslands.

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REVIEW ARTICLE

WILEY

Weed management for landscape scale restoration of global temperate grasslands

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Abstract

Globally, temperate grasslands have been significantly degraded as a result of urbanisation, grazing and agriculture. Weeds now dominate most of these ecosystems, resulting in the loss of ecosystem services, reduced carrying capacity for farmers, and reduction of habitat for native plants and animals. This paper reviews the literature relating to temperate grassland restoration efforts across the globe, noting which techniques and combinations have been used successfully to reduce weed dominance and promote native recruitment and establishment. This review concludes that, using a combination of four restoration techniques, provided the highest level of success, with the caveat that, ongoing weed management should be budgeted for in all projects. There is no single optimal method for restoration and weed control, with success depending on specific site conditions and the scope and aims of particular projects. However, any form of target plant transfer was observed to significantly enhance the restoration's success and reduce exotic plant biomass. There is clearly a need for an increase in long-term monitoring of restoration projects in order to make more confident assumptions.

KEYWORDS

grassland degradation, pampas, prairie, restoration ecology, steppe, veldt

1 | INTRODUCTION

Temperate grasslands once covered almost 9 million km^2, or approximately 8% of the Earth's surface (IUCN, 2013). They include the Prairies of North America, the Pampas of South America, the South African Veldts, the Tussock grasslands of Africa, Australia and New Zealand, and the Steppes of China, Tibet and Eurasia (Table 1). These biomes are naturally species-rich (Faber-Langendoen, Shicheng, Yang, & Meijiao, 2018), providing habitat for many plants, animals and soil biota. In addition, these grasslands offer valuable ecosystem services such as high-quality forage for herbivores (Boval & Dixon, 2012), habitat for pollinators for crops and native species (Bendel, Kral-O'Brien, Hovick, Limb, & Harmon, 2019), significant levels of carbon sequestration (Eze, Palmer, & Chapman, 2018), and places for many recreational and cultural activities (Gomez-Limon & de Lucio, 1995). They also afford many other environment stabilising services, such as soil erosion control and mitigation of flood waters (Sankaran & Anderson, 2009).

Given the significance and contribution of these ecological systems, it is concerning that they are, collectively, one of the most altered landscape areas in the world (Suttie, Reynolds, & Batello, 2005), warranting immediate action to restore their beneficial services. Estimates suggest that 70% of these ecosystems were altered or degraded before 1950, and a further 14% by 1990 (Hassan, Scholes, & Ash, 2005). This decline in ecosystem health is a direct response to rapid population growth and subsequent urban expansion (Williams, McDonnell, & Seager, 2005), as well as the concomitant conversion of these fertile ecological systems into sites for agriculture, particularly for cropping systems and livestock grazing (Bartolome, Jackson, & Allen-Diaz, 2009; Martin, Moloney, & Wilsey, 2005; Prober, Thiele, Lunt, & Koen, 2005; Sankaran & Anderson, 2009). It

TABLE 1 Current and historic temperate grassland coverage

Country/region	Grassland name	Historic (km²)	Current (km²)	Total loss (km²)	Reference
America					
North America	Prairies	2,679,900	107,196	2,572,704	Henwood, 2010
South America	Pampas and Campos	2,325,700	109,600	2,216,100	Henwood, 2010
Africa					
South Africa	Veld	360,590	234,383	126,207	Henwood, 2010; Cadman, deVilliers, Lechmere-Oertel, & McCulloch, 2013
Eurasia					
China	Steppe	3,386,000	1,794,580	1,591,420	Henwood, 2010; Ye & Feng, 2011
Mongolia	Steppe	822,760	740,484	82,276	Henwood, 2010
Eastern Europe	Steppe	440,000	43,120	296,880	Henwood, 2010; Fuchs, Herold, Verburg, & Cleavers, 2013
Russia	Steppe	600,000	50,000	550,000	Henwood, 2010; Ponomarenko, 2019
Oceania					
Southeast Australia	Tussock grassland	60,000	12,000	48,000	Henwood, 2010
New Zealand	Tussock grassland	83,700	23,300	60,400	Mark, 2007; Henwood, 2010

Note: The approximate current cover is based on most recently published works but it is postulated that this coverage is likely to have diminished below these levels in most cases given lack of restoration action.

has been well documented that humans have used fire to manage landscapes since the mid-Holocene (Snitker, 2018), however in more recent times, fire regimes have been reduced or removed, and replaced with grazing, mowing, or zero disturbance, which leads to shrub and bush encroachment (Case & Staver, 2016). While increased protection of the remaining intact grasslands is critical, this alone will not be enough to ensure the future resilience and functionality of these essential ecosystems.

The UN Decade of Restoration aims to restore 350 million hectares of degraded ecosystems by 2030, however grasslands appear to be considerably underrepresented compared to forests and aquatic ecosystems, despite the fact that functioning grasslands offer important resources for future food security (UN Environment, 2019). Further, Veldman et al. (2015) highlighted that the global restoration project, known as the Bonn Challenge, misinterpreted 9 million km² of grassland communities as degraded forest communities. This shows how inappropriate afforestation of grasslands can further threaten these biomes (Veldman et al., 2015).

Ecosystem degradation is the movement of a high functioning and healthy ecosystem into an altered state, whereby ecosystem functions are reduced or lost as a result of single or multiple disturbance events, usually due to human actions (Suding & Hobbs, 2009). Degradation may be a precursor to ecosystem collapse, where the basic character of the ecosystem is lost (Keith et al., 2013). Healthy ecosystems are often resilient to moderate disturbances, and these events can be important for maintaining biodiversity and ecosystem functionality. Indeed, many grasslands have experienced human disturbances for thousands of years, such that natural processes cannot be separated from human impacts in these systems (Wilkin et al., 2020). However, when the intensity and/or frequency of these disturbances change, an ecosystem can undergo hysteresis and pass through irreversible degradation thresholds (Suding & Hobbs, 2009). An example of this was observed in the grasslands of California where the combined factors of weed invasion by Mediterranean species and altered grazing regimes from the introduction of livestock, concurrently with multiple severe drought seasons, resulted in the severe degradation of this ecosystem, pushing it into an alternative stable state (Bartolome et al., 2009). The scale of restoration required is dependent on whether the degrading pressures are being driven by biotic factors (pest and weed invasion), abiotic factors (drought or altered fire regimes) or a combination of both.

Throughout the world, grasslands are currently subjected to multiple degrading pressures, including habitat fragmentation, altered grazing pressures, desertification, transition to cultivation, bush encroachment and climate change. These degrading pressures often act to facilitate exotic plants, which in turn create positive feedback loops that maintain the degraded altered state. In this respect, the alternative state theory explains how internal disturbances and external shocks lead to these positive feedback loops (Chisholm, Menge, Fung, Williams, & Levin, 2015). The degrading factors alter the environment to promote the exotic plants, as observed in south-eastern Australian grasslands where annual exotic grasses outcompete the perennial native grass, *Themeda triandra* by developing new positive feedback loops that increase soil nitrogen. Unless these available soil nitrogen levels are reduced, the invasive species will maintain their competitive edge (Prober, Lunt, & Morgan, 2009). Cross-facilitation of invasive plant feedback loops has been identified by observing *Agropyron cristatum* in northern United States, which alters native soil biota and the consequent soil dynamics (Jordan, Larson, & Huerd, 2008). This reduces the competitiveness of the native vegetation, promoting niche availability for the invasive *Bromus inermis* (Jordan et al., 2008). It is in this aggressive context that ecological restoration needs to first disrupt the pressures driving the change, thus preventing further degradation, and then second, to assist in the recovery of the natural ecosystem.

There is agreement, for example, that native tufted perennial grasses can be described as 'keystone' species, as they act to resist weed invasion and maintain ecosystem processes (Prober et al., 2005; Stromberg, D'Antonio, Young, Wirka, & Kephart, 2007). However, human induced disturbance of grasslands has resulted in the decline of these native grasses allowing non-native perennial grasses to become established, which significantly reduces the carrying capacity and biodiversity of the area. This has been observed in grasslands that are now dominated by the long-lived exotic perennial grass, *Nassella trichotoma* throughout SE Australia (Campbell & Nicol, 1999), South Africa (Joubert, 1984) and New Zealand (Lamoureaux, Bourdôt, & Saville, 2011; Lusk, Hurrell, Saville, & Bourdôt, 2017). Likewise, annual weeds are also highly problematic, and outcompete native perennial grasses in their early life stages. This aggressive infestation behaviour is most prevalent after disturbance, such as soil cultivation (Musil, Milton, & Davis, 2005; Bartolome et al., 2009; James, Drenovsky, & Monacvo, 2011). Because invasive plants are generally one of the main drivers for holding these ecosystems in degraded states, the main focus of restoration efforts is on reducing the dominant weed population and promoting competition from native species (Averett, Klips, Nave, Frey, & Curtis, 2004; Marushia & Allen, 2011). Passive and active restoration techniques have been used to achieve this outcome at varying levels of success throughout different temperate grasslands.

This paper investigates and analyses the literature regarding a wide range of efforts for landscape restoration of degraded, weed-invaded, temperate grasslands. The objectives of this work are to: (a) identify the leading degrading pressures acting on temperate grasslands across the world; (b) observe what restoration techniques are frequently used and assess their effectiveness; (c) identify techniques that are not effective, and understand the reasons for this; (d) make recommendations for future restoration efforts within these globally threatened communities.

2 | METHODS

2.1 | Literature search and selection

Limited English language literature was available that specifically addressed the restoration of weed invaded temperate grasslands. As only English language literature was used, important literature of large temperate grassland areas from non-english speaking regions, including South America, China and Russia may have been unintentionally excluded. Therefore, the criteria for selecting the literature for this review only required that the papers were: (a) a field-based ecological restoration, (b) a study conducted within a temperate grassland, and (c) a manipulation and measurement of the standing vegetation in the attempt to facilitate target species. The term 'target species' is used here to describe either a native or favourable species that was purposefully introduced, or promoted in a study. The English language literature search was conducted using Wiley Online Library and Google Scholar using the search terms; 'Ecological Restoration' plus one of the following, the brackets indicate where a term was searched in both its singular and plural context; temperate grassland(s), prairie(s), tussock grassland(s), veldt, veld, steppe(s), pampa(s), weeds, invasive plants, exotic plants. Papers with suitable titles were selected and their abstracts scanned for suitability. From this, 37 papers were selected to be read in full to determine their suitability for this review. Only 26 papers met the review criteria and these papers were selected for analysis.

2.2 | Analysis

The papers selected for analysis varied greatly in the extent of site degradation, restoration techniques used and data collection and monitoring. Therefore, only qualitative data were collected for the analysis. Each site's land use history and current degrading pressures were identified. The restoration techniques used in each experiment, including different combinations trialled within each study, were then categorised under the headings; herbicide (H), fire (F), physical soil alteration (PSA), chemical soil alteration (CSA), above ground biomass reduction (including; hand removal, leaf litter removal, raking, mowing and clipping), (BR), grazing manipulation (including grazing exclusion) (GM), and seed transfer or propagule introduction (ST). The key results for each experiment were summarised as shown in Table 2, and based on this summary, given a rating of high, moderate or low success. A treatment combination was considered to be highly successful if weed control (including shrub encroachment) was successful and the biomass of target species was increased, if only one of these factors were observed, the treatment was considered moderately successful, and if no change was observed to weed dominance and target species, the treatment was scored as low. The duration and locations of each experiment was also recorded.

Pie charts were created to show proportionally where the studies were conducted, leading degrading pressures and to show the frequency of restoration techniques used. Bar chats were used to show the level of effectiveness of using multiple restoration techniques, herbicides, and fire.

3 | RESULTS

3.1 | Treatments

Of the 26 studies analysed, 22 incorporated seed or plant propagule transplant, making it the most commonly used treatment (Figure 1). Often, studies that included this treatment obtained higher species richness and reduced weed biomass than those that relied on natural recruitment, therefore providing a more successful outcome. The species richness of the seed mixture varied considerable between studies, and was related to the studies budget and desired outcomes, with some studies only using one species (Page & Bork, 2005), which was observed to be successful for reducing a target weed, while some

TABLE 2 Summary of the data collected from each paper

Author	Location	Journal	Study length (years)	Degrading pressures	Treatments							Effect
					H	F	PSA	CSA	BR	GM	ST	
Ansley & Castellano, 2006	United States	*Restoration Ecology*	8	Agriculture, invasive weeds	✓	✓						Moderate
Averett et al., 2004	United States	*Restoration Ecology*	1	Agriculture, fragmentation, loss of target species from seedbank	✓		✓	✓			✓	High
Baasch, Engst, Schmiede, May, & Tischew, 2016	Germany	*Ecological Engineering*	6	Grazing					✓	✓	✓	Moderate
Blumenthal, Jordan, & Russelle, 2003	United States	*Ecological Applications*	2	Agriculture, invasive weeds				✓			✓	Low
Brown et al., 2017	Australia	*The Rangeland Journal*	1	Shrub and woody weed encroachment	✓		✓	✓			✓	High
Cuevas & Zalba, 2010	South America	*Restoration Ecology*	4	Fragmentation, grazing					✓			Moderate
Foster et al., 2007	United States	*Restoration Ecology*	6	Grazing, invasive weeds					✓		✓	Moderate
Jaunatre, Buisson, & Dutoit, 2014	France	*Applied Vegetation Science*	3	Agriculture, fragmentation, loss of target species seedbank			✓				✓	Low
John, Dullau, Baasch, & Tischew, 2016	Germany	*Ecological Engineering*	1	Invasive weeds, altered fire regimes				✓	✓		✓	High
Johnson, Catford, Driscoll, & Gibbons, 2018	Australia	*Applied Vegetation Science*	1	Invasive weeds, loss of target species from seedbank	✓				✓		✓	Moderate
Klaus et al., 2018	Germany	*Restoration Ecology*	9	Invasive weeds			✓				✓	Low
Marushia & Allen, 2011	United States	*Restoration Ecology*	2	Invasive weeds	✓		✓		✓		✓	High
McManamen, Nelson, & Wagner, 2018	United States	*Restoration Ecology*	1	Grazing, invasive weeds	✓		✓		✓		✓	Moderate
Musil et al., 2005	South Africa	*South African Journal of Science*	2	Fragmentation, invasive weeds	✓	✓			✓			High
O'Dwyer & Attiwill, 2000	Australia	*Restoration Ecology*	1	Grazing, bush encroachment, altered fire regimes					✓		✓	Moderate
Page & Bork, 2005	United States	*Restoration Ecology*	1	Altered fire regimes, agriculture, local extinction of keystone grazers (elephant and rhino)	✓						✓	High
Radloff, Ladislav, & Snyman, 2014	South Africa	*Applied Vegetation Science*	6	Altered fire regimes, agriculture, loss of native seedbank		✓				✓		Moderate
Rupercht et al., 2016	Romania	*Applied Vegetation Science*	9	Fragmentation, agriculture		✓			✓			Moderate
Sengel et al., 2016	Austria	*Basic and Applied Ecology*	3	Agriculture, altered soil nutrients			✓				✓	Moderate
Tikka, Heikkilä, Heiskanen, & Kuitunen, 2001	Finland	*Applied Vegetation Science*	3	Fragmentation, agriculture, invasive weeds					✓		✓	Moderate

(Continues)

TABLE 2 (Continued)

Author	Location	Journal	Study length (years)	Degrading pressures	Treatments H	F	PSA	CSA	BR	GM	ST	Effect
Tognetti & Chaneton, 2012	Argentina	*Biological Invasions*	2	Invasive weeds, native seedbank depletion, local extinction of large herbivores, altered fire regimes					✓		✓	High
van Dyke, Van Kley, Page, & Van Beek, 2004	United States	*Restoration Ecology*	14	Fragmentation, agriculture, invasive weeds, shrub encroachment, altered fire regimes		✓			✓			Low
Waller, Anderson, & Allsopp, 2016	South Africa	*South African Journal of Science*	2	Invasive weeds	✓	✓	✓			✓	✓	High
Wilson & Pärtel, 2003	United States	*Restoration Ecology*	7	Invasive weeds, agriculture	✓				✓		✓	High
Wohlwend, Schutzenhofer, & Knight, 2019	United States	*Restoration Ecology*	7	Grazing, agriculture	✓		✓	✓	✓		✓	High
Zhou, Wilson, Cobb, Yang, & Zhang, 2019	China	*Land Degradation and Development*	2	Agriculture, invasive weeds				✓	✓		✓	High

Note: The ✓ is used to show what treatments were used in each of the reviewed papers.

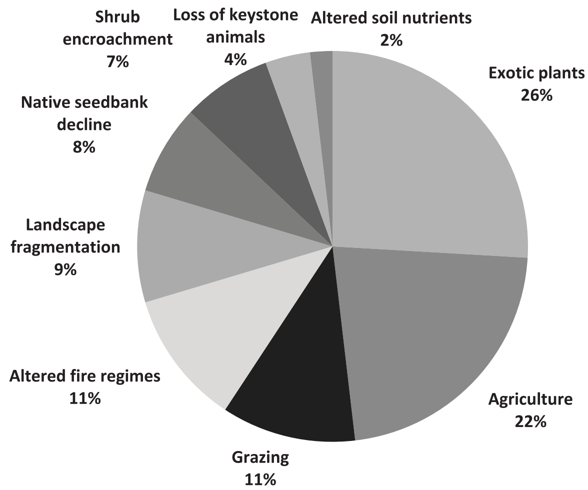

FIGURE 1 This chart highlights the different restoration techniques used and their frequency within the literature. Most experiments utilised multiple techniques, with seed/seedling transfer being the most commonly used. Mowing and clipping, a technique that is often used to mimic the effects of grazing, was the second most frequently used technique, while grazing manipulation and grazing exclusion were the least frequently used techniques

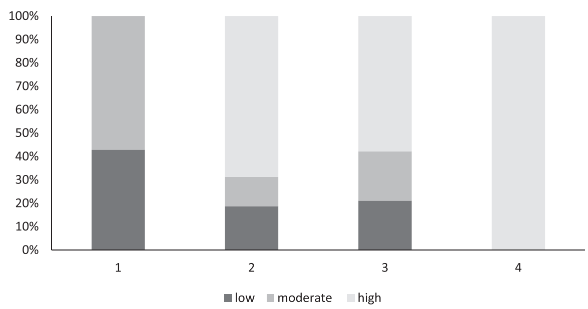

FIGURE 2 This indicates the degree of effectiveness when using 1, 2, 3 or 4 treatments. Those trials with only 1 treatment used were never highly successful. Experiments that included 2 or 3 treatments provided similar results (approximately 60% effective), however, studies that integrated 4 treatments were always highly successful

studies used over 30 species in their mix to improve species richness (Foster et al., 2007). Only four of the studies used a more passive approach to restoration. In these studies, the above ground biomass of invasive plant species was targeted using either manual removal, fire and/or grazing management. In these cases, native/target species were present or able to naturally colonise the study site, or the intervention was implemented early in the invasion process, which promoted the success of the passive restoration.

We found that studies that used only one treatment were never highly successful in reducing weed biomass (Figure 2). Using two or three treatments provided moderate effectiveness (60%), however four treatments or more where always highly successful. The combined use

FIGURE 3 Combining fire with the herbicide treatment proved to be highly effective (90%). When the fire treatments were used alone, the effect was only 40% effective

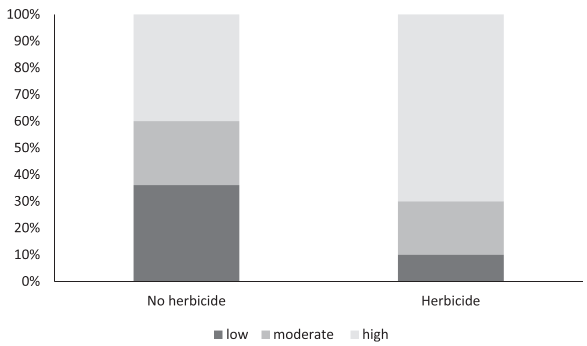

FIGURE 4 Experiments that included a herbicide treatment offered 60% effectiveness for achieving their restoration targets, while those studies that did not include a herbicide treatment were only 40% effective

of herbicide and fire (Figure 3) provided substantial control (90%) of exotic plants and promoted the establishment of target species, compared to fire and herbicide treatments being used singularly (40% and 60% effective, respectively). The experiments that incorporated herbicide with other treatments were more effective than herbicide alone experiments that did not (Figure 4).

4 | DISCUSSION

4.1 | Location of research

The majority of the selected experiments for this review were conducted in the United States and Europe. North America has degraded a considerable fraction of its intact temperate grasslands, estimate to be a loss of 2,572,704 ha, making it the region with the highest loss in the world. South America and China have lost a similar size of grasslands as the North America, but have only contributed one study each. While these areas produce literature regarding ecological restoration, there was very limited research from these regions published in English that fit the analysis criteria, particularly the criteria that required the experiments to manipulate and measure standing vegetation dynamics. It is possible that different criteria and the inclusion of non-English publications (notably Chinese, Portugese, Russian and Spanish) may alter this picture.

4.2 | Approaches to restoration

Costs associated with landscape restoration can limit the techniques available, particularly the magnitude and mixture of seed or plant additions, as well as limiting the labour and duration of a study. In cases where the restoration budget is severely limiting, passive restoration can offer some benefits, particularly if the degradation is in its early stages, or the target species are able to naturally recruit in the restoration context. Passive ecological restoration involves removing human-induced degrading pressures from a site with minimal remediation. In many cases, it is presumed that non-target species will expand without human intervention; however, many passive restorations have observed weeds to decline with sufficient native recruitment (Sinkins & Otfinowski, 2012; Valko et al., 2017). Notable vegetation shifts often occur within 10 years of rest from degrading pressures (van Dyke et al., 2004), however, it will take several decades before species richness increases, (van der Merwe & van Rooyen, 2011), and many sites may never recover their pre-degradation richness. The assemblage of species will also differ greatly from the remnant sites (van der Merwe & van Rooyen, 2011).

4.3 | Importance of site history

Understanding a site's history is critical for landscape restoration (Higgs et al., 2014). The history of a site can identify the factors that moved it into a degraded state, and whether these changes occurred rapidly or continuously over an extended interval (Prober & Thiele, 2005). Further, if known, historic vegetation cover can act as a restoration target, and guide managers on the composition of revegetation assemblages (Prober & Thiele, 2005). Given our search terms, we expected that invasive plants would be the most common degrading pressure mentioned in the literature. This was closely followed by agriculture. Agriculture often leads to other degrading pressures, including altered soil nutrient levels (which was only mentioned once) (Tikka et al., 2001), grazing intensities (Musil et al., 2005) and fire regimes (Rupercht et al., 2016). Therefore, most sites with a history of agricultural have breached both biotic and abiotic thresholds and are now held in an alternative stable state (Suding & Hobbs, 2009). This adds complexity to the restoration process needed for these sites, as there are multiple degrading forces to overcome (Foster et al., 2007; John et al., 2016). In these cases, active restoration techniques can be implemented to disrupt these new stable states. Active ecological restoration involves the integration of management techniques, such as revegetation, herbicide application or soil disturbance to take an ecosystem from a degraded state to one that is functional, self-sustaining and resilient. In weed-dominant systems, restoration efforts that focus on the removal of invasive plants and promote dense, native competition, are often the most successful. The active restoration studies analysed in this review highlighted that the restoration of a weed-dominated temperate grassland should implement one or more of the four techniques that; (a) remove the weeds biomass, (b) manipulate the soil to return it to a favourable condition for facilitating target species, (c) incorporate reintroduction of native propagules if they cannot recruit naturally, and (d) implement site specific grazing regime.

4.4 | Lessons learnt on implementing restoration techniques

4.4.1 | Targeting weed biomass

The results of this analysis show that incorporating active restoration efforts that target the above ground density of exotic plants was critical for reducing competition for naturally-recruiting native species, or those added via revegetation efforts. Weeds are often fast growing and form dense canopies, which reduces light to the soil and can thus restrict the germination and subsequent growth of native seeds or seedlings. Further, many annual grassland weeds have higher nutrient requirements than native perennial grasses, thus making abandoned agricultural sites, which have been treated many times with fertilizer, a highly competitive environment for natives to establish. The most commonly used methods for reducing weed biomass noted in the review included hand removal, mowing, herbicide application and fire.

Grubbing, or hand weeding, is a restoration technique that completely removes unwanted plants (Tikka et al., 2001). While highly effective, this method is also very labour intensive, and is usually only appropriate for small-scale projects (Gibson-Roy, Delpratt, & Moore, 2007). It has been observed to be most effective when applied repeatedly, for example, every 3 years, community efforts have successfully removed 34% of the invasive perennial grass, *N. trichotoma* throughout Canterbury, New Zealand, which has subsequently contained this invasive weed from further expansion (Bourdôt & Saville, 2019). Grubbing is one of the most effective methods to reduce competition for space, light and soil nutrients as the whole plant is instantly removed, as demonstrated by O'Dwyer and Attiwill (2000). These authors found that combining hand weeding with broadcasting seeds reduced weed density by 35%, but when grubbing was excluded, the broadcasted seeds were outcompeted. Grubbing is the best solution for sites where weeds are newly emerging and easy to remove, or where only a few individuals have established.

Herbicide application was used in 11 of the restoration experiments and was found to be an economically viable and effective solution for reducing weed competition. Herbicide works most effectively when integrated with other treatments, as seen by Johnson et al. (2018) who observed spot-spraying weeds with glyphosate significantly improved the establishment of native forbs seeds when combined with fencing, and the removal of leaf litter. Aerially spraying clopyralid (at a rate of 37.4 L ha^{-1}) was successful in reducing woody weed encroachment and enhancing plant diversity when combined with prescribed burning (Ansley & Castellano, 2006). Waller et al. (2016) also observed significantly improved native establishment when a herbicide treatment was combined with fire, tillage and rodent exclusions. In some cases, herbicides were also observed to be highly effective, and the most economically viable option, when used singularly (Huddleson & Young, 2005). Huddleson and Young (2005) identified that herbicide application on its own was effective for not only reducing annual weed competition by 40%, but increasing native establishment by a 10-fold margin.

In some cases, herbicide application was ineffective at improving native establishment (Cole, Lunt, & Koen, 2005; Conrad & Tishew, 2011). Spot-spraying Snapshot (a pre-emergent herbicide containing trifluralin and isoxaban) at 2.5 kg per 100 m^2, effectively controlled invasive annual grasses, however, native forbs were also significantly reduced (Musil et al., 2005). Herbicides can be used selectively so that weeds are killed, while the target species remain unharmed, making it the most desirable outcome. Such a situation can be achieved by understanding the physical growth parameters and plant physiology of both the weeds and native/target species, such as differences in their active growing seasons (Sutton, 1967).

It is important to note that the constant use of herbicides within an ecosystem can promote the emergence of herbicide-resistant populations (Heap, 2019), which will reduce long-term effectiveness. Resistance to arguably the world's most important herbicide, glyphosate, has already been observed in several weeds (Powles, 2008), including *Conyza* spp. (Feng et al., 2004; Urbano et al., 2007) and *Lolium* spp. (Baerson et al., 2002; Yanniccari, Vila-Aiub, Istilart, Acciaresi, & Castro, 2017). It is considered important, therefore, that herbicides should be used selectively, moderately and in combination with other control methods, in order to secure their effectiveness for the long term.

Taken together, the literature reviewed here suggests that the combination of fire and herbicide gives effective control of weeds and is an important tool for temperate grasslands restoration. Historically, grasslands are ecosystems that are accustomed to frequent fire events, and altered fire regimes across the world (Stromberg et al., 2007; Standish, Sparrow, Williams, & Hobbs, 2009; Sankaran & Anderson, 2009) have been a contributing factor to the degradation of these landscapes (Archer, Schifers, Bassham, & Maggio, 1988; Knicker, 2007). Fire quickly creates available space for heat-resistant seeds to germinate and grow relatively free of competition immediately after the fire event (Meyer & Schiffman, 1999). Lipoma, Funes, and Díaz (2018) identified fire to significantly reduce the viable number of seeds in the soil compared to pre-burnt conditions, and as most weeds have dense seedbanks, this can be beneficial in reducing at least the surface seedbanks of some species (Peltzer & Douglass, 2019). In contrast, some species, particularly broadleaf weeds such as *Echium plantagineum*, are promoted by fire (Prober, Thiele, & Lunt, 2004). This suggests that follow-up weed management of burnt sites is critical for the successful establishment of native species. Heat tolerance in seeds has been linked to seed shape, with more rounded seeds demonstrating higher resistance than thinner seeds, which are common in European temperate grasslands (Ruprecht, Fenesi, Fodor, Kuhn, & Tökölyi, 2015). Fire also offers soil manipulation services, since relevant carbon and nitrogen compounds volatize at 180 and 200°C respectively (DiTomaso et al., 2006), therefore assisting in the reduction of elevated soil nutrients. Strategically burning when problematic weeds are actively growing can also effectively prevent seed set for that season (Prober et al., 2005). The complexity of fire effects suggests that post management plans should be specific for the site in order to promote the optimum establishment of a healthy native grassland community (DiTomaso et al., 2006; Musil et al., 2005).

4.4.2 | Soil manipulation

It was observed that restoration techniques that target the soils chemical or physical composition were important for providing more effective control of invasive plants. In this review, we found that techniques that target the soil's physical characteristics were used 11-times, making it one of the most widely used techniques, while chemical modification was incorporated in only 6 of the 26 experiments. Both of these soil manipulations were important for improving native plant establishment.

Altered soil nutrients and textures resulting from agricultural practices have important consequences on the ability of vegetation to take up water and nutrients (Sankaran & Anderson, 2009). As a consequence, restoring these factors to resemble historic levels can be important for weed suppression. Soil nutrients such as nitrogen, phosphorous and potassium, are altered by agricultural practices, and even long after agriculture has ceased, the soil nutrient levels remain higher than historical levels (Prober et al., 2005). Annual weeds become problematic in environments with high nitrogen, where they are able to quickly dominate over the slower-growing native perennial grasses (Huddleson & Young, 2005). It is known however, that perennial species invest in the development of deeper root systems that allow them to store and recycle nutrients, giving established perennial species an advantage over annual weeds in areas of low nutrient availability. Therefore, integrating control methods that target soil nutrient levels should be strongly considered for those grassland restoration projects in areas that have a history of agriculture. This can be achieved with the addition of a carbon source, such as sucrose, which stimulates soil microbial activity, creating a highly competitive environment for nutrients, leaving nutrient-adapted weeds at a disadvantage. This technique has been used successfully in Australia (Hacker, Toole, & Melville, 2011; Prober et al., 2005) and the United States (Blumenthal et al., 2003), but only at small scales. In one reported prairie restoration, carbon addition reduced soil nitrogen by 86%, which subsequently reduced weed biomass by 54% (Blumenthal et al., 2003). While carbon addition has proven to be successful, it is a time and resource-demanding approach. Prober et al. (2004) used 500 g of sugar for every square metre, which was reapplied every 3 months, making this technique impossible to implement at a landscape scale. Further, it is only suitable with nitrophytic weeds (Blumenthal et al., 2003).

Another method for altering soil dynamics is through mechanical disturbance techniques, such as tilling or scalping. These techniques are effective for creating an environment that promotes the establishment of broadcasted seeds and sometimes reduces competition from established weed seedbanks (Tikka et al., 2001). Since other weed seeds respond positively to soil disturbance events, tillage can also be used to stimulate these stored seedbanks (Stromberg et al., 2007). Scalping is a technique where top soil is physically removed from a site to restore appropriate nutrient balance and remove seedbanks. This is a useful technique in highly degraded sites that are heavily infested by weeds (Brown et al., 2017). Nevertheless, scalping may result in excessive waste soil, remove remnant plants, increase erosion rates and habitat loss, and cause disrupted mycorrhizal symbiosis. It should, therefore, be implemented with caution (Brown et al., 2017; Gerlach, 2015; Gibson-Roy, Moore, & Delpratt, 2010). Further, weed reinvasion from the seedbank can still occur on scalped sites, and Gerlach (2015) identified weeds to occupy 70% of the ground cover after 3 years of scalping and revegetation. It is noted however, that scalping treatments followed by spot-spraying has proven to be successful within small scale (1 m × 1 m) plots for reducing all exotic vegetation (Gibson-Roy et al., 2010).

4.4.3 | Grazing management

Altered grazing regimes were observed to have contributed to the degradation of nine of the selected analysed papers. This includes overgrazing by livestock and the removal of important native grazing fauna. The effects of grazing alteration are complex, and may be positive or negative, depending on the grazing species and the context. One study within our analysis observed grazing to promote seed dispersal of target species (Baasch et al., 2016). Grazers play an important role in the continuous removal of leaf litter and generate space for new recruitment (Lengyel et al., 2012; Török et al., 2018), which can promote species richness (Klaus et al., 2018; Towne, Hartnett, & Cochran, 2005). Germination of the native North American prairie grass, *Nassella pulchra* was enhanced by burning and sheep grazing (Dyer, 2002). Moderate grazing (30–50 animals within a 303 ha enclosure) using Bison *Bos bison* significantly improved the species richness of Prairie grassland within its later stages of development (approximately 10 years after revegetation) (Wilsey & Martin, 2015). This result was also observed within tallgrass prairies (Towne et al., 2005). Livestock can transport seeds of important species over great distances via endo- or ectozoochory if remnant sites are available (Lengyel et al., 2012; Török et al., 2018). Further, the careful management of paddock rotations for grazing livestock has been identified to be critical in maintaining genetic diversity for plants threatened by fragmentation (Plue, Aavik, & Cousins, 2019).

Mowing and clipping are restoration techniques used to mimic the effects of grazing, and these techniques were used in 14 of the experiments analysed. John et al. (2016) observed that clipping three times within the 1-year experiment significantly improved the establishment of target species compared to only clipping once. Heavy clipping was used in the last 3 years of a 7-year restoration experiment to reduce weeds by 90%, significantly enhancing the establishment of target species (Wilson & Pärtel, 2003). The combined treatment of phosphorous fertilizer addition and mowing was also observed to enhance the establishment of seeded alfalfa species in a nutrient depleted steppe grassland, and this also improved natural succession of other target species not seeded (Zhao et al., 2018). In many cases however, the effect of mowing or clipping applied singularly or integrated with other techniques, was not a significant factor (Van Dyke et al., 2004), or was observed to be counterproductive (Marushia & Allen, 2011).

While grazing plays an important role in maintaining highly productive grasslands, those suffering excessive grazing pressure, extensive degradation or of lower productivity often benefit from the complete removal of grazing livestock. A long-term (20 and 30 years) grazing exclusion zone was developed in the steppe grasslands of China, which observed an increase in perennial grass cover, as well as higher density bud banks of favourable grasses when compared to the grazing sites (Zhao, Wang, Liang, & Wu, 2019). The effects of different degrees of overgrazing were observed by Török et al. (2018) within four different Hungarian steppe grassland communities. They found that the highest richness was achieved from low to moderate grazing (maximum of 2.5 animals per hectare), while grazing densities above this had detrimental effects of species richness. Further, different grassland communities responded differently to different grazing intensities suggesting they are grassland specific, due to differences in productivity and the consequent rate of biomass production (Török et al., 2018). Competition dynamics between forb and grass species were altered by livestock grazing in southern Argentina (Díaz Barradas, García Novo, Collantes, & Zunzunegui, 2001). Under sheep grazing, the grasses did not produce inflorescences and forbs became taller and more abundant compared to non-grazing tracts, where grass species dominated (Díaz Barradas et al., 2001). Forb cover was also observed to increase in Prairie grasslands when exposed to grazing from cattle and bison (Towne et al., 2005). Therefore, grazing intensities should be carefully managed to suit a particular system, particularly during drought periods to promote competition from native perennial grasses (Klaus et al., 2018), and resting paddocks from grazing when natives are emerging, particularly if herbage is sparse, could improve their establishment and survival (Clarke & Davison, 2004).

4.4.4 | Revegetation

Establishing dense competition from desirable species is the most effective way to reduce weeds and return natural ecosystem functionality. Revegetation was the most widely used restoration tool, with 22 of the 26 experiments incorporating this technique in some form. Grasslands have been observed to have short-lived seedbanks (Morgan, 1998), therefore, those that have been in an altered stable state for several decades are unlikely to have native viable seeds in the seedbank. Also, as a result of fragmentation, many sites are isolated from remnant grasslands, making seed transfer by migration unlikely. Where natural regeneration is unlikely, competition from desirable native species can be introduced using a variety of methods including direct seeding (Cole et al., 2005; Thomas et al., 2019), transfer of threshing material (Baasch et al., 2016) or hay (Sengel et al., 2016), direct drilling (Bakker et al., 2003) and plant plugs (Tikka et al., 2001). Hedberg and Kotowski (2010) reviewed the effectiveness of different revegetation options for fragmented grasslands and found that direct seeding (sowing and broadcasting) to be the most widely used and most effective for introducing species back to semi-natural systems. However, they specifically recommended the use of plant plugs for the establishment of rarer species (Hedberg & Kotowski, 2010).

The effectiveness of species richness in seed mixes has been explored for grassland restorations, with both high and low rates demonstrating beneficial results dependent on the projects scope (Prober & Thiele, 2005; Wortley, Hero, & Howes, 2013). The determined species mixture is often reflective of the goals of that particular restoration project; for example, Conrad and Tishew (2011) found a seed mix of 35 species achieved their goals of increasing species diversity as well as establishing target species, whilst in another area, Huddleson and Young (2005) used a mix of only three native grasses to successfully outcompete weeds. Further, it appears that high species diversity improves the establishment of native species (Barr, Jonas, & Paschke, 2017), long-term resilience to weed reinvasion (Carter & Blair, 2012; Scotton, 2016), and provide habitat for recolonisation of threatened wildlife (McDougal & Morgan, 2005). Nemec, Allen, Helzer, and Wedin (2013) demonstrated seed diversity to be a more important factor than seed rate for achieving resistance to weed invasion. While high seed rates can improve the chances in successfully outcompeting weeds (Bakker et al., 2003; Barr et al., 2017; Tikka et al., 2001), this approach can waste seeds as a result of higher intraspecific competition, and the associated high costs can make it impractical (Sheley, Mangold, & Anderson, 2006; Wagner, Pywell, Knoop, Bullock, & Heard, 2011). Seed mixes low in diversity and density can promote spontaneous secondary succession, and this can stimulate ecosystem processes more quickly (Lengyel et al., 2012). We note that the failure of sown seeds to establish can be linked to several factors, including herbivory, adverse weather conditions, and competition (Gibson-Roy et al., 2007), therefore implementing pre-sowing management that minimises these threats is critical. Whilst it is clear that the introduction of seeds or seedlings is often critical for the restoration of many degraded temperate grasslands it is also clear that the best implementation method will be dependent on the site, scale and funding available to the project (Prober & Thiele, 2005).

5 | CONCLUSIONS

The majority of papers analysed in this review demonstrated that agricultural practice inevitably alters the physical and chemical composition of the soil, reduces biodiversity and alters grazing and fire regimes. This promotes aggressive invasion and establishment of exotic plants after the land has been abandoned. In these cases, biotic and abiotic ecological thresholds are soon breached, making passive restoration associated with land abandonment unsuccessful. While there are a variety of active restoration techniques available, this review concludes that these actions are most successful when four different methods are integrated. These are: (a) removal of the weeds biomass; (b) if necessary, manipulating the soil to return it to a remnant condition; (c) incorporating revegetation of native propagules; and (d) implementing site-specific grazing management. By incorporating these techniques, feedback loops created by weeds are disrupted, and favourable plants are facilitated. A traditional meta-analysis review on a similar subject would be high advantageous for future research, once either more literature becomes available or by encompassing a broader spectrum of grasslands, as quantitative data could provide a more conclusive results.

Based on the literature reviewed, we recommend that future temperate grassland restoration efforts should:

i. Conduct a comprehensive background check on the selected site's land use history and ecological community prior to disturbance. Understanding this is critical for identifying what degrading pressures are acting on the ecosystem, which can then signal what approach should be used to remove or alter these pressures. Where possible, knowledge of a site's ecological community prior to disturbance will help to set realistic restoration goals and act as a guide when selecting suitable species for broadcasting;
ii. Identify an intact, remnant reference site for measuring what restoration success should look like;
iii. Incorporate seed broadcasting to return target species to the site, particularly if the site is isolated from remanent sites. This treatment will also provide weed control through competition;
iv. Include ongoing exotic plant management, as these species have been observed to be the most frequent degrading pressure on native sites, and are the main obstacle preventing successful restoration; and,
v. Integrate multiple, ongoing treatments that focus on standing vegetation, grazing, and soil chemistry and physiology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Chapter 3. Ecology, distribution and control of the invasive weed *Nassella trichotoma* (Nees) Hack. ex Arechav.: A global review of current and future challenges

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REVIEW PAPER

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Ecology, distribution and control of the invasive weed *Nassella trichotoma* (Nees) Hack. ex Arechav.: A global review of current and future challenges

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Abstract

Nassella trichotoma (serrated tussock) is a highly invasive perennial C_3 weed from South America. It grows in most soil conditions, can resist fire and frost, and is unpalatable to grazing animals. Each plant can produce up to 140,000 seeds annually, and together, these characteristics make it a damaging landscape weed. It has diminished the agricultural carrying capacity of pastures in south-eastern Australia, New Zealand and South Africa, and emerging populations have now been identified in Europe and the United States, and bioclimatic models suggest its distribution could significantly expand within these regions in the near future. Research into control methods for this weed has been explored, and these include herbicides applied alone and in combination, the establishment of plant competition, the introduction of seed mitigation fencing, grazing management and exclusion zones, specific biological management and alteration of soil composition. Currently, the most effective and widely used control method is the residual herbicide flupropanate (2,2,3,3-tetrafluoropropanoic acid). This review will investigate the ecology, distribution, current control techniques and past research on this species, and make recommendations for future research and management.

KEYWORDS
climate change, integrated weed control, *Nassella trichotoma*, serrated tussock, weed management

1 | INTRODUCTION

Nassella trichotoma (Nees) Hack. ex Arechav. (serrated tussock) is a hardy and competitive weed species of the family Poaceae and has been identified as being widespread in Australia (Campbell and Nicol, 1999), New Zealand (Bourdôt and Saville, 2019) and South Africa (Joubert, 1984). It is an emerging weed in the United States (USDA, 2020) and several European countries (EPPO, 2012). Tolerant to extended periods of frost and drought, it is also rejuvenated by fire (CRC, 2013) and has the ability to flourish in a wide variety of soil types (Lamoureaux et al., 2011) and fertility levels (Bourdôt and Saville, 2019; CRC, 2013).

Knowing the life cycle of a weed can help identify when a species is most susceptible to treatments that limit its reproduction and prevent further spread, and allow cost-efficient and successful management solutions to be developed (Ahmed et al., 2015). Despite extensive research into herbicide application (Grech et al., 2012; Pritchard and Bonilla, 1999), competition (Badgery et al., 2005; Miller, 1998; Vere and Campbell, 1984), biological control (Anderson et al., 2002; Briese et al., 2001; Hussaini et al., 2000), grazing (Vere and Campbell, 1984)

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and burning (Joubert, 1984), *N. trichotoma* is still globally problematic (Kriticos et al., 2004; Vere and Campbell, 1984).

Because *Nassella trichotoma* is a highly threatening perennial grass weed to agriculture and general biodiversity (CRC, 2013; Vere and Campbell, 1984), globally adaptable landscape-scale solutions for its management are essential. We suggest that a global review of the literature can focus the extant work, facilitating a balanced understanding of the problems associated with the current management strategies and reasons why previous research has failed to be implemented.

2 | BIOLOGY AND ECOLOGY

2.1 | Physical description

Nassella trichotoma is a perennial tufted grass that can live in excess of 20 years (CRC, 2013). It utilises a C_3 photosynthetic pathway, and grows at latitudes between 30^{O} and 45^{O} South (Campbell, 1982) in areas with an annual mean rainfall of 500 to 990mm (Campbell and Vere, 1995). It is identifiable by the light serrations on the leaf blades felt when stroking towards the base (Osmond et al., 2008), and by observing a 1mm, white, hairless and rounded ligule found at the leaf/base intersection (McLaren et al., 2004). It is more commonly found on southern-facing slopes due to higher plant competition on northern-facing slopes (Lamoureaux and Bourdot, 2002). In fertile soil conditions, the grass will grow to a height of 60cm and develop a dense base diameter of 25cm, which helps to protect the plant from fire, frost and drought conditions (Osmond et al., 2008). The tightly rolled leaves have low nutritional value (Campbell and Barkus, 1965; Osmond et al., 2008), and in grazing systems, it is not preferentially grazed by livestock and can quickly outcompete more palatable species (Pisani et al., 2000). The low nutritional value of its leaf litter means that nutrients are not effectively recycled back into the soil, resulting in the soil being significantly poorer than that under palatable species, promoting growth of unpalatable species (Moretto and Distel, 1997). Its yearly growth cycle is summarised in Table 1.

2.2 | Germination

Whilst *Nassella trichotoma* seeds undergo a brief non-deep dormancy period (Table 1), under favourable conditions, germination may occur at any time of the year, particularly if there is a soil disturbance or fire (Osmond et al., 2008). Germination has been observed to be significantly reduced by drought and salinity (Humphries et al., 2018), but light availability is not a significant factor since seeds can germinate under both alternating light regimes and complete darkness (Humphries et al., 2018; Lusk et al., 2008). Seeds are in highest abundance 2cm into the soil profile (Joubert, 1984) and at this depth have significantly higher emergence rates than seeds on the surface or those buried more deeply (Humphries et al., 2018). This suggests that the awn is hygroscopic, assisting burrowing of the seed into the soil profile, a strategy which protects seeds from dynamic soil moisture patterns frequently observed within temperate grasslands (Badgery et al., 2008). Whilst there is evidence that some seeds can remain dormant for several years (Campbell and Nicol, 1999), most seeds will germinate within a six- to twelve-month period, resulting in a 74-91% reduction of the annual seedbank (Campbell and Nicol, 1999; CRC, 2013).

2.3 | Reproduction and seed dispersal

Reproduction occurs via seed production, the majority of the seeds forming in cleistogamous (self-pollinating) flower heads, which is a typical weed trait that allows small populations to grow quickly (Connor et al., 1993). Cross-pollination is achieved by wind, but accounts for only 10-15% of the flowers (Connor et al., 1993; Osmond et al., 2008). In early spring, the weed will flower and seeds will mature within the seed head for up to 10 weeks. As the seeds reach maturation, the seed head changes its colour from a deep purple to a pale yellow, droops to the ground and break loose from the plant for dispersal (CRC, 2013; Osmond et al., 2008; PIRSA, 2011; VSTWP, 2017). The seed head is multi-branched, allowing it to catch the wind and tumble along the ground for seed dispersal (Osmond et al., 2008). Seed heads can travel great distances, with one observation being 16 kilometres dispersal from the parent plant (Campbell and Vere, 1995). Another study suggested that emigration rates for *N. trichotoma* are as high as 97%, which suggests most of the seeds produced by a population are not deposited into the local seedbank (Lusk et al., 2008). Seeds are also dispersed in agricultural systems via livestock, as the seeds attach to animal hair, fur or wool, or pass through their digestive tracts (DPIPWE, 2016; PIRSA, 2011). The seeds can also get lodged in mud, being thus transported by the feet

TABLE 1 The life cycle of *Nassella trichotoma*. The darker shade indicates general growth patterns, whilst the lighter shade indicates growth patterns under favourable conditions (Osmond et al., 2008; CRC, 2012; VSTWP, 2017)

	Autumn	Winter	Spring	Summer
Germination				
Bleaching				
Flowering				
Seed formation				
Seed drop				

of animals and humans, or vehicle tyres (DPIPWE, 2016). The plump seeds are approximately 1.5 to 2 mm in length and have a golden-blonde colour with white hairs at their tip with an off centre, variable length, awn (DPI, 2016; VSTWP, 2017). It has been estimated that a heavy infestation of *N. trichotoma* could result in an additional 930 million seeds being annually added to the soil seedbank per hectare (DPIPWE, 2016; Osmond et al., 2008).

3 | DISTRIBUTION, HISTORY AND CURRENT MANAGEMENT OF *NASSELLA TRICHOTOMA*

Nassella trichotoma, being an economic threat to agricultural and natural grassland systems (Jones and Vere, 1998; Lamoureaux and Bourdot, 2002), is considered to be a globally significant weed. Countries where this weed is not widespread are still on alert for outbreaks (CABI, 2020; USDA, 2020). The current global distribution of *N. trichotoma* is summarised in Table 2, and Figure 1 highlights the key historic events regarding the spread and management of *N. trichotoma* across the globe.

3.1 | Native distribution

Nassella trichotoma is native to Bolivia (CABI, 2020), Brazil (Missouri Botanical Gardens, 2003), Chile (McLaren et al., 1998), Peru (EPPO, 2012), Uruguay (Erb, 1988) and Argentina (Erb, 1988). It is not considered to be a problematic species in South America, as its low palatability, which can reduce carrying capacity for economically important animals, is controlled by natural means.

Competition studies conducted in Argentina have demonstrated that native grasses that are more palatable and productive outcompete unpalatable grasses like *N. trichotoma*, significantly reducing their growth and seed production (Moretto and Distel, 1997). Therefore, maintaining high-quality pastures to prevent *N. trichotoma* invasion is the primary control method in agricultural systems, but in times of drought and overgrazing, this weed can become problematic, and once established, it can be difficult to remove (Moretto and Distel, 2002). However, it has also been observed that the presence of other unpalatable grasses in South America can prevent it from dominating and becoming a monoculture in times of intense grazing or drought (Badgery et al., 2003). Controlling unpalatable grasses with herbicides has not been a commonly used technique until recent years (Rodriguez and Jacobo, 2010).

3.2 | Invasion in other regions

3.2.1 | New Zealand

Nassella trichotoma is one of the most economically damaging weed species in New Zealand, costing $NZ27.1 million annually in loss of agricultural production and control expenses (Saunders et al., 2017). It was introduced accidentally to New Zealand in the 1860s, and due to its similarity in appearance to native tussock species, it was not recognised as a weed until the 1930s. By this point, *N. trichotoma* invasion was prolific, with densities reaching up to 34,000 plants per hectare (Lamoureux and Bourdot, 2002). The South Island, particularly Canterbury and Marlborough, was the region most affected, and farms in these regions suffered significant reductions in carrying capacity of livestock. In 1946, government intervention was introduced in order to control this weed and to rehabilitate infested sites. The Nassella Tussock Act (1946) (NZLII, 2020) saw two government-funded boards dedicated to the broad-scale control of *N. trichotoma* throughout Canterbury and Marlborough. The funding extended from 1946 to 1990, and during this time, densities were reduced to five plants per hectare on undeveloped lands and two plants per hectare on developed lands (Lamoureux and Bourdot, 2002).

After 1990, a locally funded $NZ1.3 million annual grubbing programme was brought into action within the Canterbury region. The Regional Pest Management Strategy (RPMS) required 98% of established *N. trichotoma* to be removed prior to the time of seed set (31st of October) (Smith and Lamoureaux, 2006). Although RPMS approaches were criticised to lack clear objectives and measurable goals, distinct research questions have been instituted, and improved evaluation of control efforts has promoted research into *N. trichotoma's* seed ecology, grubbing rates and competition dynamics (Smith and Lamoureaux, 2006). It was found that seeds mature earlier within the panicles than originally thought, which resulted in compliance dates changing from late spring to early spring (30th of September) for 900 of the 1,300 invaded properties. This ensured plants are removed prior to seed maturation, in order to reduce the seedbank (Smith and Lamoureaux, 2006). The implementation of stricter penalties improved *N. trichotoma* management by land managers, with a reduction from 28% to 11% in non-compliance observed (Smith and Lamoureaux, 2006). These measures have successfully maintained population numbers to 14 plants per hectare in the Canterbury region (Environment Canterbury, 2020).

Grubbing, a control technique that removes the above- and below-ground portion of a plant, shaking soil off the roots to prevent further growth, has been observed to be the most successful means for controlling *N. trichotoma* in New Zealand. Due to the dense root system, complete plant removal can be a challenging and time- and labour-intensive task. Grubbing prior to seed set prevents fresh seeds entering the soil seedbank, which reduces recruitment in the subsequent years. Denne (1988) calculated the cost–benefit ratio for grubbing intensity versus the rate of tussock growth, concluding that reducing grubbing intensity to once every three to four years would yield higher benefits without the danger of *N. trichotoma* spreading. This was supported by Lamoureux et al. (2011) who used a logistics model to demonstrate that, as a result of improved pasture management, *N. trichotoma's* growth is slower than historic rates. Conversely, Lamoureux and Bourdot (2002) noted that land

TABLE 2 Current global distribution of *Nassella trichotoma*

Country	State/Provence/Territory	Status	Reference
Australia	NSW	Established; Widespread	Kriticos et al. (2004), Laffan (2006), EPPO (2012), McLaren and Bonilla (2012), DPI (2016)
	Victoria	Established; Widespread	Hussaini et al. (2000), Anderson et al. (2002), Kriticos et al. (2004), EPPO (2012), McLaren and Bonilla (2012)
	Tasmania	Established; Widespread	Kriticos et al. (2004), EPPO (2012), McLaren and Bonilla (2012)
	Australian Capital Territory		Kriticos et al. (2004)
Argentina		Native	Briese and Evans (1998), Briese et al. (2001), Anderson et al. (2002), Kriticos et al. (2004), McLaren and Anderson (2011), EPPO (2012)
Bolivia		Native	Kriticos et al. (2004)
Brazil		Native	Kriticos et al. (2004), EPPO (2012)
Chile		Native	Kriticos et al. (2004)
France		Established	Campbell and Vere (2016), Kriticos et al. (2004), EPPO (2012)
Italy		Established	Campbell and Vere (2016), Kriticos et al. (2004), EPPO (2012)
New Zealand		Established; Widespread	Denne (1988), Campbell and Vere (2016), Kriticos et al. (2004), Lamoureaux et al. (2011), EPPO (2012), Bourdôt and Saville (2019)
Peru		Native	Kriticos et al. (2004)
South Africa	Western Cape	Established; Widespread	Viljoen (2017), Kriticos et al. (2004), EPPO (2012), ISSA (2020)
	Eastern Cape	Established; Widespread	EPPO (2012), ISSA (2020)
	Limpopo	Established; Widespread	EPPO (2012), ISSA (2020)
	Gauteng	Established; Widespread	EPPO (2012), ISSA (2020)
	Free State	Established; Widespread	EPPO (2012), ISSA (2020)
	Mpumalanga	Established; Widespread	EPPO (2012), ISSA (2020)
UK		Established	Kriticos et al. (2004)
Uruguay		Native	Kriticos et al. (2004), EPPO (2012)
USA	Arizona	Present	USDA (2020)
	Florida	Present	USDA (2020)
	Hawaii	Present	USDA (2020)
	North Carolina	Present	Westbrooks and Cross (1993), EPPO (2012), USDA (2020)
	South Carolina	Present	Westbrooks and Cross (1993), EPPO (2012), USDA (2020)

disturbance and subsequent available space that results from chipping established plants favour recruitment of *N. trichotoma* over favourable pastures.

Registered chemical controls include glyphosate (N-(phosphonomethyl)glycine), dalapon (2,2-dichloropropanoic acid), haloxyfop-P-methyl (methyl (2R)-2-(4-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]oxy}phenoxy)propanoate) and flupropanate. The latter is a semi-selective residual herbicide that targets C_3 grasses and has shown to be effective for reducing population sizes over consecutive years (Badgery et al., 2003; Osmond et al., 2008). This herbicide is usually applied aerially, but can be effectively spot-sprayed. Recent research suggests that flupropanate negatively impacts favourable pasture species in New Zealand, with an 89% reduction in favourable grasses observed eight months after spraying at the standard rate of 1.49 ai ha^{-1}. Further, thistles were promoted by this herbicide, by taking advantage of the consequent competition release. Glyphosate (15 m L^{-1}) and dalapon are only recommended in pastures with dense stands (AgPest New Zealand, 2020), and galliant (5 m L^{-1}) (active ingredient haloxyfop-ethoxyethyl ester) is most suitable for spot-spraying between desirable broad-leaved species (Weed Busters, 2020). Burning and grazing are not suitable for control, since these techniques favour *N. trichotoma* recruitment over native grasses (Auckland Council, 2020; Weed Busters, 2020). Of concern is that bioclimatic models suggest that *N. trichotoma*

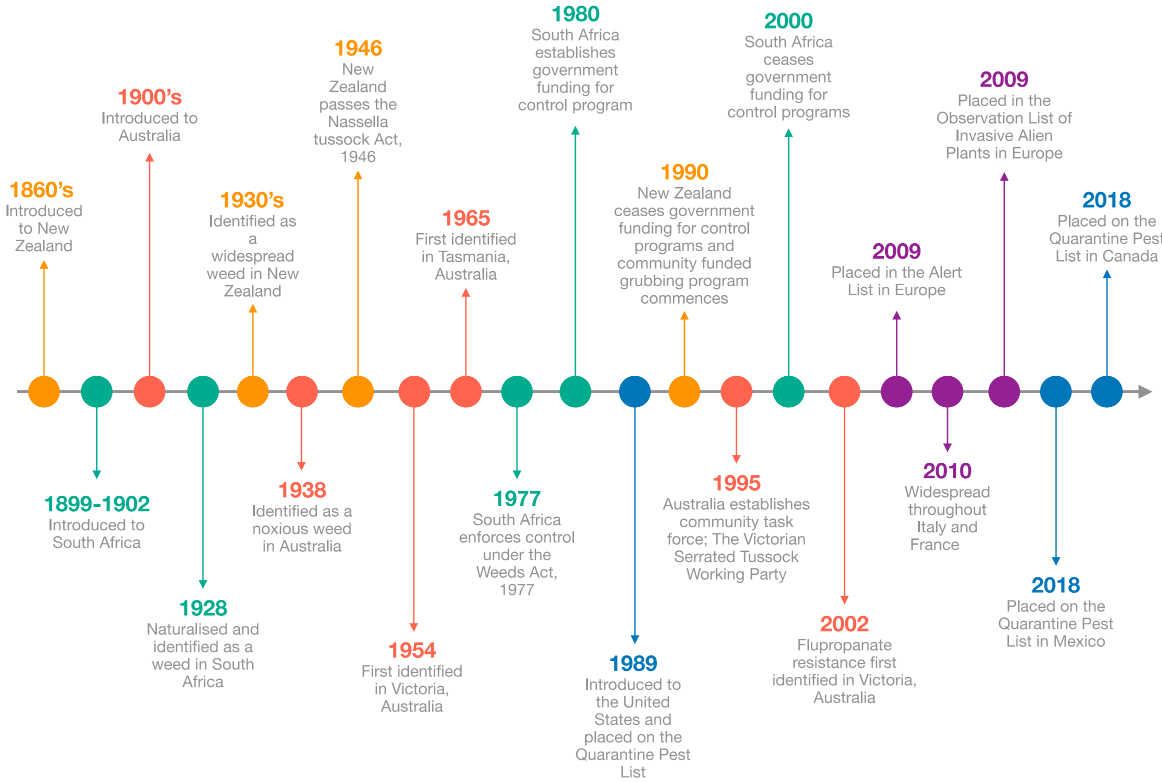

FIGURE 1 Timeline of N. trichotma's global invasion and management history. Key to timeline; orange; New Zealand, green; South Africa, red; Australia, blue; America, purple; Europe [Colour figure can be viewed at wileyonlinelibrary.com]

currently occupies 5.4% of the estimated 46% of climatically suitable land mass in New Zealand (Watt et al., 2011), making continuous control of this weed critical for preventing its spread.

3.2.2 | Africa

Nassella trichotoma is currently confined to South Africa, with small pockets of the continent being climatically suitable for its establishment (Kriticos et al., 2004). This species was presumed to have been introduced accidentally within contaminated fodder during the Anglo-Boer war during 1899-1902. It was first recorded as naturalised in 1928 (Henderson, 2017), and by 1985, it had invaded in excess of 70,000 hectares, with a further two million hectares considered at risk (ISSA, 2020; Viljoen, 1987). The Eastern Cape, Karoo and winter rainfall regions are most affected, where in some cases, the cost of control exceeds that of the land, resulting in land abandonment. It is classed as a 'Category 1b' noxious weed, and controlling *N. trichotoma* has been enforced by law since 1977 under the Weeds' Act of 1937.

During the 1980s and 1990s, Government subsidies for herbicide and task teams saw a decline in *N. trichotoma* populations. This funding ceased in the early 2000s, and enforcement of this weed's control has lapsed, despite it still being highly invasive. In 2017, it was reported that *N. trichotoma* had invaded an estimated 25% of the Golden Gate National Park (Henderson, 2017). Little research has been conducted on *N. trichotoma's* distribution or impact in South Africa since the 1990s, and these outbreaks highlight that this weed is still a significant problem and stricter measures for enforcing control are required. The herbicides flupropanate at 0.88kg per hectare (Viljoen, 1987) and glyphosate are the two currently registered control methods.

3.2.3 | Australia

In Australia, *N. trichotoma* has invaded over two million hectares, but a further estimated 32 million hectares is climatically suitable for its establishment, meaning it has the potential for considerably greater dispersal (DPI, 2016). Its distribution is currently contained to New South Wales, the Australian Capital Territory, Victoria and Tasmania, despite areas of other states having climatically suitable conditions (DPI, 2016; Kriticos et al., 2004; McLaren et al., 1998). It was firstly identified in Australia in 1935 and was suspected to have been introduced via contaminated fodder imported from South America or New Zealand, and was declared to be a noxious weed by 1938 (Campbell and Vere, 1995; Parson and Cuthbertson, 2001).

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Nassella trichotoma is one of Australia's 32 Weeds of National Significance as it has a devastating effect on the biodiversity and carrying capacity of natural and managed systems (Badgery et al., 2003; Goldson et al., 2015), and is a fire hazard within urban regions, burning hotter and faster than native grasses (Osmond et al., 2008). Jones and Vere (1998) identified that *N. trichotoma* costs the New South Wales grazing industry in excess of $AUD40.3 million per year in control and loss of production. Estimates giving the cost of control and lost production throughout Victoria, Tasmania or the Australian Capital Territory indicate that it is in the region of millions of dollars annually (McLaren et al., 2006). A mix of private and industry Australian land managers from New South Wales, the Australian Capital Territory, Victoria and Tasmania was surveyed to identify the extent of the financial impact of this weed, and of the 51 respondents, it was found that, on average, *N. trichotoma* costs $AUD12,996 in lost production, per land manager, annually (McLaren et al., 2006).

In areas that are not invaded by *N. trichotoma*, preventative methods, including good pasture management, mesh fencing and wind blocks, are recommended. The establishment of tree blocks and fencing can be a highly effective way to reduce *N. trichotoma* dispersal into unaffected properties (Miller, 1998), since the weed physically disperses its seeds within the mobile seed head, and fences act as dispersal barriers. Additionally, if native grasses or pasture are well maintained within the fenced area, *N. trichotoma* seedling establishment will be negligible (DPI, 2016). Small infestations can be managed with chipping or grubbing individual plants (Osmond et al., 2008).

Due to the broad distribution of *N. trichotoma*, the main control mechanism currently employed is herbicide treatments, applied via spot or broadacre spraying (Osmond et al., 2008). The non-selective herbicides, dalapon, glyphosate and the semi-selective herbicide flupropanate are registered for its control (Campbell and Murison, 1985; Grech and McLaren, 2010), although dalapon is rarely used as the tussocks require burning after spray, which is not always feasible (Osmond et al., 2008). Whilst glyphosate will effectively kill mature plants, reinvasion will occur if competition from perennial pasture species is not quickly established (Badgery et al., 2008; Pritchard and Bonilla, 1999). To date, flupropanate at a dose of 745 g L^{-1} is the herbicide of choice, being effective at reducing population sizes for consecutive years (Badgery et al., 2003; Badgery et al., 2008; Osmond et al., 2008). Observations indicate that flupropanate takes about a year to successfully kill an adult plant, making it a slow process to use alone (Badgery et al., 2008), but this herbicide leaves a germination-inhibiting residue on the soil that can last for up to five years, preventing any re-colonisation or successful germination within this time (Osmond et al., 2008).

It has been established that herbicide resistance to flupropanate is increasing within Australia, with approximately 20% of the *N. trichotoma* population across Victoria already requiring a dose four times the amount that was initially recommended (Grech et al., 2009; McLaren et al., 2008; McLaren et al., 2006a; Ramasamy et al., 2010). The mechanism for resistance is still unknown; however, it has been identified that the resistance can rapidly spread, and within a year of a resistant population being detected, resistant plants were identified up to 3.5km from the original source population (Ramasamy et al., 2011). Because *N. trichotoma* seeds and pollen grains can be dispersed by wind, this suggests that the resistant gene could be travelling even further (Campbell and Vere, 1995; Ramasamy et al., 2011). Pollen was not observed to be a big contributor to the heritability of flupropanate resistance since the resistance was observed to non-Mendelian and passed on more frequently on the maternal side (Ramasamy et al., 2010). Most *N. trichotoma* flowers are cleistogamous, cross-pollinating only in about 10% of flowers (Connor et al., 1993), making it unlikely that pollen will be a significant issue for resistance spread.

For the herbicide to have a continued effect on resistant *N. trichotoma*, the dose must be increased. Although flupropanate is a semi-selective herbicide, in increased doses it has been observed to negatively impact C_3 native pasture grasses, thus reducing their competitiveness, a key for successful long-term management (Badgery et al., 2008; McLaren et al., 2008). Additionally, in increased doses, the herbicide stunts the growth and increases deformities of canopy cover species including the native *Acacia aulacocarpa*, *Acacia verniciflua* and *Casuarina* sp. This makes its use in natural environments undesirable (Badgery et al., 2008; McLaren et al., 2008), and if herbicides continue to be used without the integration of other control methods, *N. trichotoma* resistance will increase and spread, requiring alternative solutions for control without delay (McLaren et al., 2014).

Due to its landscape-scale spread, more efforts into co-ordinating collaborative, community-based action are required. The community-led approach to managing this weed has been successful in Victoria, with the Victorian Serrated Tussock Working Party (VSTWP) taking significant independent control action (VSTWP, 2020). Various rural landowners throughout New South Wales described their biggest motivations for controlling *N. trichotoma* were a sense of doing the right thing, respecting the legal framework and trusting their neighbours were also controlling the weed (Marshall et al., 2016). The main concerns were the lack of efforts made by their neighbours, resulting the seeds from uncontrolled properties reinvading their own (Berney et al., 2012). Collaborative, co-ordinated control efforts are vital for reducing wind spread of this weed into treated areas; therefore, in addition to promoting individual motivation levels, community-targeted attacks are critical for establishing control (Berney et al., 2012; VSTWP, 2020).

3.2.4 | North America

In the United States, *N. trichotoma* is on a Federal Noxious Weed List (USDA, 2020). It was introduced to the United States in 1988 through contaminated *Festuca arundinacea* seeds imported from Argentina, which were recalled a year later (Westbrooks and Cross, 1993). It is believed to be present in Illinois, Kentucky, Arizona, Florida, Hawaii, and North and South Carolina (CABI, 2020; EPPO, 2012; USDA, 2020). A bioclimatic model demonstrated that no area of the United States shares a similar climate to *N. trichotoma's* current invasive regions when both precipitation and temperature were considered, suggesting it may have limited invasive potential (Patterson, 1994). *Nassella trichotoma*, as a weed, has never been reported in Canada; however, it is regulated as a pest under the Plant Protection Act, and is listed as a prohibited weed under the Seeds Act (Inspection, 2020).

3.2.5 | Europe

Nassella trichotoma has been confirmed in the United Kingdom, France and Italy (EPPO, 2012; Kriticos et al., 2004). It was introduced as an ornamental grass throughout Europe, and it is not considered a troublesome weed. It was downgraded from the EPPO Alert List to the EPPO Observation List of Invasive Alien Plants in 2012. Climatic modelling estimated that 19% of Europe's total land mass was climatically suitable for *N. trichotoma*, with the envelope mostly covering western Europe (Watt et al., 2011). In the south of France, *N. trichotoma* has been observed to have spread rapidly along roadsides. Despite this worrying trait, *N. trichotoma* is still an acceptable ornamental grass in provenances outside those at risk. The EPPO (2012) suggests prevention as the best control strategy, but in infested areas, integrating herbicide use with ploughing is important for preventing reestablishment.

4 | REVIEW OF RESEARCH INTO CONTROL TECHNIQUES

Various methods for *N. trichotoma* control have been developing for over 50 years. Research exploring preventative measures, including grazing management and identifying areas at risk of invasion using bioclimatic models, is important for preventing spread of this weed into unaffected areas. The implementation of effective preventative strategies is critical for areas at risk of invasion, as *N. trichotoma* has been observed to be a poor competitor with more palatable grasses (Moretto and Distel, 1997), being usually absent in well-managed pastural systems and intact grasslands (Miller, 1998). Research into various herbicides, biological controls and removal techniques has also been extensively explored in an attempt to provide a solution to restore invaded landscapes. Because *N. trichotoma* is a widespread weed, research into control techniques that can be applied at the landscape scale is required. These techniques and their applicability for controlling *N. trichotoma* are discussed below and summarised in Table 3.

4.1 | Preventative strategies

4.1.1 | Grazing

N. trichotoma provides low nutritional value to grazing animals as their foliage has low concentrations of proteins and nutrients. Whilst goats and cattle have been observed to graze *N. trichotoma* better than sheep (MLA, 1993), in a field occupied by 80% coarse grass cover (including *N. trichotoma*), these grasses were virtually untouched, whilst the 4% cover of soft grasses and 16% cover of shrubs were grazed heavily (Pisani et al., 2000). In addition to this finding, Distel et al. (2007) observed that after trimming *N. trichotoma's* leaves at the base to mimic grazing, they quickly regrow, which suggests it may possess grazing-tolerant traits and be able to withstand grazing pressures. This trait was speculated to have evolved as a result of frequent abiotic disturbances, particularly with frequent drought periods within its home range (Distel et al., 2007). Sheep forced to graze *N. trichotoma* suffered severe weight loss in both un-supplemented and supplemented treatments (Campbell and Barkus, 1965). The effect of grazing pressure from pest species (rabbits/deer/feral goats/insects) or native species (kangaroos) has not been explored, but it is unlikely that these fauna will graze on *N. trichotoma* directly, and high-intensity grazing on surrounding palatable grasses will promote a competition release for the weed.

Despite this objection, options for introducing grazing in *N. trichotoma*-infested sites have been explored. Research suggests that this weed provides moderate nutritional value when in its seedling stage or rejuvenation stage (MLA, 1993; Distel et al., 2005). Whilst fire has been observed to improve mineral and protein levels of unpalatable grasses in Argentina, these benefits are only short term (Distel et al., 2005). In Australia, fertilising with superphosphate and sulphate of ammonia was observed to briefly enhance the palatability of *N. trichotoma* when combined with burning (Campbell and Barkus, 1961). *Nassella trichotoma* is not a competitive weed in agricultural systems unless bare patches form from intensive grazing. When free from grazing, the palatable grass *Stipa clarazii* was observed to outcompete *N. trichotoma* and significantly reduce its seed output and growth (Moretto and Distel, 1997). Similar observations were made by Badgery et al. (2008), where *N. trichotoma* was not competitive with native grasses in rotation grazing regimes, or under grazing exclusion, particularly when fertiliser was withheld. In agricultural systems where livestock are grazing-infested sites, rotational grazing strategies should be implemented to protect pastures from being overgrazed (Miller, 1998). Animals that are grazing within infested paddocks should be quarantined for 10 days in a holding paddock to prevent distributing seeds into unaffected paddocks (Miller, 1998).

4.1.2 | Bioclimatic model predictions

The effect of climate change on future *N. trichotoma* distribution in Australia and around the world has been explored using bioclimatic models. These techniques identify the current distribution of a chosen species and use algorithms to predict changes in its distribution as a response to selected environmental parameters (Heikkinen et al., 2006). Bioclimatic modelling systems have demonstrated that 17% of Australia is currently climatically suitable for *N. trichotoma*, but this number is expected to reduce by 2050 in response

TABLE 3 Summary of past research into *Nassella trichotoma* management approaches

Control type	Treatment	Effect	References
Herbicide	Butoxydim	125 g ha^{-1} killed 80% of mature plants.	Pritchard and Bonilla (1999)
	Clethodim	90 g ha^{-1} killed 100% of mature plants.	Pritchard and Bonilla (1999)
	Flupropanate	745 g L^{-1} is recommended for control. Not suitable for areas with native pasture as they are highly susceptible reducing competition and *N. trichotoma* is favoured. Resistance is becoming widespread due to overuse. No significant effect to resistant populations.	Badgery et al. (2003), McLaren et al. (2006), Badgery et al. (2008), Osmond et al. (2008)
	Glyphosate	450 g ha^{-1} killed 100% of mature plants. Seedlings survived a concentration of 225 g ha^{-1}. Resistant populations are emerging.	Pritchard and Bonilla (1999)
	Imazapyr	188 and 250 g ha^{-1} killed 80 and 100% of mature plants respectively.	Pritchard and Bonilla (1999)
	Tetrapion	0.88 kg ha^{-1} was effective in killing mature plants after 18 months.	Campbell and Murison (1985), Viljoen (2017)
	2,2-DPA	20.8 kg ha^{-1} is recommended for effective control.	Campbell and Murison (1985)
	Addition of adjuvants to flupropanate	Reveal 9 improved foliar uptake of flupropanate.	Viljoen (2017)
	Addition of adjuvants to glyphosate	Meteor improved the effect of glyphosate and reduced the required rate of the chemical.	Pritchard and Bonilla (1999)
	Addition of adjuvants to clethodim	Hasten, Kwickin and Uptake significantly improved the effect of clethodim	Pritchard and Bonilla (1999)
	Addition of adjuvants to 2,2-DPA	BS 1,000 and Pulse improved the uptake of 2,2-DPA	Pritchard and Bonilla (1999)
Essential oils	Orange oil and sugar	Can substantially reduce *N. trichotoma* seedbank.	McLaren and Butler (2015)
	Pine oil and sugar	Can substantially reduce *N. trichotoma* seedbank.	McLaren et al. (2014)
Competition	Establishment of perennial pasture	Dense competition will outcompete *N. trichotoma*.	Vere and Campbell (1984), Miller (1998), Badgery et al. (2005), Badgery et al. (2008)
	Grazing	*N. trichotoma* is unpalatable to livestock, competitive grazing will favour its establishment.	Vere and Campbell (1984); Badgery et al. (2008)
	Use of fertilisers	Promotes *N. trichotoma* over native pasture.	Miller (1998), Badgery et al. (2005)
Seedbank destruction	Fire	Burning will not kill mature plants, but will prevent seeding, stopping additions to the seedbank. Fire promotes *N. trichotoma* recruitment as it leaves open spaces.	Joubert (1984), CRC (2012)
	Tillage	Majority of seeds are located in the top 2.5 cm of soil, tillage of 2.5 cm or more will substantially reduce seedling emergence. Germination has been observed to significantly reduce when seeds are buried 18 mm or more.	Joubert (1984), Miller (1998)
Manual Removal	Grubbing/Chipping	Effective for reducing seedbank.	Denne (1988), Bourdôt et al. (2020), Lamoureaux et al. (2011)
Biological control	*Alternaria* sp.	Ascomycete fungal species observed on New South Wales and Tasmanian population.	McLaren and Bonilla (2012)
	Corticiaceseae sp.	Pathogenic fungus observed in Argentina. Host specificity was inconclusive.	Anderson et al. (2002), McLaren and Anderson (2011)
	Corticium sp.	Crust fungal pathogen observed in Argentina, attacking the weed roots. Broad host range makes it unsuitable.	Briese et al. (2001)
	Dinemasporium sp.	Natural fungal pathogen to *N. trichotoma*. Unlikely to kill mature plant, but significantly reduce its competitiveness. Large genetic diversity of *N. trichotoma* makes the effect of the pathogen variable.	Briese and Evans (1998), Hussaini et al. (2000)

(Continues)

TABLE 3 (Continued)

Control type	Treatment	Effect	References
	Fusarium oxysporum	Ascomycete fungal pathogen observed in Victoria, Tasmania and New South Wales attacking mature plant base. Only had significant die back on Victorian populations. Not host-specific.	Anderson et al. (2003), McLaren and Anderson (2011)
	Mucor sp.	A mould species observed on New South Wales and Tasmanian populations.	McLaren and Bonilla (2012)
	Paratrichodorus sp.	This nematode was observed in New South Wales and Tasmania. It is not host-specific.	McLaren and Bonilla (2012)
	Phytophthora cryptogea	A water mould species observed on New South Wales and Tasmanian populations.	McLaren and Bonilla (2012)
	Puccinia graminella	Observed as a natural rust pathogen in Argentina.	McLaren and Anderson (2011)
	Puccinia nassellae	Observed as a natural rust pathogen in Argentina. Effect is strongly linked to the environment; under favourable wet conditions, it will kill adult plants; under drought conditions, it has no effect. More information is needed regarding this species' life cycle as basidiospores were inactive under laboratory conditions. Difficult and time-consuming to produce in high quantities. Not host-specific and will affect all *Nassella* species.	Briese and Evans (1998), Briese et al. (2001), Anderson et al. (2002), Anderson et al. (2003), Anderson et al. (2011), McLaren and Anderson (2011)
	Rhizoctonia sp.	Anamorphic fungal species observed on New South Wales and Tasmanian populations.	McLaren and Bonilla (2012)
	Rotylenchus sp.	This nematode was observed in NSW and Tasmania. It is not host-specific.	McLaren and Bonilla (2012)
	Tranzscheliella sp.	Pathogenic fungus observed in Argentina. Prevents infected plants from forming viable seeds. Very low infection rates; less than 1% in large-scale inoculation experiment.	McLaren and Anderson (2011)
	Ustilago sp.	Smut fungus observed as a pathogen on Argentinian populations. Reduces flowering and seed set. Not host-specific.	Briese and Evans (1998), Hussaini et al. (2000), Briese et al. (2001), Anderson et al. (2003)
	Uromyces pencanus	Observed as a natural rust pathogen in Argentina.	McLaren and Anderson (2011)
	Ustilago sp.	Smut fungus observed as a pathogen on Argentinian populations. Reduces flowering and seed set. Not host-specific.	Briese and Evans (1998); Hussaini et al. (2000); Briese et al. (2001), Anderson et al. (2003)
	Uromyces pencanus	Observed as a natural rust pathogen in Argentina.	McLaren and Anderson (2011)
	Zinzipegasa argentinensis	Pathogenic fungus observed in Victoria to reduce flowering and seed set	Hussaini et al. (2000), Anderson et al. (2002)
Prevention	Fencing	Effective at stopping seed spread.	Miller (1998)
	Tree blocks	Effective at stopping seed spread.	Miller (1998)

to accelerated climate change (Gallagher et al., 2012; Kriticos et al., 2010; Watt et al., 2011). Predictions suggest that Queensland and northern New South Wales environments will become unfavourable for *N. trichotoma* and its distribution will be limited to the lower south-eastern region of Australia (Watt et al., 2011). Whilst the models further predict a decline in suitable habitats for *N. trichotoma's* distribution in Australia, the same models have predicted an increase in its spread throughout Europe (Watt et al., 2011). Bioclimatic models are therefore useful to determine areas that may be at risk of invasion so adequate preventative measures can be implemented. Whilst these models are advantageous for considering a variety of different parameters, they do not consider all climatic variability that may influence a species' distribution, nor other non-climatic factors (Heikkinen et al., 2006).

4.2 | Active control strategies

4.2.1 | Herbicides

Flupropanate, also referred to in the literature as Tetrapion or Frenock, has the commercial name *TaskForce* and is globally the most used herbicide for *N. trichotoma* management (CABI, 2020). It is a residual herbicide that is taken up through the roots and the leaves of a target plant, and will diminish the target weed's soil seedbank for up to three years (Osmond et al., 2008). *Nassella trichotoma* is highly susceptible to this semi-selective herbicide, whilst more favourable species, particularly those that utilise the C_4 photosynthetic pathway, are often unharmed (Campbell et al., 1978; Grech et al., 2009). In Australia, a study showed the recommended rate of 1.49 L ha^{-1} of this herbicide resulted in a complete kill of *N. trichotoma*, whilst the six native grasses also tested were unaffected (Grech and McLaren, 2010; Grech et al., 2009). Lower rates of 745 g a.i/l (Grech and McLaren, 2010) and 0.88 active ingredient (ai) ha^{-1} (Viljoen, 1987) have also provided significant reductions. An application rate of 0.88 kg ai ha^{-1} was found to give similar control efficacy at higher rates (1.76 and 3.52 ai ha^{-1}) in 6-, 12- and 18-month-old plants, but it was observed that the time to kill the more mature plants was up to a year longer than for the young plants (Viljoen, 1987).

Due to over-reliance of flupropanate, resistant *N. trichotoma* strains have emerged (McLaren et al., 2006), initiating the search for alternative herbicides for control (Prichard and Bonilla, 1999; Grech et al., 2012). Flupropanate is a member of the J-group herbicides, which act to inhibit lipid synthesis, but does not target ACCase (CropLife Australia, 2019), and resistance to other herbicides in this group has also been observed (Grech et al., 2012). Clethodim ((5Ξ)-2-[(1Ξ)-N-{[(2E)-3-chloroprop-2-en-1-yl]oxy}propanimidoyl]-5-[(2Ξ)-2-(ethylsulfanyl)propyl]-3-hydroxycyclohex-2-en-1-one) is a group A, selective herbicide which inhibits acetyl-CoA carboxylase action for some grasses in certain cropping and pasture systems (Herbiguide, 2019). Imazapyr (rac-2-[(4R)-4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-imidazol-2-yl]pyridine-3-carboxylic acid) is a non-selective residual herbicide from the B group which acts to prevent the synthesis of branched-chain amino acids (Tu et al., 2001). Due to its non-selectivity, boom-spraying imazapyr should be used in areas where total vegetation control is required; otherwise, it should be applied directly to target species via spot-spraying (Tu et al., 2001). Grech et al. (2012) studied the effect of eight different herbicides which were applied at low, moderate and high rates on flupropanate-susceptible and flupropanate-resistant *N. trichotoma* strains, and found haloxyfop-P methyl (A group), imazapyr (B group) and hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) (C group) to give a complete kill of resistant strains at all tested rates. The effect of 12 herbicides on *N. trichotoma* seedlings and mature plants was conducted by Prichard and Bonilla (1999). Clethodim (60 g ha^{-1}), imazapyr (at 250 g ha^{-1}, but 188 gha^{-1} was almost as effective), haloxyfop-P methyl and glyphosate were effective at killing all tested mature plants, and clethodim and imazapyr were also successful at completely killing the seedlings when applied at a lower rate (36 g ha^{-1} and 25 h ha^{-1} respectively). Several other herbicides have been trialled, including butoxydim and dalapon, and their doses and effects are summarised in Table 3 (Campbell and Murison, 1985; Pritchard and Bonilla, 1999). Despite the herbicides like imazapyr and clethodim showing high kill rates for *N. trichotoma*, they have not been registered for its control in Australia, where this research took place.

The addition of adjuvant chemicals to herbicides can increase their efficacy thus benefitting land managers by reducing the required rate, which has the further advantage of reduced damage to pasture and can reduce costs. When combined with 0.44 ai ha^{-1} of flupropanate, the adjuvant *Reverseal 9* improved foliar absorption of the chemical (Viljoen, 1987). The addition of the adjuvants *Hasten*, *Kwickin* and *Uptake* significantly improved the effect of clethodim, and the effect of 2,2-DPA was improved by the adjuvant *BS 1,000* and *Pulse* (Prichard and Bonilla, 1999). The adjuvant *Meter* reduced the required rate of glyphosate from 450 g ha^{-1} to 338 g ha^{-1} (Prichard and Bonilla, 1999). We also note that synthetic herbicides are considered socially unfavourable due to the potential harm they can cause to human health; thus, there is an increasing pressure to move towards natural and integrated solutions for weed control (Batlla and Benech-Arnold, 2014; Lu et al., 2006). In addition, herbicide application alone is not sufficient for effective year-round weed control, and the development and implementation of integrated management appear to be the key for long-term solutions.

4.2.2 | Biological control

Biological control agents can play an integral role in managing widespread invasive weeds. Within its native range, *N. trichotoma* populations are kept in check by various microbial pathogens (Briese et al., 1999). To date, no successful biological control has been introduced to any invaded continents, but its feasibility is high for New Zealand and Australia where no grasses of the *Nassella* genus are native (Jacobs and Everett, 1996). At least 19 different bacterial and fungal pathogens have been isolated from the weed in its natural habitat in South America and throughout south-eastern Australia (Table 3). None of these species have yet been passed as a suitable biological control (Anderson et al., 2002; Anderson et al., 2003; Briese and Evans, 1998; Briese et al., 2001; Hussaini et al., 2000; McLaren and Anderson, 2011; McLaren and Bonilla, 2012). For the potential control species, *Dinemosporium* sp., *Puccinia nassellae* and *Tranzscheliella* sp., inoculating *N. trichotoma* under controlled conditions was unsuccessful, which was attributed to the lack of knowledge regarding *N. trichotoma's* genetic diversity and ecology of the target pathogens (Anderson et al., 2002; Anderson et al., 2003; Briese and Evans, 1998; Briese et al., 2001; Hussaini et al., 2000; McLaren and Anderson, 2011). Other potential control species, including *Corticium* sp., *Ustilago* sp. and *Corticiaceseae* sp., were deemed unsuitable as they lacked host specificity (Anderson et al., 2002; McLaren and Anderson, 2011). Due to the large genetic variation between populations, it is unlikely that any one pathogen would be effective for the whole Australian population, let alone the global population, but it is anticipated that if a suitable candidate could be found, it could play a significant role in the future integrated management of the weed. Further research into developing a better understanding of the ecology of the identified potential biological controls and ways to improve inoculation must be explored as this would assist greatly in a sustainable approach to non-chemical weed control (Hussaini et al., 2000; McLaren and Bonilla, 2012).

4.2.3 | Essential oils

Essential oils are a natural alternative to synthetic herbicides and can have a similar effect on reducing weed emergence (McLaren and Butler, 2015). A solution of pine oil and sugar reduced the seedling emergence of *N. trichotoma* by 98%-100% (McLaren et al., 2014). The addition of sugar promotes bacterial fermentation in the soil, which depletes the available soil oxygen for respiration by plant roots (McLaren et al., 2014). Further, the influx of soil microbes can outcompete weeds for nutrients, but as *N. trichotoma* can grow successfully across a range of soil nutrient levels, this technique may not be entirely successful (Moretto and Distel, 2002). The pine oil acts by damaging the seeds' coating which then enhances their susceptibility to pathogens (McLaren et al., 2014). Pine oil and orange oil both devitalised 91% of tested *N. trichotoma* seeds at a concentration of 1.25%, suggesting essential oils could be utilised effectively to reduce this weed's seedbank (McLaren and Butler, 2015). Whilst these methods were successful in reducing *N. trichotoma* germination and consequent emergence, there is no evidence to suggest that the solution was selective for the weed, which suggests that it would also be damaging to the potential competitive pastures, and would therefore be ineffective as a management strategy in the long term.

4.2.4 | Fire

The use of fire is ineffective at killing adult *N. trichotoma* plants, as they soon re-sprout and regrow vigorously, outcompeting re-establishing native grasses. Fire has been observed to enhance seedling recruitment and may play a role in breaking seed dormancy (CRC, 2013; Joubert, 1984). Exposure to a radiant heat of up to 100°C was observed to significantly enhance seed germination under controlled conditions (Humphries et al., 2018). Fire has been observed to prevent the plant from seeding if conducted in Spring, before seeds have matured (CRC, 2013; DPIPWE, 2016). It is only recommended to use this technique in areas with dense infestations (Osmond et al., 2008).

5 | CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

It is widely agreed that the control and containment of *N. trichotoma* must be enforced through legislation to prevent its spread. Due to the significant threat this weed poses to biodiversity and agriculture, government action must be implemented to assist with control and management. Government-funded taskforces have proved to be highly effective throughout New Zealand and South Africa, with significant declines in *N. trichotoma* populations being observed during the funding period. These government-funded taskforces allowed this weed to be targeted at the landscape scale, which was economically unachievable for many landholders. Of key importance here is that the preventative strategies of maintaining high levels of complex competition, manual removal of the plant prior to seed set and establishing wind stops to reduce spread of the seed heads have all been demonstrated to be effective control measures for *N. trichotoma*. Once established, this weed spreads aggressively and is difficult to remove. In areas with high densities of this weed, the registration of additional herbicides for control, such as clethodim or imazapyr, could assist in reducing the increasing risk of herbicide resistance. Despite this species being a prolific seed producer, only a few studies have explored the effect of control techniques on the longevity of this weed's seedbank. Clearly, the development of techniques that can target the seedbank should be further explored to promote long-term control, and further research should explore the benefits of combining different control techniques that are shown to be applicable at the landscape scale and which are transferrable to other invaded grasslands or pastures.

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Chapter 4. An integrated approach for the landscape scale management of the invasive tussock grass; *Nassella trichotoma*

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An integrated approach for the landscape scale management of the invasive tussock grass; *Nassella trichotoma*

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Abstract

Invasive plants are considered to be one of the biggest threats to environmental assets, and once established, they can be immensely difficult to control. *Nassella trichotoma* is an aggressive, perennial grass species, and is considered one of the most economically damaging weeds to grazing systems as well as one of the leading causes of biodiversity loss in grassland communities. This species produces high density seedbanks, and is able to survive disturbances such as fire, frost and grazing. The present study explored the effect of 13 different combinations of herbicide, fire, a second application of herbicide, grazing exclusion, tillage and broadcasting native seeds in order to reduce the above and below-ground density of *N. trichotoma*. Above-ground vegetation data was collected over five sampling periods, with the soil seedbank being surveyed using core samples taken over three sampling periods. The results were assessed using a hierarchy analysis, whereby treatments of increasing complexity were compared by their efficacy in reducing *N. trichotoma* above- and below-ground, while simultaneously increasing the establishment of the broadcast species. Whilst all integrated treatments effectively reduced *N. trichotoma's* seedbank, the treatments that included fire performed significantly better at providing a combination of reducing *N. trichotoma* and increasing the establishment of the broadcasted seeds.

Keywords: *Nassella trichotoma*, Grassland restoration, Grassland management, Weed management

Introduction

Invasive plant species are considered to be one of the most important threats to natural environmental assets such as grassy ecosystems [1-3]. Once established, invasive species can (i) alter the quality of the pasture available for grazing, (ii) change the fire regime characteristics of the above-ground material [4-5], (iii) degrade the soil quality [6-7], and (iv) modify the soil hydrological processes [8-9]. Together, these pressures can cause significant environmental state changes, often leading to reduced habitat [10] and altered food webs for higher tropic levels [11], and consequently resulting in severe changes in biodiversity and ecosystem functionality [12].

The perennial grass *Nassella trichotoma* (Nees) Hack. ex Arechav. (Serrated tussock) is considered one of the most destructive invasive grass species in Australia [13], South Africa [14] and New Zealand [15], being also an emerging weed in the USA [16] and several European countries [17]. It is considered to be amongst the most economically damaging weed species in Australia and New Zealand, with conservative cost estimates of $AU 40.3 million [18] and $NZ 27.1 million [19], based on reported costs of control procedures and loss of production. In South Africa, particularly within the Eastern Cape (Karoo), the control cost often exceeds that of the economic potential of the land, resulting in land abandonment [21].

Nassella trichotoma is native to South America and has evolved multiple biological and ecological strategies that give it a substantial competitive coverage advantage, which makes it difficult to control. It possesses a dense tussock growth form that acts to protect the plant's base from frost and fire damage [21], which allows the plant to survive severe climatic events. In addition, moderate fire events have also been observed to promote germination [14, 22], and the shallow and fibrous root-system allows for effective moisture uptake in areas of sporadic rainfall. This means that whilst this tussock grass normally requires between 500 to 900 mm of annual precipitation for optimum growth [23], adult plants are tolerant to osmotic stress in times of moisture scarcity [21-22] and populations of this weed have been observed in areas that receive below optimal rainfall (<500 mm annually) [24]. Drought tolerance is further assisted by tightly rolled leaves, which act to reduce transpiration [25]. With regard to its physical properties, the leaves are high in fibre and extremely low in protein, making this grass unpalatable to grazing animals [21, 26], and this allows it to avoid grazing pressures [27]. An adult plant's canopy cover reaches approximately 50 cm in diameter, and together with its dense population, this means that the leaves of individual plants overlap, effectively shading out competing species [22]. Additionally, as a perennial grass, it can live for up to 20 years [22], and each plant can produce in excess of 100,000 wind-dispersed seeds per year [21]. When taken together, these properties make *N. trichotoma* a very aggressive exotic grassland and pasture weed, which also effectively resists many attempts at management and control.

Despite these aggressive biological and ecological attributes, *N. trichotoma* is rarely observed to be a problematic weed in more intact, highly diverse, functional ecosystems [28]. In this context, the seedlings have been found to be weak above-ground competitors [22], and this presents an opportunity for control, since this species is not competitive until rival cover is reduced. However, once established, *N. trichotoma* forms a self-facilitating negative feedback loop that excludes favourable grasses, a situation that can only be broken through active intervention.

Testament to the importance of this problem is that management efforts for dealing with *N. trichotoma* have been developing since the 1930s in Australia [28-29], South Africa [30] and New Zealand [31]. In each of these locations, *N. trichotoma* was unintentionally introduced before becoming widespread. The most widely used control tactic currently used in Australia is spraying with the herbicide fluropropanate, which can be used to semi-selectively kill *N. trichotoma* since many Australian native grasses are not significantly harmed [32]. This residual herbicide provides effective control of *N. trichotoma* for up to five years before follow up treatments or reapplication is required. While this herbicide has been successful for reducing *N. trichotoma* in some parts of Victoria, fluropropanate is not suitable for all landscapes and, in many cases, the weed returns to the site once the effect of the herbicide has worn off, which throws into question its suitability as a long-term solution [33]. Whilst this herbicide provided similar efficacy in South Africa, lower doses were recommended in order to maintain a high *Eragrostis* spp. cover [34]. Fluropropanate is not recommended for boom-spraying in New Zealand, as native grasses were found to be significantly reduced [35]. Indeed, in these areas, manual removal and spot spraying with glyphosate have been found to be more efficient, and therefore this is widely used as a control method in New Zealand [36].

In many cases, *N. trichotoma* occupies non-arable landscapes, and the costs associated with managing this weed often exceed that of the land's capacity to generate value, making control methods unfeasible to implement [20]. This is relevant to the increasing problems with this weed, in that unmanaged populations undergo a prolific seeding event in early summer, and the seeds can be widely dispersed into important agricultural and ecological landscapes [21-22]. This dispersal then results in repeating high yearly costs to land managers for ongoing local control of this species [37].

As indicated earlier, *N. trichotoma* is not competitive in its juvenile stage, which provides a window of opportunity for systematic control. Consequently, it is suggested that, if mature plants are removed, introducing early competition for emergent seedlings from desirable perennial grass species is a critical step for helping to supressing regeneration from the seedbank [28]. Whilst this strategy is a key to the current investigation, it has been further noted that the *N. trichotoma* seedbank is particularly robust, and can yield germinants for many years [38-39]. This implies that a more complex integrated control strategy, involving modifications to the seedbank and repeated treatments, will be necessary to gain control over this species. In this respect, tillage could provide a solution, by physically burying a large proportion of the small *N. trichotoma* seeds to a depth which will prevent them germinating. While many natural landscapes may not be suitable for tillage, for example sites that are steep or with rocky terrain of natural or cultural significance, in suitable areas this treatment has been demonstrated to improve the establishment of broadcast seeds [40].

Areas where *N. trichotoma* has been abundant for some years will have likely passed significant biotic and abiotic ecological thresholds, and thus, active intervention is required to restore ecosystem structure and natural processes [41]. In addition, understanding the soil history of an invaded site can provide a conceptual reference for setting soil quality control activities, as well as assisting in breaking plant-soil feedback loops established by invasive plants [42].

It has been clearly identified that many native and other grass species have relatively short-lived persistent seedbanks (up to five years), therefore important species, which could provide competition for *N. trichotoma*, may have depleted seedbanks. In addition, normal migration of native seeds from surrounding areas is also limited by landscape fragmentation and scarcity of remnant grasslands, suggesting that relying on natural recruitment for establishing control in invaded grasslands is not a feasible strategy [43-44]. As a result of this seed deficit, implementing seed broadcasting, as an integral part of the weed management process, is likely to be important to the reduction of invasive plants long-term [45]. In this situation, it is suggested that management techniques should not only engage in the removal of above-ground *N. trichotoma* mass, but also improve abiotic conditions for the establishment of desirable and competitive grass species, particularly during the early stages of development of *N. trichotoma*.

The complexity of the problem suggests that *N. trichotoma* control will require approaches that deal with multiple aspects of the invasion, including disposing of the above-ground weed biomass, protecting native species biomass and maintaining soil seed banks of native species. The present study uses field trials to investigate the effect of integrating a series of available control tactics for reducing *N. trichotoma* in a degraded grassland. An increasingly integrated series of proven control activities have been sequentially tested for *N. trichotoma* control under the aegis of a hierarchy analysis.

Table 1: The 13 integrated treatment combinations.

Treatment Number	Treatment Combination	Treatment Abbreviation
1	Control (no treatment)	NT
2	Herbicide + Seed Addition	HS
3	Herbicide + Grazing Exclusion + Seed Addition	HGS
4	Herbicide + Soil Disturbance + Seed Addition	HTS
5	Herbicide + second Herbicide + Soil Disturbance + Seed Addition	HH*TS
6	Herbicide + Grazing Exclusion + Soil Disturbance + Seed Addition	HGTS
7	Herbicide + Grazing Exclusion + second Herbicide + Soil Disturbance + Seed Addition	HGH*TS
8	Herbicide + Fire + Seed Addition	HFS
9	Herbicide + Fire + Grazing Exclusion + Seed Addition	HFGS
10	Herbicide + Fire + Soil Disturbance + Seed Addition	HFTS
11	Herbicide + Fire + Grazing Exclusion + Soil Disturbance + Seed Addition	HFGTS
12	Herbicide + Fire + second Herbicide + Soil Disturbance + Seed Addition	HFH*TS
13	Herbicide + Fire + Grazing Exclusion + second Herbicide + Soil Disturbance + Seed Addition	HFGH*TS

The objectives of this Australian-based research are to (i) find a cost-effective, long-term, integrated method for reducing *N. trichotoma's* above-ground cover as well as its seedbank density, (ii) identify which of these control methods best enhances the establishment of two Australian native grasses, and (iii) as a result of these findings, make recommendations of how these methods could be adapted by land managers for similarly affected grasslands in other global areas.

Results

Soil conditions

Table 2 presents the results of soil nutrient analysis. It demonstrates that soil nutrients at Little Raven are generally significantly higher than that at the reference site.

Table 2: Soil nutrients and other parameters of the Little Raven study site. Significance was set to *p*=0.05 and these values are in bold. The soil data for the reference site was provided by Steve Sinclair (Victorian State Government). The Cowell method is described in Cowell [50].

Soil parameter	Little Raven	Reference site	Significance
Texture	Clay-loam		
Ammonium nitrogen	25.00	4.38	**0.05**
Nitrate nitrogen	90.25	8.63	0.09
Phosphorus (Colwell method)	28.38	10.63	**<0.01**
Potassium (Colwell method)	807.25	419.38	**0.01**
Sulphur	14.90	5.58	**<0.01**
Organic carbon	4.61	2.46	**<0.01**
Conductivity	0.22	0.09	**0.02**
pH Level ($CaCl_2$)	4.91	4.85	0.66
Ph Level (H_2O)	5.84	5.88	0.84

Note: Significance of difference was set to p=0.05 and these values are in bold. The soil data for the reference site was provided by Steve Sinclair (Victorian State Government). The Cowell method is described in Cowell [50].

The hierarchy analysis

The results of the hierarchy analysis for the cover of *N. trichotoma*, *A. scabra* subsp. *falcata* and *Rytidosperma* spp., as well as *N. trichotoma's* seedbank density are shown in Table 3, and more detailed figures regarding the steps of each analysis are available as supplementary data (referenced in the text by figure numbers starting with S).

No step in the analysis observed a significant reduction in *N. trichotoma* cover at the final sampling period between any treatment (Figure S1), despite significant reductions in cover being observed within many of the treatments (Table 4). Time and the time-and-treatment interactions, however, were observed to be significant. When comparing the effect of the HS treatment with the control (NT), it was observed that the HS treatment caused a significantly lower (p=0.002) above ground density of *N. trichotoma* compared to NT in 2019 (Figure 1). The HS treatment was then compared to HGS, HTS and HFS treatments, where it was observed that the HFS had significantly reduced cover in 2018 (p<0.001) compared to the unburnt plots. The HFS treatment was compared to the HFGS and the HFTS treatments, and it was found that the HFTS treatment was significantly better at reducing *N. trichotoma* cover than the HFGS treatment (p<0.001), but not significantly better than the HFS treatment. No further significant differences were observed. The HFS treatment provided a significant reduction in *N. trichotoma* compared to the unburnt treatments only during the 2018 sampling period, which was directly after the fire treatment. However, in the following sampling periods, *N. trichotoma* cover was not significantly different. Therefore, it was concluded that the HS treatment could provide the most effective solution for *N. trichotoma* control.

The broadcast species were analysed separately, and this showed that, for both treatments, the effect of the fire treatment was significant for their establishment (Figure 1). For *A. scabra* (Figure S2), the time, treatment, and their interaction were significant (p<0.001) in 2020 for the HS treatment compared to NT. The treatment and time factors, as well as their interaction was significant (p<0.001) in the second step of the analysis, with HFS having the highest cover. The third step did not observe any significant interactions between the treatments. No significant difference was observed between the NT and HS treatments for *Rytidosperma* spp. (Figure S3). Despite no significant results, the flowchart was followed to Step 2, where time, treatment and the interaction of these factors observed a significant result (p<0.001). The HFS treatment had the highest cover of *Rytidosperma* spp. so Step 3 compared HFS, HFGS and HFTS, and no significant difference between the treatments was observed. For both of the broadcast species, HFS was determined to be the most effective treatment.

Figure 1 shows the changes in the three analysed species for each treatment over the five sampling periods. From this figure, it is evident that *N. trichotoma* cover is reduced most effectively by the fire and the tillage treatments. In 2019, one-year post-treatment, the cover of *N. trichotoma* is at its lowest across all the treatments. The broadcast species did not successfully establish in the unburnt plots, while all the fire treated plots experienced a significant increase in both species. Figure 2 shows photographs of the HS and the HFS treatments compared to the NT, (i) prior to implementing treatments (2018), (ii) and at the end of the final sampling period (2021).

The analysis of *N. trichotoma's* seedbank (Figure S4), again found no significant difference between the treatments for reducing the seedbank density, however significant reductions in *N. trichotoma'*s seedbank were observed over the three sampling periods across all the treatments (including NT) (Figure 3).

The hierarchy analysis demonstrated that fire was the most significant factor for increasing the establishment of the broadcast species. For this reason, the vegetation community data focused on the effect of the treatments with and without fire for the native species, the invasive grasses, and the invasive 'other'. This was achieved by finding the percentage of total cover of each species in each treatment over the five sampling periods. Each species was sorted into the native, invasive grass or invasive other category, and the sum of percentages of species in each category for each treatment at each sampling period was recorded.

Table 3: The results of the hierarchy analysis using the developed flowchart (Figure 5). Each species was analysed separately and the analysis of the effect of the treatment combination, sample period and the interaction of these factors are shown. Significance of difference was set to p=0.05.

Nassella trichotoma			
Hierarchy Step	**Time**	**Treatment**	**Time*Treatment**
1	0.017	0.103	0.002
2	<0.001	0.251	<0.001
3	<0.001	0.096	0.216
***Nassella trichotoma* seedbank**			
Hierarchy Step	**Time**	**Treatment**	**Time*Treatment**
1	0.028	0.44	0.304
2	<0.001	0.639	0.669
3	<0.001	0.639	0.006
***Rytidosperma* spp.**			
Hierarchy Step	**Time**	**Treatment**	**Time*Treatment**
1	0.105	0.451	0.140
2	<0.001	<0.001	<0.001
3	<0.001	0.477	0.980
Austrostipa scabra			
Hierarchy Step	**Time**	**Treatment**	**Time*Treatment**
1	<0.001	0.006	<0.001
2	<0.001	0.001	<0.001
3	<0.001	0.470	0.919

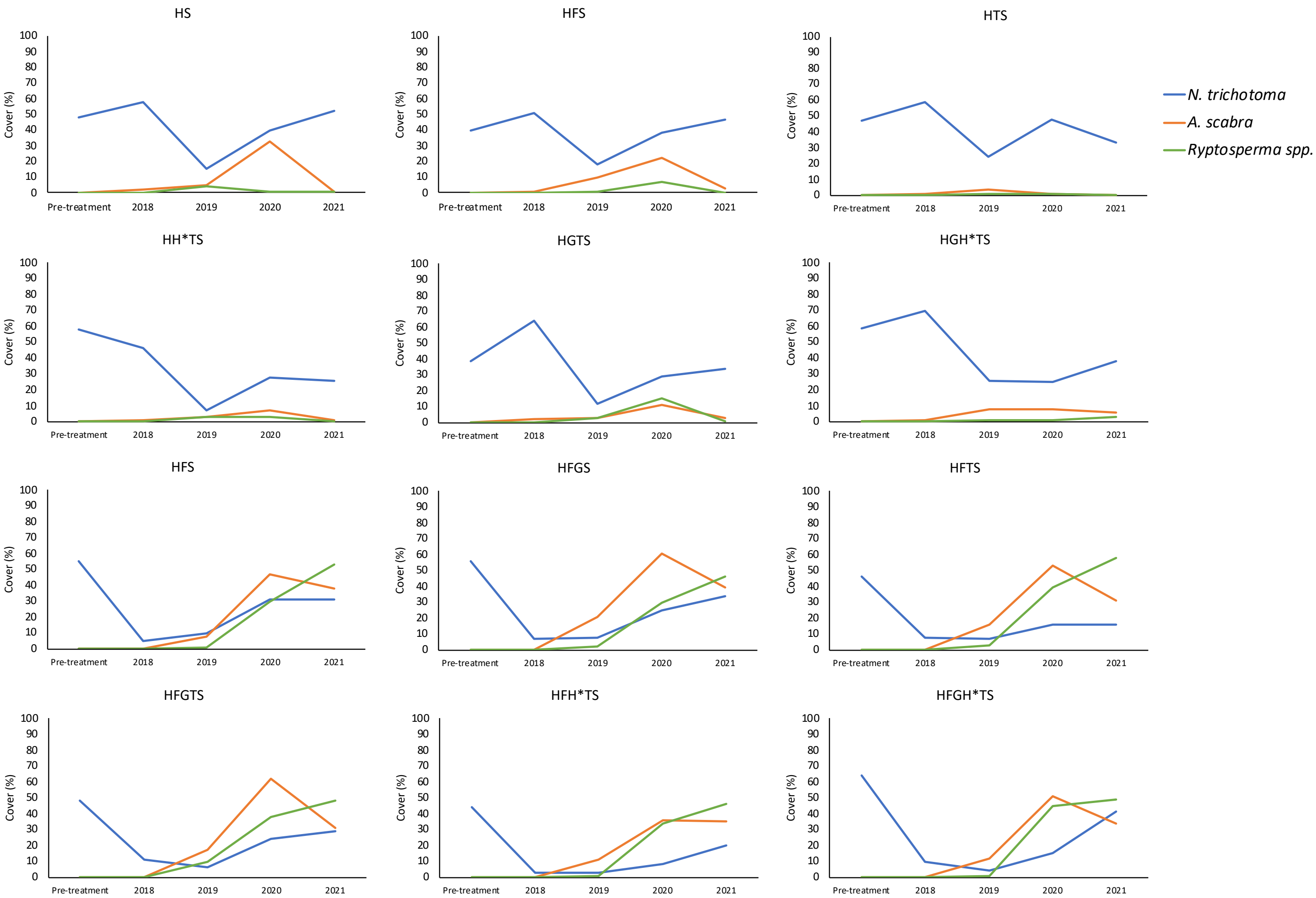

Figure 1: The changes in cover of *N. trichotoma* and the two-broadcast species: *A. scabra* and *Rytidosperma* spp. for each treatment over the five sampling periods.

Table 4: The difference between the mean *N. trichotoma* density from the first sampling period to the fifth sampling period for the treatments with cost assessments in Table 6. The negative numbers signify a reduction of *N. trichotoma* cover, while positive numbers signify an increase in cover. Differences were considered significant when $p<0{\cdot}05$.

Treatment	Mean difference	*p*-value
NT	+2.33	1
HS	+4.16	1
HTS	-14	1
HHTS	-32.33	<0.001
HFS	-24.5	<0.001
HFTS	-30.33	<0.001
HFHTS	-19.67	0.26

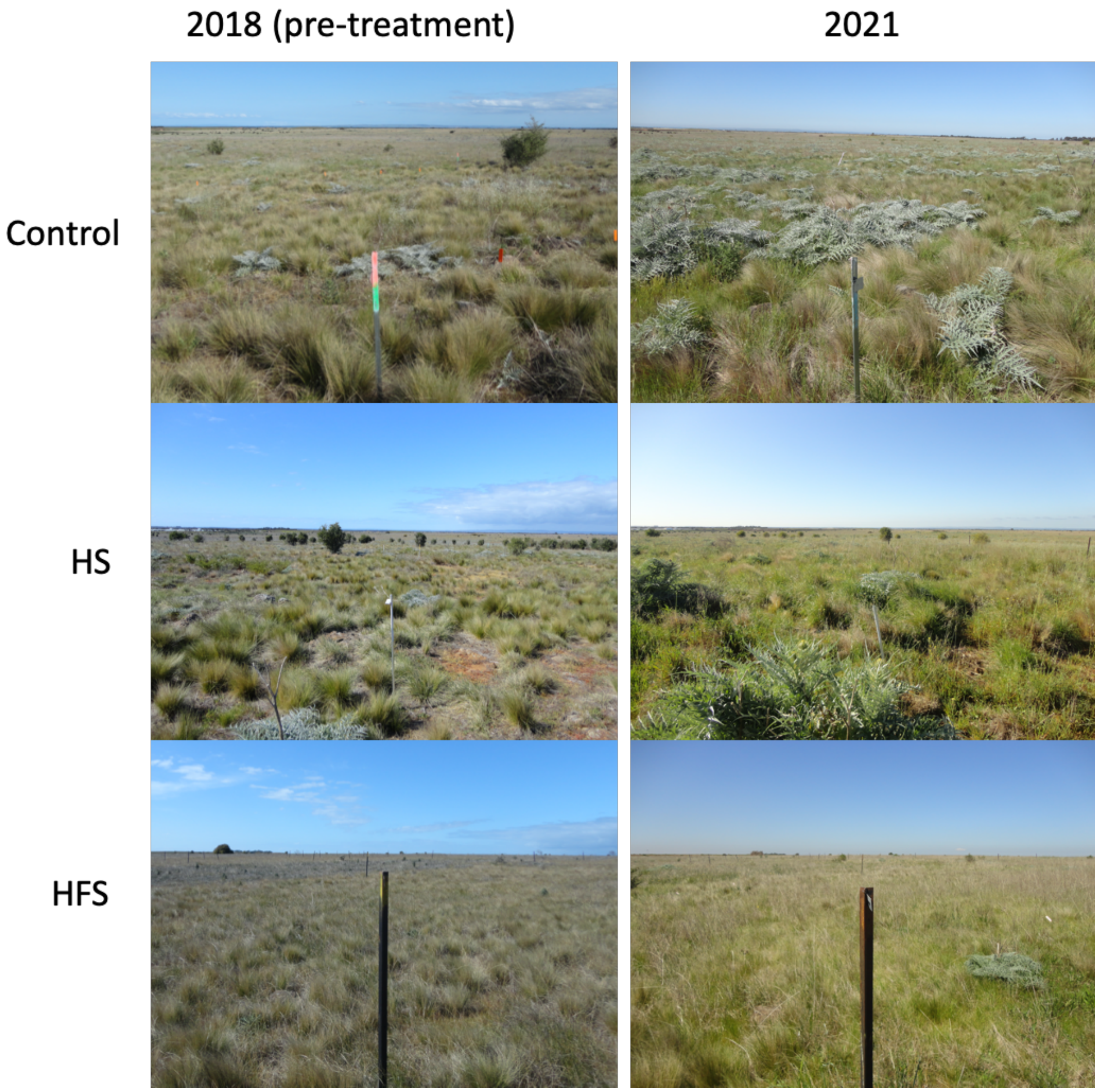

Figure 2: Photographs comparing the same plots at the first sampling period (left) and the fifth sampling period (right) for the HS and the HFS treatments*.* The control is included as a reference.

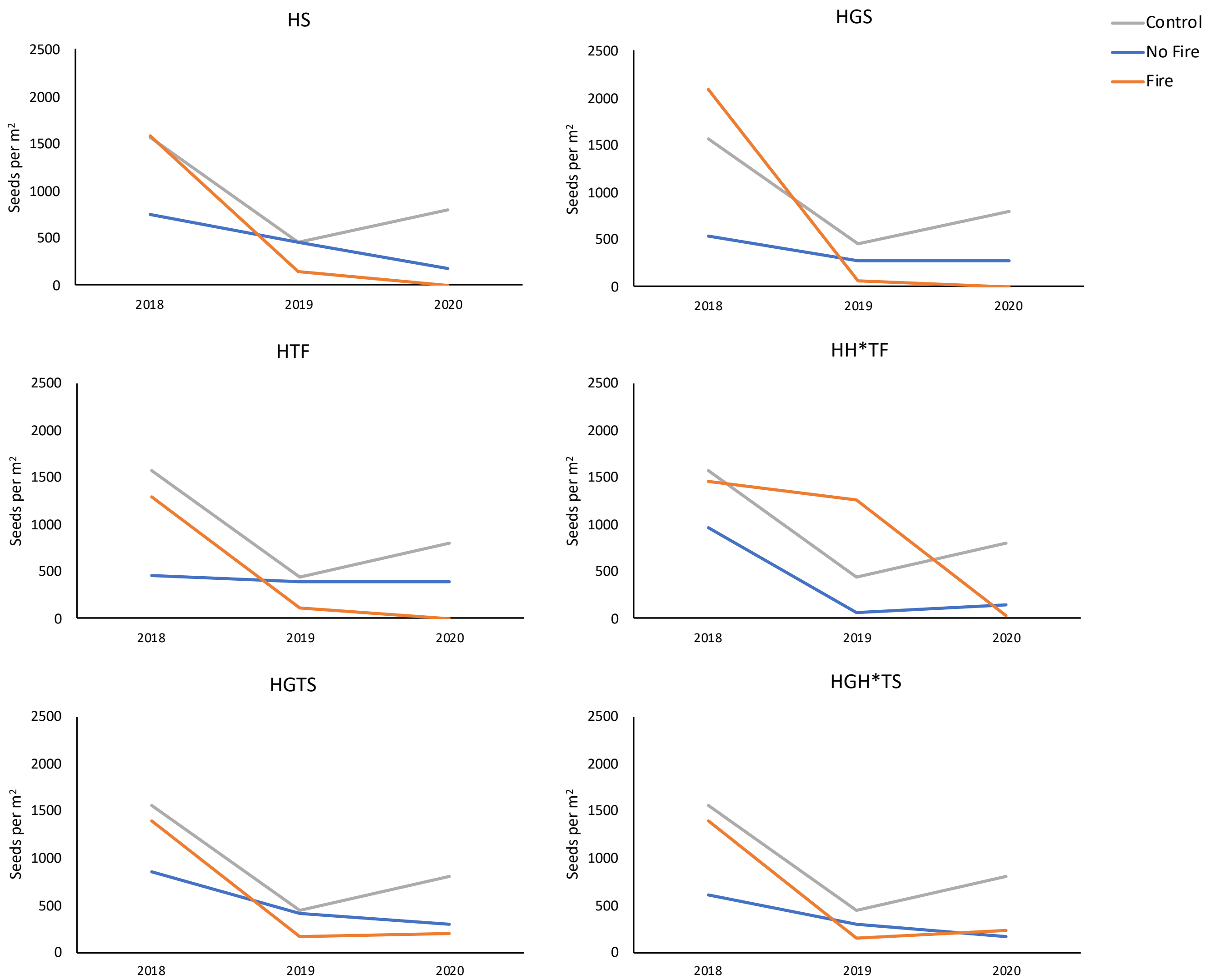

Figure 3: Changes in *N. trichotoma's* seedbank over three sampling periods. Soil was collected pre-treatment in 2018, then post-treatment in 2019 and 2020. Each graph shows the control (no treatment) line for comparison in grey, the unburnt plots are represented in blue, and the fire treated plots are represented in orange.

Analysis of the vegetation community

As fire was the most important factor at reducing *N. trichotoma* above- and below-ground and also for increasing the cover of the broadcast species, we compared the effect of the treatment combinations on the vegetation community with or without fire. The vegetation was divided into three categories; natives, invasive grasses, and invasive other. The effect of the treatments, particularly fire, on the vegetation community, is visually shown in Figure 4. In all cases, the fire treatment significantly increased the native species, particularly the broadcast species, compared to the same treatment without fire. The fire treatment was also observed to lower invasive grasses to a greater extent than that observed in the same treatment without fire. No treatments were effective at reducing the cover of the other invasive species, included forbs, herbs and shrubs.

In 2018, *N. trichotoma* was the most abundant species surveyed in all the plots (Table 5). In 2021, invasive grasses and invasive herbaceous species remained dominant for the unburnt plots, with the same invasive grass species; *N. trichotoma, Avena* spp., and *Nassella neesiana* being the top three species surveyed (Table 5). In the unburnt plots, the native broadcast grasses; *A. scabra* and *Rytidosperma* spp., did not successfully establish to provide effective competition, it being observed that there was an increase in invasive grasses including *Avena* spp. and *N. neesiana*. In contrast, the broadcast grass species were dominant in 2021 for the treatments that included fire (Table 5).

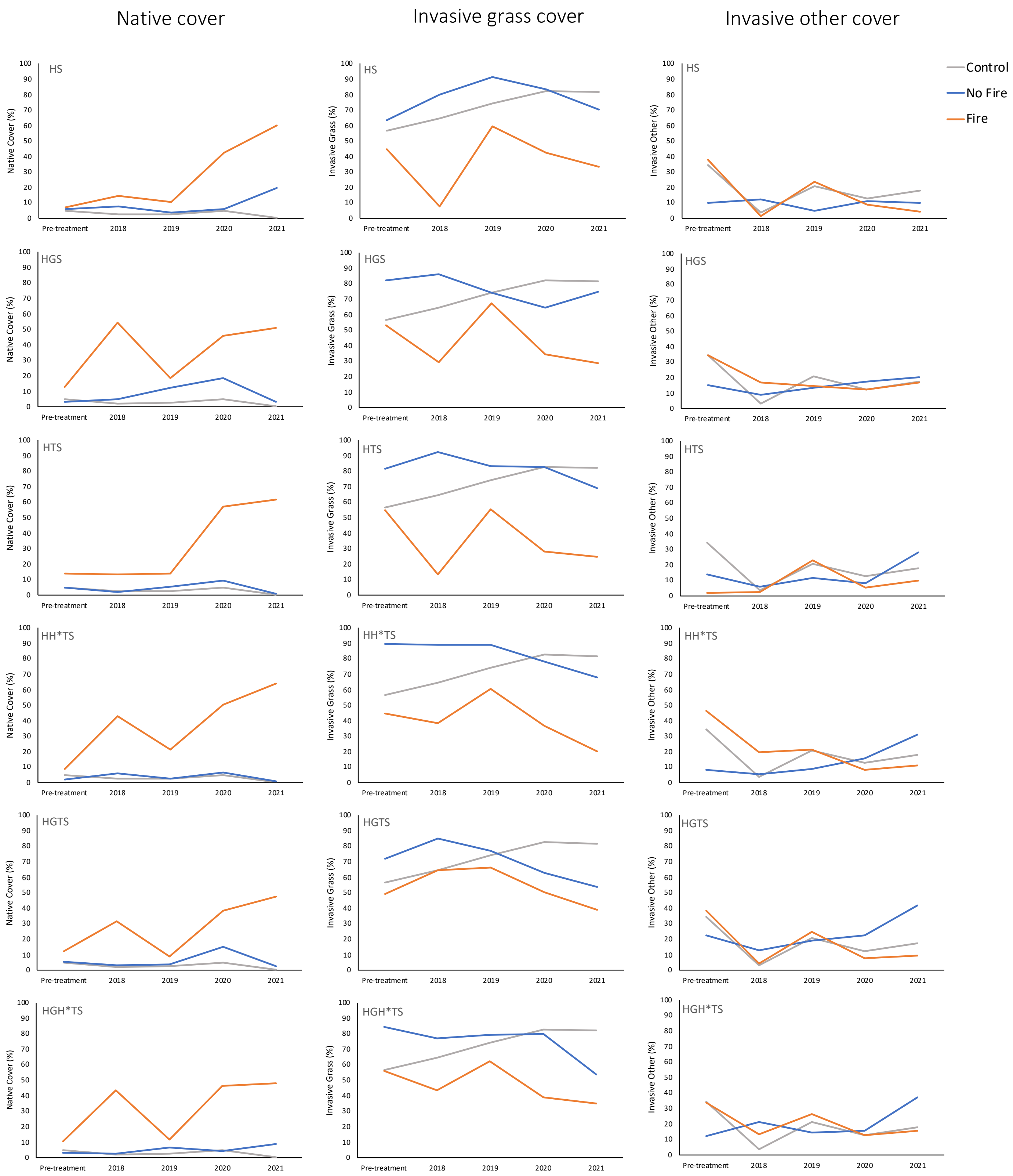

Figure 4*:* The effect of the treatments on the vegetation community (mean cover). The left column shows the change in native vegetation cover (%), the middle column shows the changes in invasive grass cover (%), and the right column shows the changes in other invasive vegetation cover (%). Each row represents

a different treatment, and the treatment code is listed beside each row (refer to Table 1). The control (NT) treatments are represented by the grey line, the unburnt treatments are represented by the blue line, and the fire treatments are represented by the orange line.

Table 5: The top five species surveyed in the first and fifth sampling periods for the fire treated and fire excluded plots. The native species are highlighted in bold font.

Fire exclusion 2018	Fire exclusion 2021	Fire treated 2018	Fire treated 2021
Nassella trichotoma	*Nassella trichotoma*	*Nassella trichotoma*	***Rytidosperma***
Avena spp.	*Avena spp.*	*Romulea rosea*	***Austrostipa scabra***
Nassella neesiana	*Nassella neesiana*	***Austrostipa scabra***	*Nassella trichotoma*
Romulea rosea	*Cynara cardunculus*	*Nassella neesiana*	*Nassella neesiana*
Cynara cardunculus	*Lolium rigidum*	*Avena spp.*	*Sonchus oleraceus*

Cost estimate

We found that the costs for implementing different treatments varied greatly, and were different between sites of different size (Table 6). The herbicide application is the most expensive treatment to implement in sites 10 ha or larger. For the treatments that used a second herbicide application, the cost for the herbicide application was doubled. Due to the costs associated with controlled burns, fire is a more economical action for large scale sites. Tillage is not often a feasible management action in grasslands that maintain their rocky composition, unless conducted manually as done in the present study, making this technique better suited to small plot scale sites. While ploughing machinery could be used to generate a similar effect, this would only be a feasible option in de-rocked sites. Broadcasting seeds becomes increasingly expensive with increased landscape size. As the seeds were only observed to establish when used in conjunction with the herbicide and fire treatments, it would be important to consider using these treatments to increase the rate of establishment.

Table 6: An estimate of the cost to implement each individual control method from the plot scale to increasing size. These figures were then combined to estimate the cost of implementing each treatment over increasing size scales.

Cost ($AUD) of action for sites of varying size (Ha)						
Individual action	0.01	1	5	10	50	100
Spot spray	35	3453	17265	34539	172648	345295
Apply fire	23000	23500	24000	25000	32000	40000
Till soil	40	90	450	900	4500	9000
Broadcast native seeds	44	440	2200	4400	22000	44000
Multi-action strategy						
HS	79	3893	19465	38939	194648	389295
HTS	119	3983	19915	39839	199148	398295
HHTS	154	7436	37180	74378	371795	743590
HFS	23079	27393	43465	63939	226648	429295
HFTS	23119	27483	43915	64839	231148	438295
HFHTS	23154	30936	61180	99378	403795	783590

Discussion

This investigation shows clearly that the combination of herbicide spraying, fire and the broadcasting of native seeds significantly increased the establishment of the broadcast native species as well as reducing the above and below-ground density of *N. trichotoma*. As a consequence, we recommended this approach to be the most effective treatment combination.

The HHTS treatment had the largest reduction in *N. trichotoma* cover of all the treatments, with an average cover reduction of 32.33% observed at the fifth sampling period compared to the first, and this treatment was the only treatment without fire to see a significant change in *N. trichotoma* cover. Unlike the fire treatments, the reason for the reduction was not due to competition from the broadcast native seeds, as these did not establish in the HHTS treatment and native grass cover remained similar to that observed for the control treatment. Overall, whilst invasive grasses declined for the HHTS treatments, other invasive vegetation increased. This is exemplified by *C. cardunculus*, which had an average increase from four to 14 plants from the first to the last sampling period. In other words, the success of HHTS in reducing *N. trichotoma* was offset by the disadvantage of allowing other invasive species to prosper.

It is important to note that establishing robust competition provides sustainable, long-term control of invasive species [52]. To this end, the pre-seed broadcasting fire treatment was the most important control factor since, in this study, it facilitated the establishment of the broadcast seeds whilst the identical treatment with seeds failed to establish competition in the unburnt treatments.

A reason the broadcast seeds were only successful in the fire treated plots could be attributed to the restorative properties of fire, including rapid nutrient cycling and decreased competition, which in turn increases light and solar heating to the soil surface [51-52]. Altered levels of soil nutrients are often found in degraded landscapes, which significantly favours the dominance of invasive plants. Soil analyses at Little Raven consistently indicated elevated soil nutrients. Fire has been observed to reduce elevated soil nutrients by volatising N, P and S, as the nutrients containing these elements have low temperature thresholds [53]. Fire also adds charcoal to the soil [54-56], and this can act as an important stimulant for the seed germination of many native Australian grassland species [57]. In the unburnt treatments, invasive annual species outcompeted the broadcast species.

Removing the biomass of invasive plants prior to broadcasting seeds is critical for improving establishment rates of broadcast plant propagules [58-60]. The fire treatment cleared not only *N. trichotoma*, but also reduced the cover of other invasive plants, and the broadcast seeds in these treatments had lower competition than in the unburnt treatments. In the unburnt treatments, the dead *N. trichotoma* plants continued to occupy a high proportion of these plots despite the herbicide effectively killing them. The dead plants were slow to decompose, and subsequently continued for some time to reduce space and light for the broadcast grasses. This provided a competition advantage for invasive annual plants, such as *Avena* spp. and *C. cardunculus*, which generally establish well in nutrient-rich and shady places, and were observed to increase in cover in the unburnt treatments over the five sampling periods. In the fire treated plots, the broadcast seeds had high rates of establishment and provided effective competition against *N. trichotoma* and invasive annuals.

Tillage and fencing did not have a significant effect on the establishment of the broadcast seeds in the unburnt plots, nor did it significantly improve their establishment compared to the HFS treatment. The fencing was included to identify the effect of macropod grazing on the seed establishment. Fencing has also been used as a preventative strategy in *N. trichotoma* control for reducing seed immigration, as the fence line can trap the panicles and therefore reduce the spread of the seeds. However, the herbicide treatment applied in the present study prevented panicle formation and subsequent seed production of *N. trichotoma*, and the fencing treatment, therefore, did not significantly reduce the seedbank density compared to the unfenced treatments.

Tillage was used to soften the soil, which was anticipated to assist the establishment for broadcast native seed, while also acting to reduce competition by burying the invasive seeds already present further into the soil profile. Tillage provided better reductions in *N. trichotoma* cover, in both the burnt and the unburnt treatments, suggesting tillage buried the seeds in the topsoil to a depth that reduced seedling emergence. The second application of herbicide did not observe to improve *N. trichotoma* control, or establishment of the broadcast species.

All the treatments reduced *N. trichotoma's* seedbank, including the control. While small numbers of *N. trichotoma* seeds are capable of long-term persistence, the majority of this species' seeds germinate or decay within the first year [38, 61]. In this respect, the timing of the treatments was critical for the prevention of seed addition in 2018, as seed set begins at the start of Summer for this species. Therefore, by applying herbicide in Spring, the plants failed to produce fresh seeds for the year. The fire treatment was implemented in early Summer, which not only stopped seed set from mature plants, but could have flushed the seedbank through the promotion of germination or the devitalizing of any remaining seeds [62-63]. The seeds rely on wind for dispersal, and the reduced seed production from the treatments surrounding the untreated control plots (NT) may have inadvertently resulted in the density reduction observed within these plots, despite these plots retaining a high cover of *N. trichotoma*. Due to the treatments only being applied within the plots, healthy *N. trichotoma* plants surrounded the plots and continued to set seed each year, therefore the seed density results reported in this study may be higher than if the treatments were applied uniformly across a larger site.

The combination of herbicide, fire and broadcasting seeds should be implemented at a larger scale to better identify the effectiveness of these treatments on reducing *N. trichotoma* at a paddock and landscape scale. As these treatments are globally adaptable, they could be trialled in grasslands and grazing systems with adjustments being made to the seed mix to reflect the location and objectives of the site. Low diversity seed mixes have been observed to encourage natural succession of a vegetation community [64]. As many degraded grasslands are fragmented and isolated from remnant sites, native propagules cannot readily recolonize naturally through migration [65]. Also, many grassland species have short-term seedbanks, and thus sites that have been in a degraded, weed-dominant state for an extended period of time may not have sufficient viable seeds available for seedbank recruitment [66-67]. Therefore, changes in the vegetation community will require human intervention [68].

In this study, the objective of broadcasting seeds was to provide direct competition with *N. trichotoma* and prevent this species' re-establishment. This study used two C_3, perennial species that provided excellent competition with the target weed. However, it is argued that a more diverse mix, that included C_4 native grasses and native herbs or forbs, could have provided better year-round control of non-target invasive species [68-69]. Native species other than the broadcast species were rarely detected, and thus a more diverse seed-mix could be expected to enhance species richness [60]. To transfer these methods to international grassland communities, the species selected for the seed-mix should be adapted to include suitable native species that reflect the site, but it is cautioned that at least one grass species with similar growth parameters to *N. trichotoma* is recommended for effective competition [21]. The methods are also transferrable to grazing systems, and clearly the broadcast seed mix should be altered to include palatable pasture grasses.

The two key factors that will assist land managers in deciding the most economical treatment implementation are; (i) the size and, (ii) the rocky composition of grassland. The size of the site is important when selecting an appropriate control strategy, because the cost of employing different strategies will vary with the scale of the treated area. Some treatments increase steeply in cost as the site area increases (for example herbicide delivered by backpack and seed broadcasting by hand), while other treatments increase relatively slightly as site area increases (such as the application of fire, which can cover a larger area with relatively little extra input). This means that a strategy that may be recommended for small sites may be unfeasible or uneconomical for larger areas. The complexity of this issue is also a factor to be considered. Whilst with increasing scale it is expected that the cost to implement the herbicide treatment becomes more expensive due to the number of hours and staff required to cover the area, if the site is actually unsuitable for post treatment by fire due to nearby infrastructure for example, the herbicide application alone would not provide effective control of *N. trichotoma's* seedbank. Our results showed that the HS treatment was not effective at reducing *N. trichotoma* above-ground cover alone, and it would thus be recommended that follow up with soil tillage and spot-spraying of remerging seedlings would be needed to see a significant reduction ($p<0.001$).

In addition, the option to incorporate tillage will be determined by not only the size of the site, but also by the level of rockiness in the soil. If the site was small but rocky, it could be tilled manually using the same technique as applied in this study, but this however would not be a suitable solution for moderate sized sites due to the intensity of physical labour required. It would rather therefore be best suited to address localised rocky patches. Overall, tillage would be most economically feasible for areas that have been previously cropped, and therefore no longer display a rocky composition. It is noted that ploughing equipment for tillage is easily damaged by rocks, and careful manoeuvring through rocky terrain will clearly slows down the tillage process and subsequently increase the costs.

It would not be recommended to add seeds without the prior application of herbicide and fire as this treatment was critical for their establishment. Therefore, if seed addition is required for a site, we recommend budgeting to include the herbicide and fire treatments. It becomes increasingly effective to apply fire over a large scale. If fire is implemented in late-spring or early-summer, it can be effective for preventing *N. trichotoma's* seed set for the year, as well as remove excess biomass of standing plants to allow competition from native species [21]. However, the inclusion of herbicide would be required to effectively kill the adult *N. trichotoma* plants, as they are able to reshoot and grow following fire disturbance.

Another problem that can arise is in the acquiring large quantities of native seeds of local provenance for grassland restoration, which can prove to be very difficult [70]. This seed acquisition can be a limiting factor on the scale of a weed-invaded area that can be reasonably targeted for treatment. However, the literature suggests that if seeds are able to recruit to a site naturally, applying a combination of herbicide with fire [71] or other biomass removal techniques [72] can reduce weed cover and promote the passive succession of native plants. Therefore, to overcome limited seed availability, as well as the high associated costs involved in large scale re-vegetation programs, applying the HFS treatment within a strategically selected area of a site could provide a seed source for the surrounding, untreated area [73]. This could promote natural succession of the native species out of the treated area into the surrounding untreated areas.

Conclusion

We suggest that this study has indicated a tractable pathway for the control of *N. trichotoma,* while simultaneously restoring native species to degraded grassland, in a range of suitable areas. The combination of herbicide, fire and broadcasting seeds, or in some cases tillage of the area prior to seed broadcast, has been shown to provide a significant reduction in *N. trichotoma* cover and the establishment of a significant increase in cover by the broadcast species. There is reason to believe that these integrated control methods are also clearly adaptable to international grasslands and grazing systems where this species is dominant and difficult to control, and where the application of herbicides are permitted.

Methods

Site Description

The study took place at Mambourin, Victoria, Australia (37 ^{O} 55' 18.12'' S, 144 ^{O} 32' 43.079'' E), which is west of Melbourne on the Werribee Plains. This site has an average annual rainfall of 468 ml, with the highest rainfall occurring during Autumn. The average highest temperature of 26 ^{O}C occurs in January, and an average low of 5 ^{O}C occurs in July [46]. This site has a history of sheep grazing, which facilitated the encroachment of invasive grasses through the reduction of native vegetation. Whilst historically, this site was dominated by *Themeda triandra*, cover of this species is now depleted regionally due to grazing. In addition, a vegetation survey conducted by the Department of Environment, Land, Water and Planning [47] identified that 54 different weed species belonging to 20 different families had established within this site, with the most notable dominant species being *N. trichotoma*.

Site Setup

Experimental plots were established in areas that had high densities of *N. trichotoma* (80% or greater foliage cover), which had not been recently treated with herbicide. A total of 78 plots measuring 10 x 10 m were set-up for the trials, and each was marked with a unique metal tag. Each plot was separated from the next by a one metre buffer of untreated area. The 36 fire-treated plots were clustered in the south of the site, while the remaining 36 treatment plots and six no treatment (control) plots were clustered together in the north-east of the site. These plots were grouped together (i) to ensure that the fire, and fire parameters including smoke and radiant heat, did not interfere with the control plots, and (ii) to make the large-scale burn manageable. Within the two locations, treatments were randomly assigned to each plot.

Soil analysis

To provide data for comparison, eight soil cores were collected at random locations within the site. Samples were taken prior to implementing treatments at the study site. The soil analysis was conducted by the CSBP Soil and Plant Analysis Laboratory. The results of the soil tests were compared to those collected from a reference site, which is a nature conservation reserve located at nearby Mt. Cottrell (37 ^{O} 55' 18.12'' S, 144 ^{O} 32' 43.079'' E). This reference site is approximately 20 km from Little Raven and shares the same soil type and original vegetation. The data was analysed using ANOVA on Microsoft Excel.

Description of treatments

The treatment activities selected for this work are:

(i) herbicide spot-spraying with glyphosate to destroy all above-ground matter, code H;
(ii) seed addition using native Wallaby-grasses (mixed *Rytidosperma* spp.) and Slender Spear-grass (*Austrostipa scabra* subsp. *falcata*) seeds of local provenance, code S;
(iii) burning of residual above ground matter, code F;
(iv) establishment of fencing to prevent grazing damage to the broadcast seeds, code G;
(v) second respraying with glyphosate to remove sprouted *N. trichotoma* from the seedbank, code H*;
(vi) soil disturbance by tillage to bury *N. trichotoma* seeds, code T.

In total, there were 13 integrated treatment combinations which provided data for the hierarchical analysis. Table 1 lists the treatments and provides abbreviation codes for the integrated treatments used throughout this work.

For treatment element code H, all *N. trichotoma* plants were sprayed using a backpack spot-spraying applicator with the recommended rate of glyphosate (100 ml to 10 L of water). Each plant was thoroughly sprayed to ensure all leaves and the bases of the plants were covered. Using this method, the plots selected to be fire treated were sprayed on the 25th of September, 2018, to allow sufficient time for the plants to dry out before implementing the fire treatment. As a consequence of weather conditions, the unburnt plots were sprayed over two days, half on the 31st of October and the other half on the 17th of December, 2018.

The fire treatment, code F, was implemented on the 4th of December, 2018 by Parks Victoria and the Victorian Country Fire Authority (CFA).

The treatment of Grazing exclusion, code G, was achieved by fencing each selected plot by securing 'stocklock' wire fencing to 150cm stock fence posts. While this site no longer is used for livestock grazing, Eastern Grey Kangaroos (*Macropus giganteus*) are frequently observed grazing at the site. High visibility bunting was secured to the top of the fence post to deter them from jumping the fences of the plots. The fencing was installed in the control plots during late November, and in January for the fire plots, after the burn had been conducted.

For the second herbicide application, code H*, selected plots were thoroughly examined and any emerging *N. trichotoma* plants were sprayed with the glyphosate solution, at the same concentration and application method as the first application, on the 8^{th} and 9^{th} of March, 2019.

Selected plots were tilled, code T, between the 17^{th} and the 23^{rd} of May, 2019, using a hand-held rotary hoe to depth of approximately 5 cm.

For the seeding treatment, S, *Rytidosperma* spp. and *Austrostipa scabra* seeds of local provenance to Little River were purchased from Seedling Australia and transferred to Federation University, Australia. These native species are both C_3 grasses, and share a similar growth period as *N. trichotoma.* As a result of this, it has been observed that C_3 species offer a higher level of competition [48]. For each plot, a seed mixture consisted of 160 g of *Ryptosperma* spp., and 40 g of *A. scabra* were weighed and combined within paper bags. To prepare the seeds for broadcasting, the bagged seeds were mixed with sawdust (to reduce the seed clumping due to the intertwining of awns). The seeds were scattered by hand, and then raked lightly into the soil with a garden rake. The seeds were broadcast between the 25^{th} to the 31^{st} of May, 2019.

Data Collection

Above-ground vegetation

The above-ground vegetation was surveyed on the five sampling periods in Table 7, using point intercept transect lines [49]. For each plot, five evenly spaced 10 m transect lines were surveyed at 0.5 m intervals, starting at 0 m and ending at 10 m. The 0.5 m interval was selected as this length is slightly wider than a typical *N. trichotoma* plant. A narrow pole was placed on the ground at each 0.5 cm point, and the vegetation that it touched was identified and recorded.

Table 7: Dates for each sampling period

Sampling period	Start date	End date
1	28/08/2018	16/10/2018
2	4/02/2019	18/02/2019
3	12/12/2019	24/01/2020
4	2/11/2020	20/11/2020
5	28/9/2021	18/10/2021

The seedbank

To sample the seedbank, a soil corer (5 cm diameter and 5 cm depth) was used to collect cores from the centre of the plot, and then from an additional three randomly selected locations within the plot. The four soil samples were combined into a zip lock plastic bag and labelled with the plot's tag number. The soil samples were transported to a glasshouse at Federation University Australia, Mt Helen, and the bags were left open to allow the soil to air dry for two weeks. Plastic punnets (14 cm x 8 cm x 5 cm) were prepared by placing a sheet of absorbent towelling at their base, and then adding 50 g of river sand and 160 g of the collected dry soil. The sample from each plot was divided into five replicates, with two punnets from each plot randomly selected to be initially watered with 10% smoke water solution and then subsequently only with tap water. The punnets were placed into butcher's trays (28 cm x 44 cm x 5.5 cm) to allow bottom watering. Tap water was added to the butcher's trays three to five days a week, and emerging seedlings were identified and removed weekly. Seedlings that could not be identified immediately were replanted separately and grown until identification could be made with confidence. Soil cores were taken for the pre-treatment survey on the 13th and the 20th of August, 2018. Cores were collected from the fire plots on the 20th of December to assess the effect of the fire treatment on the seedbank. Two further sampling periods were conducted; one in 2019 and one in 2020. A research permit to handle *N. tichotoma* ex situ, as well as other nationally significant weeds that may be present within the soil seedbank was obtained prior to taking soil corers. Permission from the land managers and cultural heritage partners was also granted prior to conducting this experiment.

Estimating costs of treatment application at different scales

In order to examine the efficiency of the various treatments, we estimated the costs ($AU) of implementing each of the treatments over areas of different sizes. To calculate herbicide application costs using backpack spraying, we used an unpublished cost model held by the Victorian Department of Environment, Land Water and Planning (DELWP) which considers labour and chemical costs, and assumes that effort is proportional to weed cover and site area (C. Hauser, DELWP, pers. comm.). To calculate the costs of implementing fire, we used unpublished cost estimates compiled by DELWP and Parks Victoria, that include planning, equipment and labour costs at pre-burn, burn and mop-up phases, and list examples for fires of different sizes (S. Sinclair, DELWP, pers. comm.). Cost estimates for tilling were based on the cost estimates that Parks Victoria use for ploughing. The cost for broadcasting the native seeds was determined by the purchase cost for the seeds used in the present study.

Data Analysis

The hierarchy analysis

In order to reduce the number of treatments being compared at the one time, the analysis of the treatments was strategically implemented using a hierarchy analysis, which allowed elimination of non-significant treatment combinations (Figure 5). This process assisted with understanding management implications, as it helped to highlight the most successful result with the least number of control methods. The comparative analysis was conducted using the statistical analysis program SPSS (IBM®). *Nassella trichotoma* vegetation cover, *N. trichotoma* seedbank density, *A. scabra* cover, and *Ryptosperma* spp. cover, were analysed separately. The flowchart followed the path of statistically significant (p=0.05) reduction for *N. trichotoma* cover and seedbank density, and the path of significant increase for the two broadcast species cover. At each step in the analysis, the changes in above-ground cover were analysed over five sampling periods (i) within each treatment on its own, and (ii) between each treatment within the given step of the analysis. The same process was performed for the seedbank density analysis over three sampling periods. When no significant results are observed between treatments, or the analysis reaches the final step, the analysis stopped.

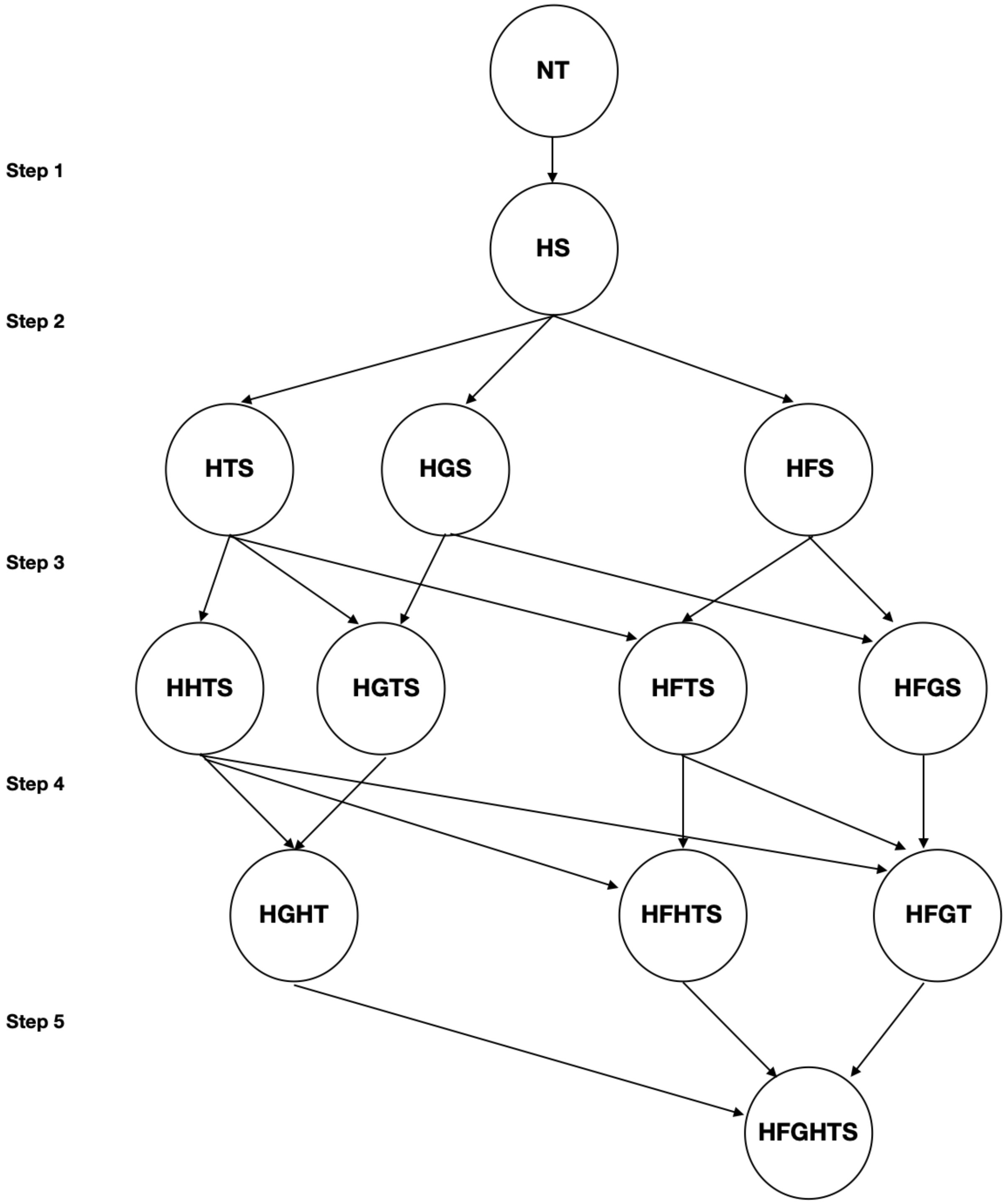

Figure 5: The hierarchy analysis process used to analyse the effect of the treatments on *N. trichotoma* and the establishment of the broadcast seeds. The treatment codes shown in the flowchart are defined in Table 1. The presented flowchart shows the possible analysis pathways for the increasing complexity of integrated treatments used in this study. The analysis is conducted in a series of steps:

- *Step 1* compares the control (code NT) to the simplest treatment combination used in this study; herbicide and seeding (code HS). If the HS treatment was seen to produce a significant result, Step 2 was invoked.

- *Step 2* compares the HS treatment to the treatments that added fire (code HFS), grazing (code HGS) or tillage (code HTS). If a significant result was observed in Step 2 of the analysis, the analysis continued to Step 3.

- *Step 3* follows the path of significance indicated by the flowchart. There are three possible paths for analysis depending on what treatment produces a significantly better result:

    1. The HTS treatment is compared to the HH*TS, HGTS, and HFTS treatments,

    2. The HGS treatment is compared to the HGTS, and the HFGS treatments,

    3. The HFS treatment is compared to the HFGS and the HFTS treatments.

- *Step 4* again follows the path of significance with four possible pathways:

    1. The HH*GS treatment is compared to the HGH*T, HFH*TS, and the HFGT treatments,

    2. The HGTS treatment is compared to the HGH*T treatment,

    3. The HFTS treatment is compared to the HFH*TS and the HFGT treatments,

    4. The HFGS treatment is compared to the HFGT treatment.

- *Step 5* compares the significant treatment from Step 4, either the HGH*T, HFH*TS, or the HFGT treatment to the HFGH*TS treatment.

Analysis of the vegetation community

The total cover of each species was recorded within each treatment at each sampling period. The vegetation community data was analysed by categorizing the recorded species into one of three groups; (i) Australian native species, (ii) invasive grasses, or (iii) invasive "other", which included invasive herbs, forbs and shrubs. This was done separately for each treatment at each sampling period. Cover was assigned and calculated on the basis of groups rather than for their constituent species. Figures were created using Microsoft Excel.

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Conflict of interests

The authors declare no conflict of interests.

Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

Author Contributions

Experimental design and implementation: Talia Humphries and Singarayer Florentine. Literature review and writing: Talia Humphries. Statistical analysis: Talia Humphries and Chris Turville. Data interpretation: Talia Humphries and Steve Sinclair Editing: Talia Humphries, Singarayer Florentine and Steve Sinclair.

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Supplementary Data

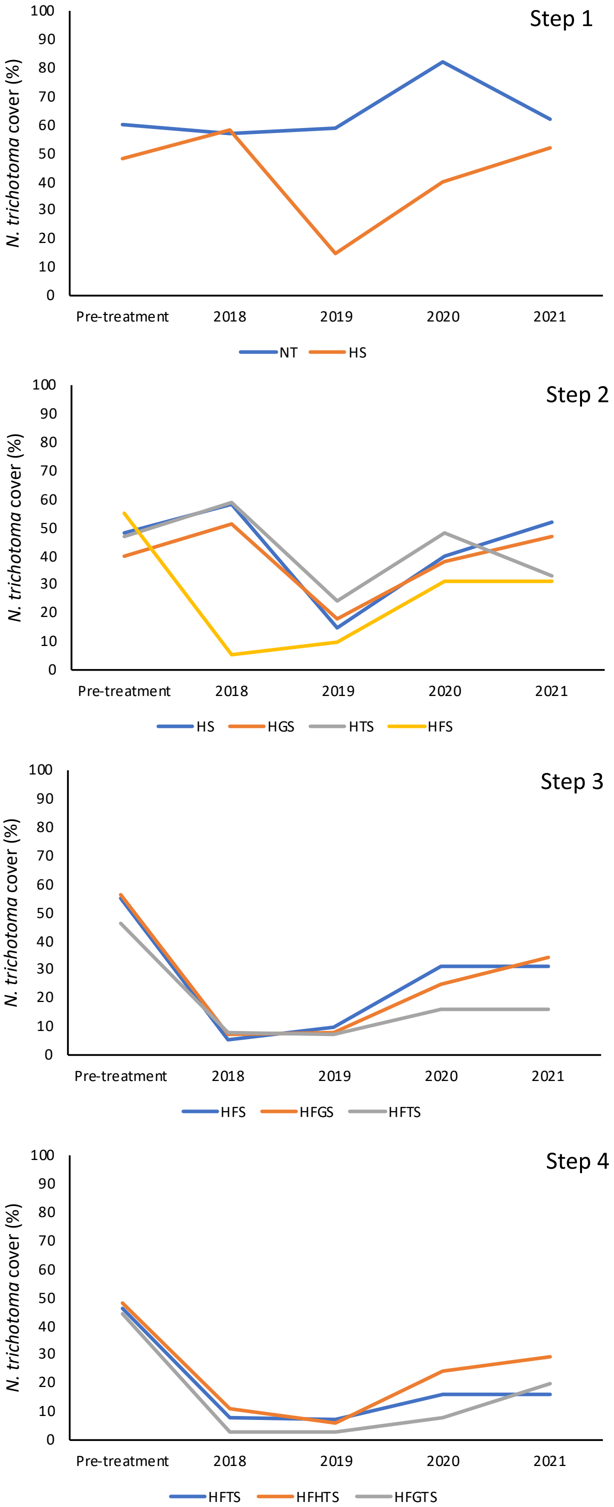

Figure S1: The result of the hierarchy analysis on *N. trichotoma* cover. The individual graphs show each step of the analysis until no further significant differences were observed between the treatments.

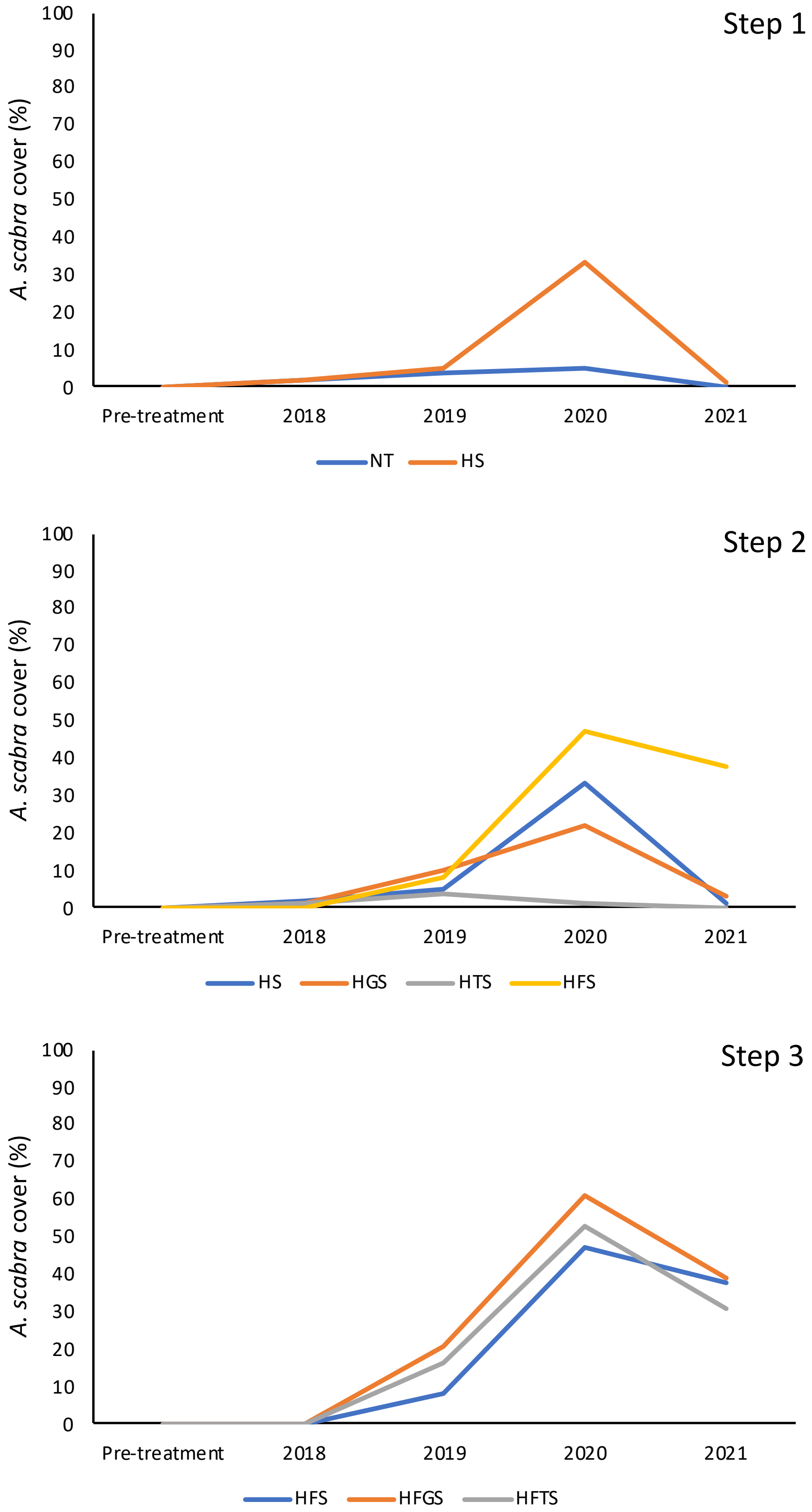

Figure S2: The result of the hierarchy analysis on *A. scabra* cover. The graphs show each step of the analysis until no further significant differences were observed between the treatments.

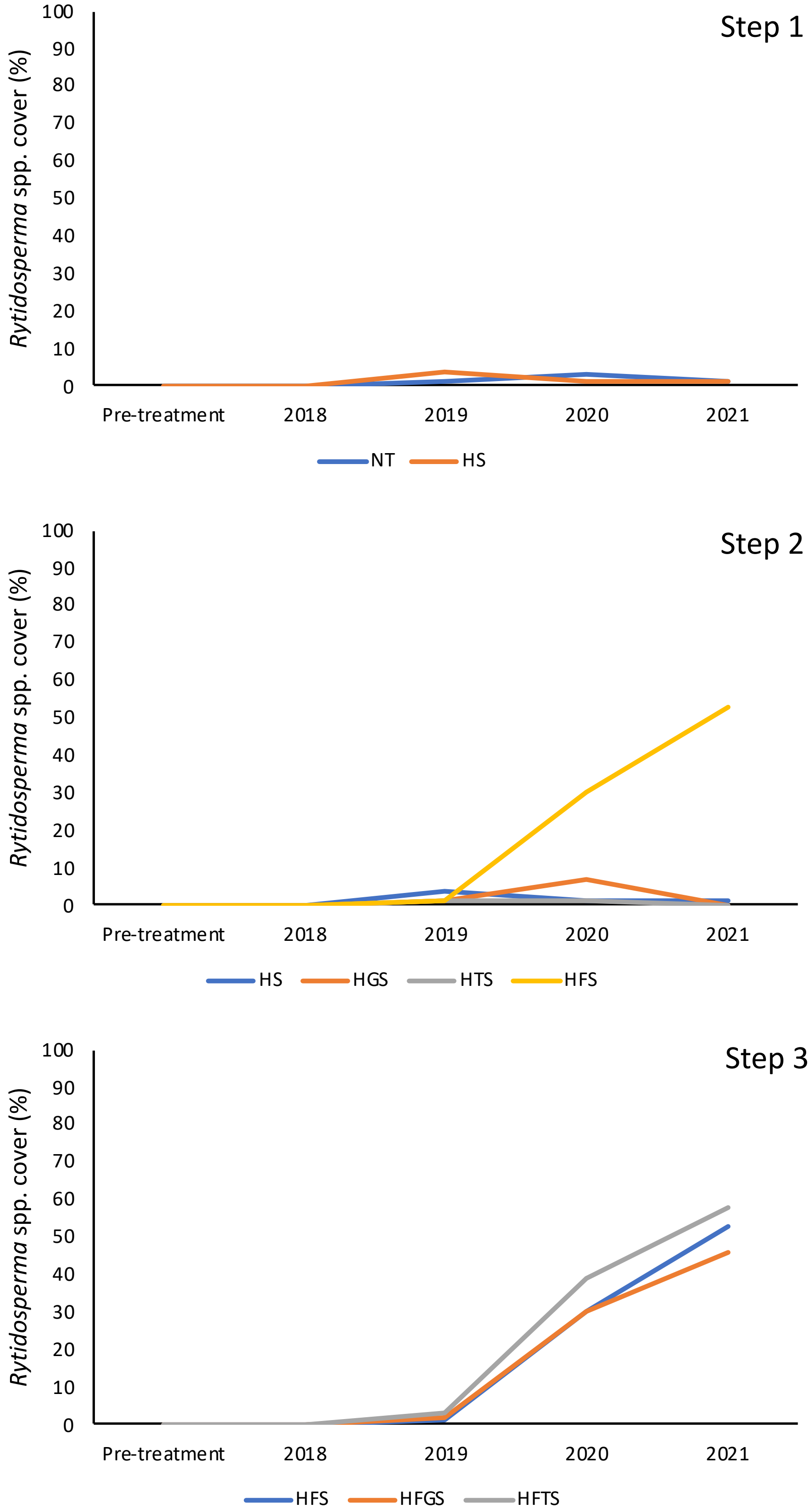

Figure S3: The result of the hierarchy analysis on *Rytipsperma* spp. cover. The graphs show each step of the analysis until no further significant differences were observed between the treatments.

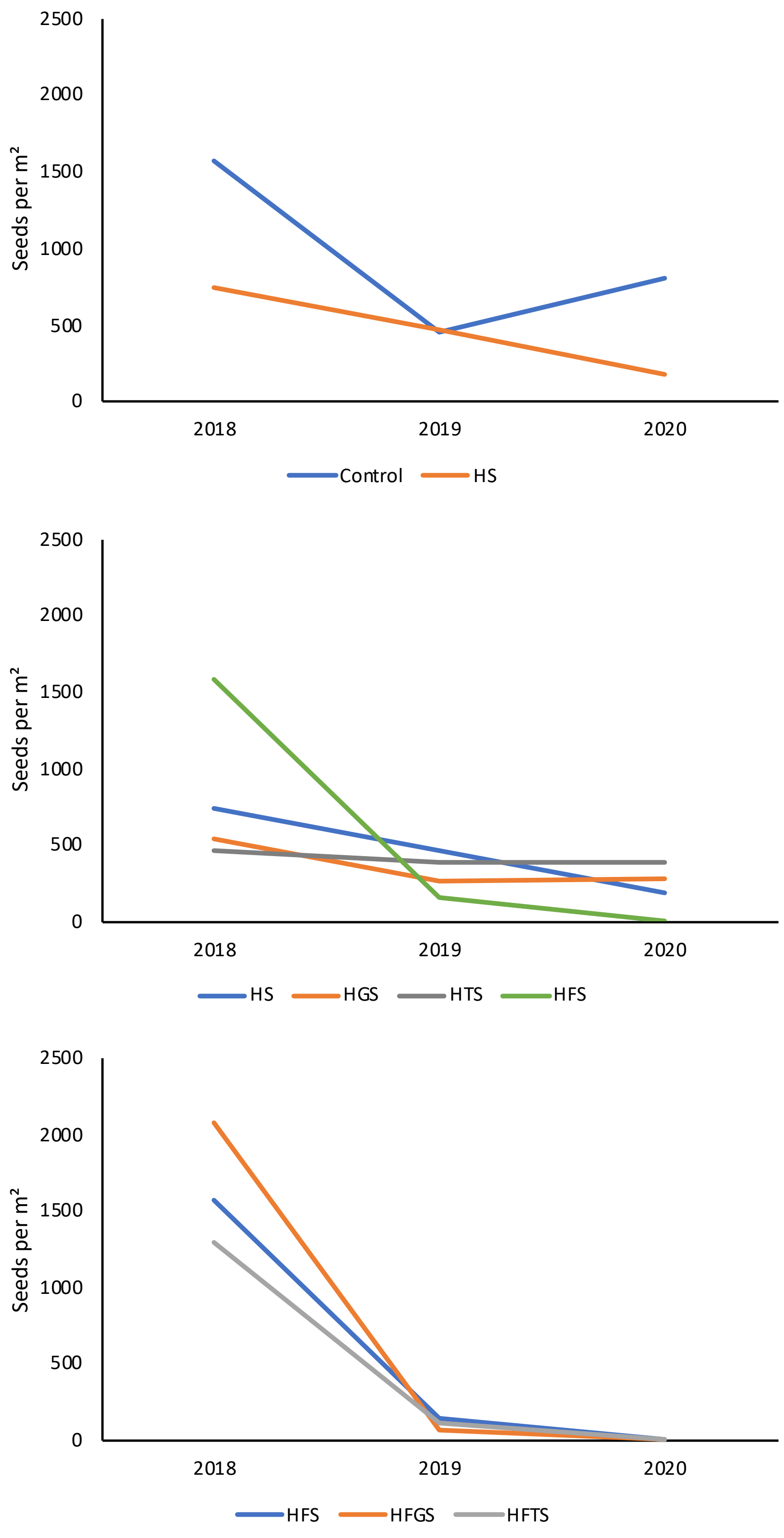

Figure S2: The results of the hierarchy analysis for *N. trichotoma's* seedbank. Soil was collected pre-treatment in 2018, then post-treatment in 2019 and 2020. Each graph shows each step of the analysis until no further significant differences were observed between the treatments.

Chapter 5. Assessing seedbank longevity of the invasive tussock grass; *Nassella trichotoma*, through in-field burial and laboratory-controlled ageing

Humphries, T.; Florentine, S (2022) assessing seedbank longevity and seed persistence of the invasive tussock grass *Nassella trichotoma* using in-field burial and laboratory- controlled ageing. *Plants* 11: 2377. https://doi.org/10.3390/ plants11182377 *plants*

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Article

Assessing Seedbank Longevity and Seed Persistence of the Invasive Tussock Grass *Nassella trichotoma* Using in-Field Burial and Laboratory-Controlled Ageing

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Abstract: The ability to produce highly dense and persistent seedbanks is a major contributor to the successful widespread establishment of invasive plants. This study seeks to identify seed persistence and seedbank longevity for the invasive tussock grass *Nassella trichotoma* (Nees.) Hack. ex Arechav in order to recommend management strategies for preventing re-emergence from the seedbank. To determine the seedbank longevity and persistence, two experiments were conducted: (i) seeds were buried at four depths (0, 1, 2, and 4 cm) and collected and assessed for viability, seed decay, and in-field germination after 6, 9, 12, 15, and 18 months of field burial; and (ii) seeds were exposed to artificial ageing conditions (60% RH and 45 °C) for 1, 2, 5, 9, 20, 30, 50, 75, 100, and 120 days, and viability was determined through germination tests and tetrazolium tests. Less than 10% of the seeds collected after 12 months of in-field burial were viable. The artificial ageing treatment found germination declined to 50% after 5.8 days, further suggesting that *N. trichotoma* seeds are short lived. The results from both experiments indicate that *N. trichotoma* has a transient seedbank, with less than 10% of the seeds demonstrating short-term persistence. It is likely the persistent seeds beyond 12 months were exhibiting secondary dormancy as viable seeds did not germinate under optimal germination conditions. The "Best Practice Guidelines" recommend monitoring for seedbank recruitment for at least three years after treating *N. trichotoma* infestations. The results of this study support this recommendation as a small proportion of the seeds demonstrated short-term persistence.

Keywords: invasive species; seed ecology; seed longevity; seedbank persistence; invasive species management; *Nassella trichotoma*

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1. Introduction

Human interactions with many grassland ecosystems have led to the significant alteration of their natural state, and as a consequence, there is a severe threat of ongoing degradation to grasslands ecosystems in many parts of the world [1]. As an example, the Greater Western Plains, located in central Victoria, Australia, provides a case illustration of the arising degrading pressures acting on these endangered grasslands, including: increased grazing regimes, habitat fragmentation, increased urban expansion, and the introduction and widespread establishment of invasive plant species [2,3]. To date, approximately 1% of the once extensive Greater Western Plains remains in its historic condition [4]. It is now understood that for ecosystems which have been reduced to a degraded state, conservation and restoration efforts must be prioritised and implemented in order to assist them in remaining resilient in the face of changing climate conditions and projected increases in natural disasters [5]. In this regard, conservation and restoration efforts are often thwarted by the presence of invasive plant species, and, of key importance to this investigation, while efforts to reduce the aboveground cover of significant weeds is regularly implemented, efforts to control their seedbanks are often overlooked.

In terms of degraded grasslands, the presence of large, persistent seedbanks of invasive species is a key indicator of the precarious state of an ecosystem [6]. When these invasive seedbanks are high in density, they have a significant competitive advantage over native species, since (i) they physically displace germinating species in the search for water, nutrients, and light, and (ii) they can alter the biotic and abiotic composition of the ecosystem in favour of the invasive species [7]. Some work on controlling the seedbank of a weed-dominated site has demonstrated that this action improves restoration success [8,9], and it has been suggested that restoration efforts that seek to understand seedbank density and persistence of the dominant invasive species can significantly assist in the ongoing management of degraded areas [10].

Understanding the seedbank dynamics of invasive species can uncover important ecological factors that can better equip land managers with information for developing effective and sustainable control strategies [11,12]. Therefore, research that provides a greater understanding of an invasive species' seedbank longevity and persistence should be prioritised [10,12–14]. Although these two factors are similar, seedbank longevity refers to the length of time a seed can remain viable and germinate successfully [15], whereas seedbank persistence refers to the ability of a seed to resist various environmental pressures before losing viability [16]. Seed longevity studies are often conducted ex situ, where the seeds are exposed to elevated humidity and temperature to rapidly age the seeds [17–19]. This process of artificial ageing testing for seed vigour and longevity provides rapid results, and this can provide insight for understanding issues such as invasive potential, soil seedbank eradication programs, and future monitoring in a short timeframe [10]. Seed persistence studies are required to be conducted in situ, where seeds are buried to observe the effects of different seasons, climates, duration of burial, or burial depths on seed viability [20,21]. Seed burial trials are useful for identifying how real-world factors such as burial depth, soil moisture, and pathogens influence seed longevity [22,23]. Additionally, the proportion of seeds that germinate under field conditions can be observed [24]. The comparison of these two methods may provide enhanced insight for identifying the seedbank longevity and persistence of an internationally significant, aggressive, invasive grass species: *Nassella trichotoma* (Nees.) Hack. ex Arechav. (known commonly as serrated tussock) of the Poaceae family [17]. Wide variations in the seed longevity for this species have been reported in the literature, with claims of seeds aged between 1 to 13 years germinating successfully under soil conditions [25,26]. It is widely reported in publicly available management material that this species' seedbank can persist for up to three years [26].

It has been determined that seed longevity is related to the availability of stored macromolecules such as nucleic acids, lipids, and proteins together with their ability to protect and repair these resources under stressful conditions [27]. The current categorisation of seedbank resilience refers to (i) transient seedbanks, which do not persist in the soil for more than one year, (ii) short-term persistent seedbanks, which can remain viable from one to five years, and (iii) long-term persistent seedbanks, which are viable for at least five years [28]. Notwithstanding the attraction of this somewhat simple framework, observed persistence varies greatly between species and biotypes, and is affected by different environmental cues and disturbances, which may differ from year to year [7,21]; although the development of a simple categorisation system for the seedbank of an invasive species is a powerful and rapid management tool, it overlooks the various environmental pressures that alter seedbank persistence.

The position of the seed within the seedbank can alter its experienced microhabitat, significantly influencing the seed's persistence [21]. A common example of this microhabitat trend is that shallow burial depth and increased interred duration result in a significant decline in seed viability, as seen in *Andropogon gayanus* [29], *Raphanus raphanistrum* [30], *Erigeron canadensis* [31], and *Artemisia tridentate* [14]. In this regard, seeds located on or just below the soil surface are exposed to more intense fluctuations in soil moisture and temperature compared to deeper buried seeds. These fluctuations can result in shallow covered seeds experiencing more intense wet and dry cycles, as was observed in *A. tridentate,* where it was found that after two years only 0-11% of viable seeds remained at the soil surface compared with almost half of the seeds maintaining viability at a 3cm depth [14]. Seed predation is also increased at shallow depths [32]. Due to these factors, it has been observed that seeds that are buried deeper into the soil profile are better protected from a range of devitalising disturbances and persist in the seedbank for a longer period of time compared to those buried closer to the surface [14,29]. The trade-off, however, is that at increased depths, seed dormancy is usually prolonged, particularly for photoblastic seeds [33], leading to observations that with increased depth, successful emergence is reduced, particularly in smaller seeds with less energy reserves [34].

One of the most aggressive and difficult to manage weeds in the Greater Western Plain's grasslands is *N. trichotoma*. This South American native is considered a significant weed in southeast Australia [35,36], New Zealand [37], and South Africa [38]. It is also problematic within its native range due to its low palatability for grazing animals and livestock [39,40]. Individuals of this species can produce over 100,000 anemochory seeds annually, which are dispersed within the panicle up to 20km from the parent plant [41]. As a consequence of the high fecundity, seedbank densities of up to 50,000 seeds per square metre have been recorded [38,40], and rapid germination has been observed following disturbance events and heavy rainfall [41].

The majority of the seeds remain within the top 2.5 cm of the soil layer [38] and the optimal emergence occurs within this depth, whereas the emergence of seedlings buried at 4 cm was significant reduced [42]. The rounded plump seeds are 3 mm in length when measured from the hooked tip to the base, contain an off-centred 25–35 mm long awn, and weigh 76–86 mg [42]. The seed drop begins in early summer when the panicles containing seeds droop over and detach from the plant, and subsequently, they are dispersed by strong winds. Under favourable conditions, the seed drop may begin as early as October and continue up until May [41,43]. Under favourable years, a single plant can produce up to 2000 panicles [44].

Nassella trichotoma has non-deep physiological dormancy, which is broken by increased rainfall in Autumn, and some research suggests that the seeds can initiate dormancy at any time of the year to avoid unfavourable growing conditions [26]. As seeds dropped late in the seeding period, for example, in late April or May, will miss the optimal germination cues provided in autumn, these seeds may contribute to extending the seedbank longevity for this weed [45]. The combining factors of uneven germination, a potentially long-lived seedbank, and a large annual seed set with wide dispersal make recruitment from the seedbank one of the most challenging aspects for managing this species and achieving grassland restoration goals.

This paper aims to integrate seedbank persistence and longevity methodologies to make confident recommendations for the management of *N. trichotoma's* seedbank. With the wide variability in seedbank longevity reported for *N. trichotoma*, a study combining and comparing the results of in situ (seed burial) and ex situ (artificial ageing) techniques should provide greater clarity regarding the seedbank ecology for this species. Long et al. [17] compared the results of artificial ageing to field persistence data of 27 invasive plants, and found the two techniques to provide a positive correlation. The benefits of using both techniques allow for the consideration of the real-world factors on seed longevity; however, as these experiments are limited by time, the rapid ageing technique can assist in identifying any seeds that persist beyond the burial treatment timeframe.

2. Methods

2.1. Seed Collection and Preparation

Panicles containing seeds were collected from over 100 mature *N. trichotoma* plants in early December 2018. Seeds were collected from a *N. trichotoma*-dominated grassland in Mambourin, Victoria, Australia (37°55′18.12″ S, 144°32′43.079″ E). The panicles containing the seeds were sealed within a plastic zip-lock bag and transported to Federation University Australia, Mount Helen Campus, located approximately 100 km from the collection site, where they were stored in darkness at room temperature (approximately 20 °C) until being placed into nylon mesh bags approximately three months after collection. The mesh bags containing the seeds were stored within a drawer at the Federation University Australia's seed ecology lab until they were required for the subsequent experiments. Prior to use, a germination test confirmed the average seed viability was 75.32%.

2.2. Seed Longevity

In order to assess the longevity of *N. trichotoma's* seedbank, the artificial ageing technique, developed by the Millennium Seed Bank Project, Royal Botanical Gardens, Kew, UK [18], was implemented. To prepare for this treatment, 45 plump, viable seeds (viability was determined by gently squeezing the seeds with forceps) were placed into bags made of fine (0.05 mm), semipermeable nylon mesh, which were sealed using hot glue. For the rehydration process, the mesh bags containing seeds were placed on a stand above a 47% relative humidity (RH) lithium chloride (LiCl) solution (370 g/L deionised H_2O) within a sealed electrical box (300 × 300 × 102 mm), which was then placed in an incubator set to 20 °C and 24 h darkness for 14 days. After this time, the seed eRH was assessed using a hygrometer (HygroPalm HP23-A/HP23-AW-A handheld indicator, Rotronic) and considered successful with a reading of 47%. After the seeds were considered rehydrated, the seed bags were moved to a second electrical box set to 60% RH (LiCl, 300 g/L) and placed in an incubator set to 45 °C for the ageing treatment. At each sampling period, six seed bags were randomly selected and removed from the ageing box on days 1, 2, 5, 9, 20, 30, 50, 75, 100, and 120. At each sampling period, 25 seeds were randomly selected from each of the six replicates to undergo a standard germination test. For the germination test, the selected seeds were plated into Petri dishes lined with a single layer of sterilised Whatman 1 No. 10 filter paper and then moistened with 10 mL of sterilised RO water. The Petri dishes were sealed with parafilm and placed into an incubator (Thermoline Scientific, temperature and humidity cabinet, model: TRISLH-495–1-s.d., Vol. 240, Sydney, Australia), which was fitted with cool-white fluorescent lamps that provided a photosynthetic photon flux of 40 mmol m^{-2} s^{-1}. The incubators maintained alternating temperatures of 25/15 °C, with 12 h of light and 12 h of darkness [42]. Seeds were observed for germination twice a week, and the seeds were incubated for 42 days, or until all seeds had successfully germinated. The remaining 20 seeds in each replicate were assessed for viability using only a tetrazolium test.

2.3. Seedbank Persistence

To identify the seedbank persistence of *N. trichotoma*, 50 viable seeds were placed into 5-centimetre square bags made of fine (0.05 mm), semipermeable nylon mesh that allowed for the natural flow of water and micropathogens while keeping the seeds contained. The mesh bags were sealed using hot glue. One bag was placed into a hole that was dug to a depth of either 0 (surface), 1, 2, or 4 cm, and then covered with the excavated soil.

As it was mentioned in the introduction, the seed set of *N. trichotoma* normally occurs in December for the southern hemisphere; however, in the case of climatically favourable seasons, seed set has been observed to continue throughout autumn. To determine if this time variation of the seed entering the soil seedbank influences the seedbank persistence, half of the seed bags were buried in January at Mambourin (37°55′18.12″ S, 144°32′43.079″ E), whilst the remainder were buried in May at a privately-owned site dominated by *N. trichotoma* that is located in the Pentland Hills, Victoria (37°39′50.515″ S, 144°24′10.334″ E) (Figure 1). The sites are located within 50 km of each other, and share similar rainfall and air temperature conditions.

At 6, 9, 12, 15, and 18 months after burial, five replicates from each depth were randomly exhumed from the soil and placed into labelled plastic zip-lock bags for transporting the mesh bags containing seeds to Federation University Australia's seed ecology laboratory, located at Mt. Helen, Victoria, for examination. Seeds that germinated under field conditions (determined by visible radical emergence, intact root, or cotyledon) were recorded and discarded. The condition of the remaining seeds was assessed using the crush test, whereby gentle pressure is applied to the seeds by tweezers, and seeds that collapsed were deemed to have decayed [46]. Seeds that maintained their integrity were considered intact and were tested for viability via germination trials. These intact seeds had excess dirt removed using tap water and were assessed using the germination method previously described. At the completion of the 42-day incubation period, the remaining seeds were assessed by the crush test and any seeds that maintained their integrity underwent a tetrazolium test for viability.

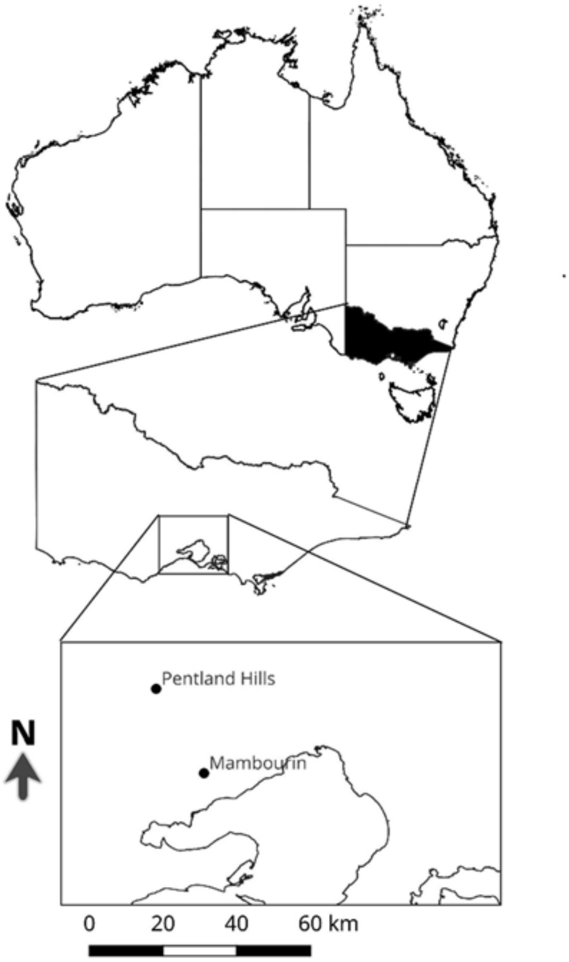

Figure 1. The location of the two sites used to investigate the seedbank persistence of *N. trichotoma*. The two sites are located within the Greater Western Plains grasslands, located to the west of Melbourne, Victoria.

2.4. Data Analysis

The effect of seed burial depth and duration on seed longevity of *N. trichotoma* was determined by transforming the data to an averaged percentage. The data were graphed on a cluster bar graph to show the effect of burial on time and burial depth on the longevity factors of viability, in-field germination, and seed degradation under field conditions.

For the artificial ageing treatment, a probit analysis to find the P_{50} value was performed on the data using SPSS statistical software [46]. Seed viability was plotted against time (days) and a seed survival curve was fitted to the data. The number of final germinated seeds was converted into a percent of germination (%) [47]. The number of germinated seeds at 14 days of incubation was used to find the germination energy. Mean germination time was calculated using the equation:

$$\text{Mean germination time} = \Sigma Dn/\Sigma n \quad (1)$$

where n is the number of seeds, which were germinated on day Dn, and n is the number of days counted from the beginning of germination [48].

The germination rate index was calculated as described by the Association of Official Seed Analysts [49] by using the following formula:

No. of germinated seeds/day of first count + . . . + No. of germinated seeds/day of final count.

3. Results

3.1. Seed Longevity

The effect of the artificial ageing experiment found that germination (%) steadily declined with increased exposure to the ageing treatment. The control viability (shown in the Section 2) determined germination (%) to be 75.35, and therefore, no significant variation was observed after one day of exposure to the ageing conditions. After two days of exposure to the ageing conditions, the germination (%) was reduced to 63.33%, and, with the exception of day nine, germination (%) continued to decline at each sampling period (Table 1). No germination was observed at or after 75 days of exposure to the ageing treatment. The seeds exposed to the ageing treatment for 1 and 9 days had higher germination energy compared to all other days (52.67% and 57.33%, respectively), which indicates over half of the seeds had germinated within the first 14 days of the germination trial. The seeds recovered at the same sampling periods were also observed to have a higher germination rate index (3.76 and 3.96, respectively) compared to those retrieved on day 5, 20, 30, and 50, and the germination rate for the latter three sampling periods was considerably low. With the exception of the seeds exposed to the ageing conditions for 50 days or more, the mean germination time remained steady throughout the treatments, with the average germination occurring at approximately 24 days of incubation. The germination trials achieved P_{50} by 5.8 days, whereas this value increased to 31.2 days in the tetrazolium test for viability (Figure 2). The probit analysis for the germination trials suggests that approximately 10% of the seeds would survive at least 100 days of the artificial ageing treatment.

Table 1. The effect of increased exposure time to artificial ageing conditions on *N. trichotoma* seeds. The table shows the mean and standard error for germination (%), germination energy (%), germination rate index, and mean germination time at each duration of exposure to the treatment. As no germination was observed for 75, 100, and 120 days under artificial ageing conditions, these results were not included.

Artificial Ageing Time (Days)	Germination %	Germination Energy %	Germination Rate Index	Mean Germination Time
1	72.67 ± 7.62	52.67 ± 6.65	3.76 ± 0.46	23.64 ± 0.40
2	63.33 ± 4.89	33.33 ± 3.21	3.11 ± 0.28	24.02 ± 0.51
5	56.67 ± 5.6	32 ± 3.27	2.34 ± 0.25	25.01 ± 0.28
9	66 ± 5.34	57.33 ± 5.33	3.96 ± 0.34	22.87 ± 0.32
20	28 ± 8.13	25.33 ± 6.08	1.33 ± 0.32	24.59 ± 0.49
30	30.67 ± 9.78	22.67 ± 7.13	1.25 ± 0.36	24.76 ± 1.04
50	22.67 ± 6.59	0	0.36 ± 0.1	30.25 ± 0.62

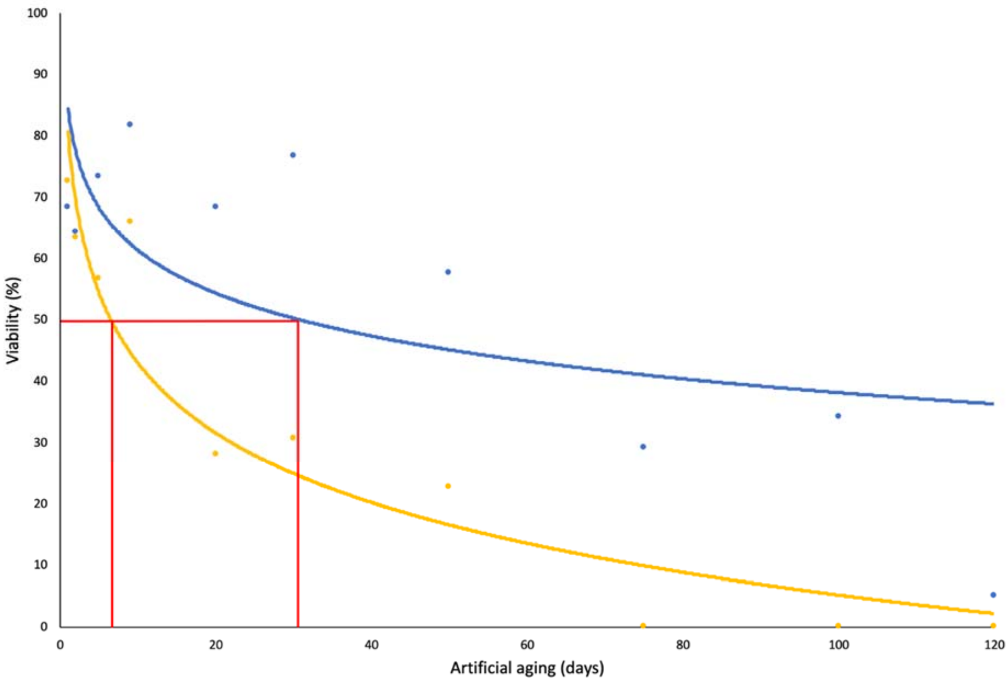

Figure 2. The effect of artificial ageing on viability (%) for *N. trichotoma* determined by germination (yellow) and tetrazolium (blue). Survival curve was fitted to each data set to represent the confidence limits of the probit analysis. The probit analysis determined P_{50} = 5.8 days and P_{50} = 31.3 days for the germination and tetrazolium trials, respectively, and these values are highlighted in red.

3.2. Seedbank Persistence

The climate conditions, in terms of temperature and monthly rainfall, were similar at the two selected sites, Pentland Hills and Mambourin, for the duration of the burial trials (Figure 3). The sum of rainfall at Pentland Hills was higher (940 mm in total) than that observed at the Mambourin site (857 mm). Similar maximum temperatures were observed at both sites, with the average summer temperature reaching 25 °C at the Mambourin site and 24 °C at the Pentland Hills site, with both sites averaging a top temperature of 14 °C during the winter months. The Mambourin soil had a dark-brown colour and a clay to clay-loam texture, and the site was relatively flat in topography. The Pentland Hills site had soil with a loam texture, was dark reddish-brown in colour, and the seeds were buried on a slight slope close the top of the hill with a southern aspect. It is known that *N. trichotoma* can germinate under a wide range of temperatures, with a high germination (%) observed under alternating temperatures from 17/7 °C to 30/20 °C [42].

The depth of seed burial did not impact the seedbank persistence for *N. trichotoma*, as similar observations in viability were observed across all burial depths (Table 2). The seedbank persistence appeared to reduce as a result of an increased time of burial (duration) and the season the seeds were buried, with those buried in May exhibiting greater persistence than those buried in January. The proportion of seeds to have decayed under the field conditions was high across all the retrieval times for both the January and May burial times. It was observed that seed viability (%) decreased with an increased burial duration for the seeds buried in May, but this was not the case for the seeds buried in January. The highest proportion of viable seeds recovered (15.2%) were buried in May at 1cm for nine months. Overall, only a small proportion of the seeds were intact and viable at any of the sampling periods, suggesting that seed persistence rapidly declines in *N. trichotoma*.

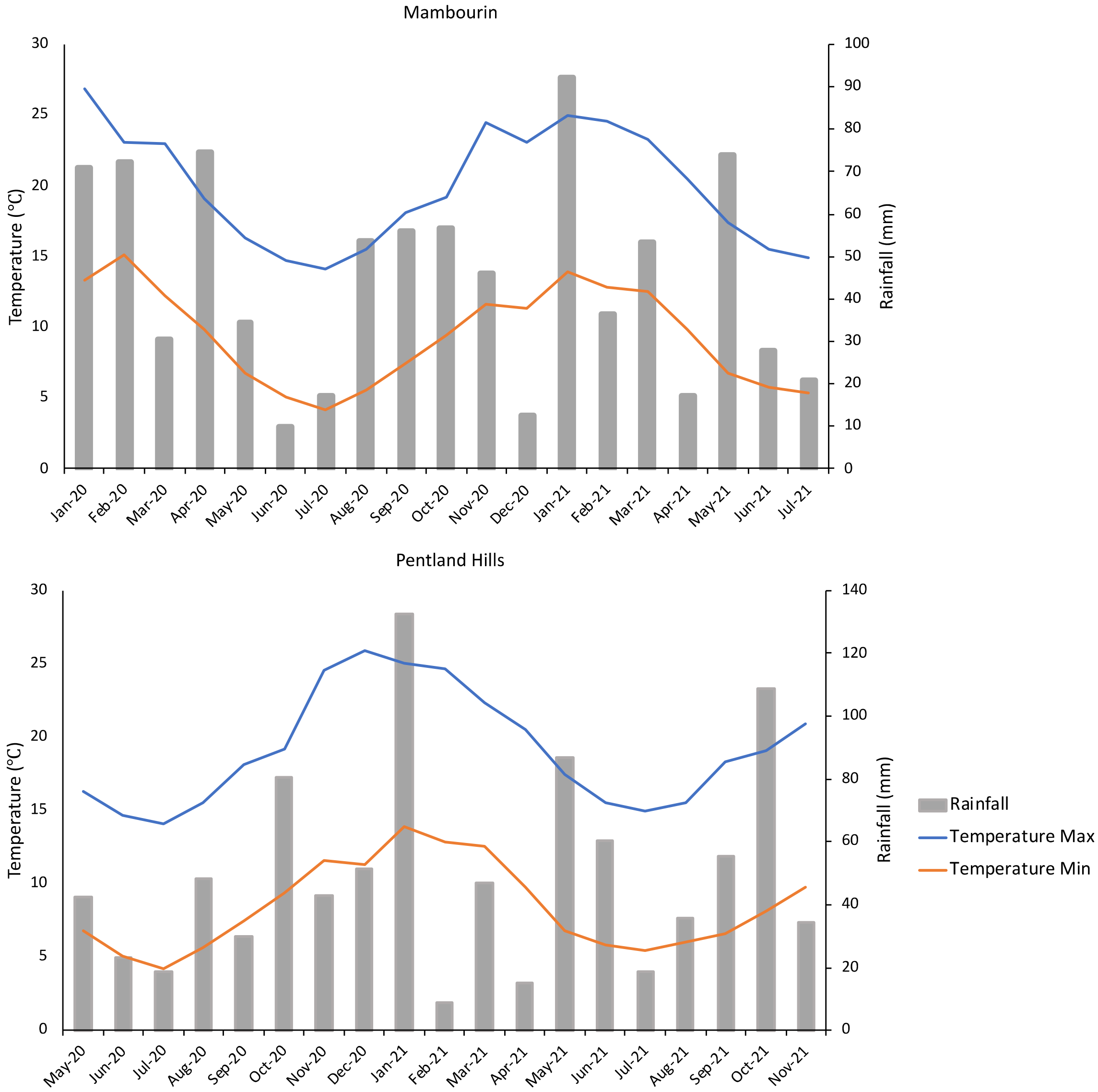

Figure 3. The climate data for Pentland Hills and Mambourin for the duration of the seed burial trials at each location. Data were accessed from the Bureau of Meteorology [50]. Temperature and rainfall data for the Mambourin site were taken from Mt. Rothwell weather station, which is located approximately 15 km from this site. The temperature data for Pentland Hills were collected from the Durdidwarrah weather station, located approximately 40 km from the site, and the rainfall data were collected from the Merrimu Reservoir weather station, located approximately 13 km from the Pentland Hills site.

Table 2. Effect of burial depth and time on *N. trichotoma* seed persistence for seeds buried in January and May.

	Time of Burial									
	6 Months		**9 Months**		**12 Months**		**15 Months**		**18 Months**	
Burial depth (cm)	Germination (%)									
	Jan	May	Jan	May	Jan	May	Jan	May	Jan	May
0	0	11.2	0	10	0	1.6	2	0	2	3.20
1	0	12	3.2	15.2	0	14.8	0.4	5.2	0.4	0.40
2	0	14	0.8	5.2	0	11.2	0.4	9.2	0.4	0.40
4	0.4	12.8	1.6	12	0.8	7.6	6.4	6.8	6.4	0.40
Mean	0.1	12.5	1.4	10.6	0.2	8.8	2.3	5.3	1.2	1.2
SEm±	1.08	1.26	1.46	1.51	2.15	2.07	2.53	2.24	3.12	2.92
Burial depth (cm)	Seed decay (%)									
	Jan	May	Jan	May	Jan	May	Jan	May	Jan	May
0	80.4	74.8	87.2	76.8	88.8	72.4	92	70.8	66.4	78.4
1	80.8	78.4	88.8	77.2	57.6	63.2	77.6	57.6	81.2	79.2
2	77.6	74	90.4	82.4	86.4	76.4	84.4	66.8	82.8	82.4
4	94.8	72	93.2	83.2	96.8	69.6	80.4	74	78.8	86.8
Mean	83.4	74.8	89.9	79.9	82.4	70.4	83.6	67.3	77.3	81.7
SEm±	14.39	12.60	14.80	13.00	14.21	10.84	12.72	9.87	11.14	11.71
Burial depth (cm)	Field germination (%)									
	Jan	May	Jan	May	Jan	May	Jan	May	Jan	May
0	19.6	14	12.8	13.2	11.2	26	6	29.2	30.4	20.4
1	19.2	9.6	8	7.6	42.4	22	22	37.2	18.4	19.2
2	22.4	12	8.8	12.4	13.6	12.4	15.2	24	16.8	15.2
4	4.8	15.2	5.2	4.8	2.4	22.8	13.2	19.2	20.8	10
Mean	16.5	12.7	8.7	9.5	17.4	20.8	14.1	27.4	21.6	16.2
SEm±	3.4	1.5	1.11	1.41	6.21	2.62	2.33	3.54	2.25	1.69

SEm± refers to the standard error of the mean.

4. Discussion

4.1. Seedbank Longevity

Understanding key biological traits of invasive species, such as seed longevity, is critical for developing effective management policies and strategies for long-term control and, potentially, local eradication [51]. The artificial ageing technique has proven to be an effective and rapid tool for determining if a species can produce transient, short- or long-term persistent seedbanks [10,17]. The artificial ageing treatment determined that *N. trichotoma* has a short-lived seedbank, with a P_{50} value of 5.8 days. It was previously determined that a P_{50} value < 20 days corresponds to short-lived, transient seedbanks [17,28]. The results of the seed longevity experiment are consistent with our findings in the seedbank persistence experiment, whereby 99% of the January seeds and 91% of the seeds buried in May had either germinated under field conditions or had decayed within the first year.

Germination energy refers to the ability for seeds to germinate fast and in unison, as the more seeds that germinate within a pre-defined timeframe can indicate the level of seed vitality [52,53]. The *N. trichotoma* seeds demonstrated decreased germination energy with increased exposure to the artificial ageing experiment. This can indicate that the emergence vigour of seedlings declines with increasing age [54]. There was little variation in the mean germination time for seeds exposed to the ageing conditions between 1 and 30 days; however, this time increased after 50 days of exposure. A higher mean germination time was observed to reduce the mean emergence time of subsequent seedlings [55], which further supports the claim that the vigour of the emerging seedlings reduces with increased seed age, making them less competitive.

Viability (%) determined by the tetrazolium test was higher (P_{50} = 31.3 days) than that recorded through the germination test, suggesting the seeds may have short-term persistence in the seedbank (between 1 and 3 years). Tetrazolium tests can overestimate the viability (%) of seeds when compared to germination (%), but this is often by a margin of 5% [56]. The seeds tested via the germination test could have demonstrated low germination (%) as a result of stress caused from the artificial ageing treatment. Exposure to a high constant temperature, such as that experienced in the artificial ageing treatment, has been observed to induce secondary dormancy in *N. trichotoma* seeds [26], and therefore, the tetrazolium test may have accounted for these dormant seeds. Secondary dormancy is a protective strategy for mature, hydrated seeds, which is induced by prolonged exposure to unfavourable environmental cues [57,58]. Secondary dormancy has proven difficult to break for other species such as *Coreopsis lanceolata*, even after optimal temperature and light cues return [59].

4.2. Seedbank Persistence

The ability for an exotic plant species to develop dense and persistent soil seedbanks contributes to its invasive potential [60]. The ability for seeds to persist for over one year is an important bet-hedging strategy to protect the plant's population from various disturbance events and unsuitable conditions for germination [61]. Further, having seeds of multiple ages within a seedbank can enhance the genetic diversity within the population [62]. Consequently, these factors make the management of invasive plants with persistent seedbanks complex, time- and cost-consuming, and often, the weeds return after the invaded areas have been treated [63].

Whilst the literature suggests that *N. trichotoma* is able to establish short-term persistent seedbanks (up to three years), it is found, however, that most of the seeds germinate within the first autumn following seed set [24,26], and seedbank density has been observed to be reduced by approximately 40% between March and August [24]. The results of this research support this claim, as only 9% of the seeds buried in May and <1% of the seeds buried in January maintained viability after one year. A similar figure was observed after six months of burial, with only 12.5% and < 1% of the seeds buried in May and January, respectively, being intact and viable. The literature also observes that the germination for *N. trichotoma* predominantly occurs in autumn and winter [35,41], and it was observed in the present study that field germination (%) was higher within the first six months (equivalent to autumn and winter) for the seeds buried in January compared to those buried in May, which initially experienced winter and spring conditions.

The average monthly temperatures observed during this experiment were within the ideal range for germination, ranging from a maximum average daily temperature of 25 °C in the summer months and 14 °C in the winter months [42]. Rainfall was consistent over the 18-month period, with exceptionally high rainfall observed in January 2021 at both sites. Despite a high soil moisture content previously being determined as one of the most important factors for initiating *N. trichotoma* germination [26,42], no variation in in-field germination (%) was observed as a result of the rainfall event, nor was there any significant variation between the two sites despite the Pentland Hills site receiving higher rainfall. The variation in soil type between the two sites may have contributed to seed decay (%), as clay soils observed at Mambourin can hold higher water content compared to the loam soil types observed at Pentland Hills.

A longevity study conducted in New Zealand found a similar proportion of seed decay (%) for *N. trichotoma*, whereby 90% of the seeds were recorded as decayed after 19 months of in-field burial [64]. The presence of an intact cotyledon or radical was required for seeds to be classified as germinated under field conditions. As the burial time increased, the ability to detect these structures prior to their deterioration reduced, and it is likely that the majority of the seeds classified as decayed did, in fact, germinate under field conditions. Further, the seeds considered as germinated under field conditions are likely to have done so recently for these structures to remain intact [65].

Our analysis of the artificial ageing data suggested that a small proportion of the seeds would exhibit long-term persistence, which agrees with other accounts which have identified small quantities of *N. trichotoma* seeds capable of persisting for up to 15 years under field conditions [44,66]. After 18 months of burial, 1.2% of the seeds in both the January and May burial periods remained viable. As *N. trichotoma* can produce as many as 140,000 seeds per plant annually, and seedbank densities have been recorded as high as 50,000 seeds m^{-2} in South Africa [38] and Australia [67], and up to 42,000 seeds per m^{-2} in New Zealand [68], even a proportion as low as 1.2% of the seeds demonstrating persistence could equate to a significant number of germinants that would require ongoing management.

4.3. Implications for Management

Understanding longevity can assist with understanding persistence potential, which is a critical element for the effective development of management policies and control strategies. The use of the artificial ageing technique provided an effective method for rapidly estimating the seedbank longevity for *N. trichotoma*, and these results complimented the data obtained by the burial treatment. Although the seedbank persistence categories currently associated with the artificial ageing technique are quite broad, this technique offers a simplistic tool to assist in the development of weed management programs. As a basis, knowing if a species can produce transient, short- or long-term persistent seedbanks can allow managers to make decisions regarding a plant species invasive potential [51]. This research suggests that a high proportion of the seedbank either germinates or decays within the first six months of seed set, regardless of what time of year the seeds are dropped. Therefore, control methods should be implemented in late winter to early spring to maximise control strategies targeting the emerging seedlings, as the majority of the seedbank will have either germinated by this point, or have decayed.

It is suggested that achieving local eradication is more feasible for invasive species that produce transient seedbanks [61], yet *N. trichotoma* is considered very difficult to control and remains widespread at a landscape scale [36,37]. In order for *N. trichotoma* to remain competitive, it must rely on producing high volumes of seeds annually. These seeds are able to travel great distances and recolonise previously treated areas. This indicates the importance for community groups to organise a collaborative, land-scale management for *N. trichotoma*, rather than land managers treating only their own property. This research shows promise in that the seedbank density can be significantly reduced after one year by implementing strategies that prevent seed set.

5. Conclusions

Nassella trichotoma has a transient to short-term persistent seedbank, with less than 10% of the seeds demonstrating viability after 12 months. After six months of burial, 75 to 83% of the seeds had decayed, and a further 13 to 16% had evidence of in-field germination. Although this species is able to produce exceptionally high seedbank densities, our results demonstrate that to maintain its viability, the species requires continuous seed input annually. The difference in viability observed in the germination trials and the tetrazolium test could indicate secondary dormancy under unfavourable environmental conditions. For this reason, it is recommended that the seedbank should be monitored for at least three years and efforts to prevent seed set and seed migration into treated areas should be prioritised by land managers.

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Chapter 6. The response of *Nassella trichotoma* (serrated tussock) seeds and seedlings to fire

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The response of *Nassella trichotoma* (serrated tussock) seeds and seedlings to fire

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Abstract

Context: Fire is an important disturbance regime in grassland communities for regeneration and maintaining biodiversity. When invasive plants, such as *Nassella trichotoma* (commonly known as serrated tussock), establish and become widespread in a grassland community, these important fire regimes can be altered in intensity and/or frequency to facilitate the establishment of the exotic species. Therefore, before fire can be recommended as a suitable control technique, or integrated into grassland restoration programs, understanding the response of the seeds to high temperature, such as that experienced during a fire, should be well understood.

Aims: Our aim was to identify the response of *N. trichotoma* seeds and seedlings to a gradient of temperatures associated with fire and how increased duration of exposure effected the response. We also wanted to identify if seed age or seed moisture content affected the germination response.

Methods: To gain an understanding of the fire response of *N. trichotoma's* seedbank, seeds were collected in 2016, 2017, 2018 and 2019 and then stored until the commencement of the experiments in 2020. The seeds were first subjected to an increasing temperature gradient (80, 100, 120, 140^{O}C, and a control), and an increasing duration of exposure (1, 3, 6, and 9 minutes). In the second experiment, one population was selected to test these same temperatures and duration of exposure after the seeds were hydrated to 15, 50, or 95%. Lastly, seedlings were grown for three months under glasshouse conditions and then exposed to increasing temperatures (20, 60, 80, 100, and 120^{O}C), and increasing duration of exposure (3, 6, and 9 minutes). The seedlings were assessed two weeks after the heat exposure for signs of damage.

Key results: Increased temperatures and duration of exposure had a subtle negative effect on germination parameters, including reduced total germination and increased time to 50% germination. The 140^{O}C treatment was significant at killing the seeds. The age of the population was not a significant factor on germination (%), and the variations in germination (%) was rather influenced by seed weight. Seed germination (%) was significantly reduced for seeds hydrated to 95% compared to the control treatment, while no significant difference was observed for the seeds hydrated to 15 and 50%. For the seedlings, damage to the leaves was observed in the 80, 100, and 120^{O}C treatment, with some plants in the 120^{O}C treatment experiencing extensive damage and re-sprouting. No seedlings were killed at the tested temperatures.

Conclusions: The results of this study indicate that fire may be a useful tool for reducing seedbank density by killing a high proportion of the seeds at the soil surface, or located within the top 1cm of the soil profile. The efficacy of fire on surface and shallow buried seeds is improved with high seed

moisture content. Seeds buried below this depth are protected by the soil from the lethal temperatures.

Implications: Fire implemented either before seed set, or immediately after seed set could effectively kill the seeds. It would be recommended to integrate a chemical treatment with the fire treatment to improve its efficacy on *N. trichotoma's* canopy.

Summary: Grass species have adapted strategies to survive and respond rapidly in fire prone environments. This study investigates the response of *N. trichotoma* seeds and seedlings to temperatures associated with fire. The 140^{O}C treatment was lethal, however germination was observed across all lower tested temperatures. While all the seedlings survived, the 120^{O}C treatment observed extensive damage. Our findings suggest that fire alone may not be a suitable method for killing the seedlings, however it may kill seeds at the soil surface.

Introduction

Fire is an important ecosystem disturbance in grassland communities for removing excess biomass and stimulating the regeneration of vegetation (Price *et al*. 2019; Yan and Liu 2021), which corelates to improved habitat structure for maintaining the diversity of higher trophic levels (Price *et al*. 2019; Beal-Neves, *et al*. 2020). Fire plays an important role in breaking seed dormancy of many grassland species, through the scarification of seed coats (Moreira and Pausas 2012); and/or the triggering of a response to elevated temperature, smoke or charcoal (Franzese and Ghermandi 2010; Hodges *et al*. 2021). Further, fire parameters, such as heat and smoke, are known to increase germination rate and germination uniformity, which increases the vigour of emergence (Zirondi *et al*. 2019; Hodges *et al.* 2021).

Increased urban and agricultural expansion into grassland communities has resulted in the reduction of burn frequencies (Florec *et al*. 2020). As a consequence, the biomass accumulates to increase fuel loads, often resulting in fire intensities that are higher than historically recorded (Price *et al*. 2021). The fire regimes in many grassland communities have also been altered through the introduction and spread of invasive plants, and once established, the invaders are able to alter the fire regimes to facilitate their dominance (Fusco *et al*. 2019). Consequently, if the intensity and/or frequency of fire events are altered, the germination responses for the native species either will not be activated if the intensity and frequency is reduced, or they may be killed by fires that are more frequent or of higher intensity (Fusco *et al*. 2019; Hodges *et al*. 2021). Once established, invasive grass species establish negative feedback loops that alter fire regimes, as well as other environmental parameters, to facilitate their dominance (Suding and Hobbs 2009). Therefore, understanding how a dominant weed responds to fire events can assist in more effective management through the breaking the dominance cycle and reintroducing fire regimes that represent the ecosystems historical state (Price et al. 2021).

Nassella trichotoma (Nees) Hack. is considered one of the most economically damaging invasive plant species in Australia (Campbell and Nicol 1999), New Zealand (Bourdot and Saville 2019) and South Africa (Joubert 1984). This species has high reproductive output, with an individual plant able to produce over 100,000 viable seeds per year, and up to 50,000 seeds per square metre have been recorded in areas with heavy infestations, (Joubert 1984; Campbell and Nicol 1999). Within its native range, a significant proportion of the seeds germinate between autumn and spring, with only approximately 25% of the seeds remaining in the seedbank for longer than one year (Garcia 2021). Within its invasive range, the seeds have been observed to persist in the soil for up to three years (Osmond *et al*. 2008), however the majority of the seedbank is considered transient and germination will occur within the first 12 months (Ruttledge *et al*. 2020: Humphries and Florentine 2022). Research into developing solutions to reduce *N. trichotoma's* seedbank density and longevity, is therefore clearly important for its long-term control.

One such method that merits exploration is incorporation of prescribed burning in areas invaded by *N. trichotoma*. Fire is an important ecosystem process that can selectively influence the recruitment of plant communities, and understanding how the seedbank of exotic plants responds to fire cues has important implications for their management (Mack *et al*. 2000; Franzese and Ghermandi 2010). If the required temperature to flush the seedbank or devitalise the seeds can be determined, managers can then strategically implement fire accordingly (Vermeire and Rinella 2009; Emery *et al*. 2011; Riveiro *et al*. 2019). It is noted that fire intensity is influenced by the interactions between fuel load and soil moisture, and these factors vary seasonally and across the landscape (Bradstock *et al*. 2010; Kreye *et al*. 2013).

High soil moisture often reduces the intensity of fire, while the intensity is increased with dry burns (Kreye *et al*. 2013). Soil moisture also has a direct influence on the moisture content of seeds which lack physical dormancy structures (Tangney *et al*. 2018). Physiological processes within the seed become more active with increasing seed moisture levels (Walters *et al*. 2005), and subsequently, seeds become more susceptible to devitalization when exposed to high temperatures (Tangney *et al*. 2018). It has been observed that high seed moisture contents can reduce the seed's ability to resist fire, demonstrating that lower intensity burns can still provide effective control of invasive plants (Fer and Parker 2005). Therefore, understanding the interactions of seed moisture levels and fire intensity can be important for knowing when to implement strategic burns in weed-dominant areas.

It has been observed that seedling recruitment increases in volume and uniformity when *N. trichotoma* is exposed to fire (Joubert 1984; Hamilton 2012). Fire has also been observed to destroy approximately 18% of this species seedbank (Wells and De Beer 1987). Another adaptive trait seen in fire-prone ecosystems is the ability for established plants to re-shoot rapidly after a fire event (Pausas and Keeley 2014). This trait has been observed in *N. trichotoma*, which is a result of the plant's base being well protected by its dense tussock growth form (Osmond *et al*. 2008). Once established, this weed alters fire regimes for its self-facilitation by producing higher than historic fuel loads through slow nutrient cycling and grazing avoidance (Moretto and Distel 2002). Because high fuel loads negatively affect native seeds and plants during fire events, fire has not been recommended for *N. trichotoma* control programs despite it successfully flushing the persistent seedbank (Osmond *et al*. 2008).

The objective of this paper is to identify the response of *N. trichotoma* seeds of different (i) ages and (ii) moisture content levels to increased time of exposure to radiant heat. The ability of established plants to recover from these factors will also be explored. The findings of this research will provide important information better understand how *N. trichotoma* responds to fire .

Methods

Seed collection and preparation

Seeds were collected from mature *N. trichotoma* plants in Mambourin, Victoria, Australia (37 ^{O} 55' 18.12'' S, 144 ^{O} 32' 43.079'' E), located within the Greater Western Grasslands, located in central

Victoria, Australia (Figure 1) in December 2016, 2017, 2018 and 2019. The seeds were stored within their panicles in zip locked bags at room temperature until use. When the study was about to commence, seeds were removed from the panicles and gently squeezed with forceps to test for seed fill. Seeds that collapsed easily were discarded. Seed mass was determined for each age class weighing 100 seeds for each age group.

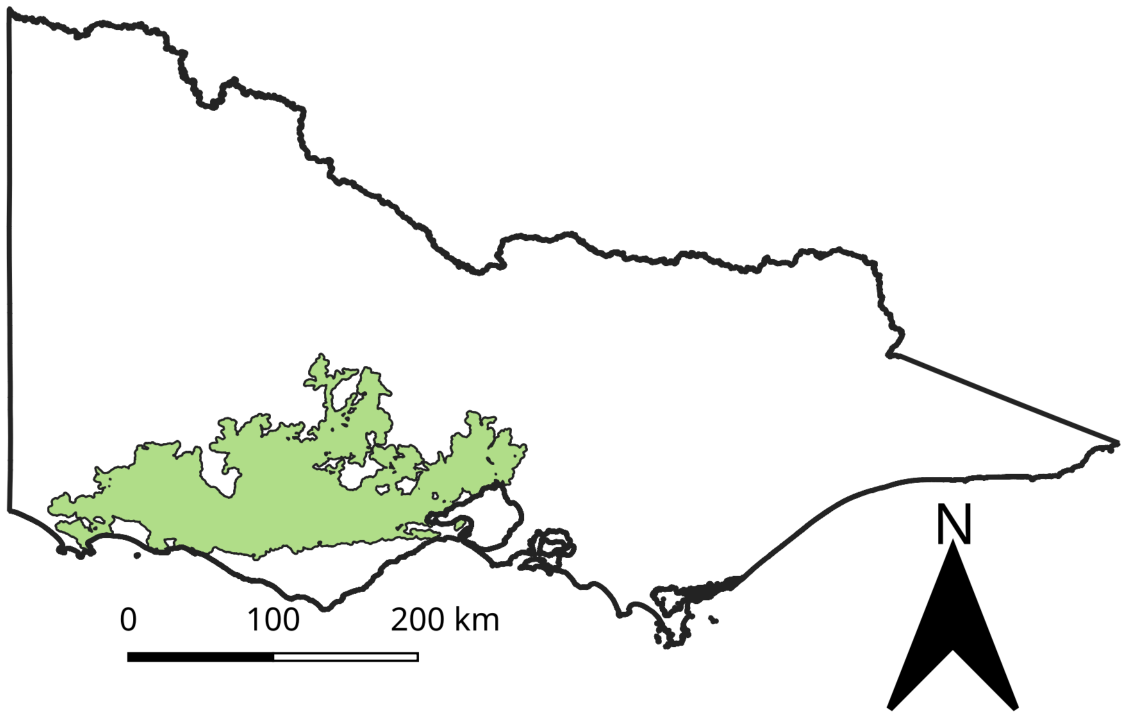

Figure 3: A map depicting the location of the Greater Western Grasslands (in green) within Victoria, Australia.

Effect of radiant heat on seed germinability

For each treatment combination, 25 seeds were counted and placed into aluminium bowls (10cm diameter), and were then exposed to temperatures of either 80 ^{O}C, 100^{O}C, 120^{O}C or 140^{O}C for 1, 3, 6 or 9 minutes inside a temperature-controlled oven (Memmert, Type No. ULE500). This multifactor experiment contains four seed ages, four heat treatments, and four exposure durations for a total of 64 treatment combinations, plus a control for each seed collection year. Each treatment combination was replicated six times.

Immediately following heat exposure, seeds were placed into Petri dishes lined with sterilised filter paper and moistened with sterilized reverse osmosis (RO) water, with parafilm being used to prevent drying. The treatments were placed into incubation chambers set to alternating 25/15^{O}C, 12-hour light and dark conditions, which has been pre-determined as the optimal growth conditions for *N. trichotoma* (Humphries *et al*. 2018). The treatments were checked twice a week for 35 days and germination was determined by protrusion of the radicle.

Effect of moisture content and radiant heat on seed germinability

It was observed that the seed collected in 2016 provided the highest germination across all tested temperatures, and was therefore selected for the rehydration treatments. To prepare for the experiments, nylon mesh bags containing filled seeds (determined by the squeeze tes described previously) were secured within an airtight polycarbonate electrical enclosure box (28 x 28 x 14cm) containing LiCl solutions adjusted to either 15% RH (740g/L), 50% RH (364g/L), or 95% RH (48g/L). The relative humidity (RH) levels were selected as these moisture contents are associated with the three distinct seed hydration levels that have previously been shown to promote different physiological activity within the seeds (Walters, et al., 2005). The boxes were stored at 20^{O}C for two weeks, and seed hydration was assessed at the end of this period using a digital hygrometer. After the rehydration period, the seeds were exposed to the methods previously described for heat exposure and subsequent germination monitoring.

Effect of radiant heat on established plants

To assess the effect of radiant heat on established plants, 100 plastic garden pots (140mm in diameter and 140mm in height) were lined with absorbent towelling, then filled with a fine layer of river sand and then topped with commercially purchased soil. Five viable *N. trichotoma* seeds were placed into each pot and lightly covered with soil. The pots were placed into watertight plastic trays (28cm x 44cm x5.5cm), and were placed into a glasshouse at ambient temperature on the 24th of February 2022. Pots were bottom watered at least once a week and seedlings were thinned to one plant per pot after the first month of growth. The final plants were grown under glasshouse conditions for three months.

The heat treatments were conducted on the 22nd on May 2022. To prepare the plants for the heat exposure, the plant and soil was were carefully removed from the pots and placed into heat-safe aluminium trays. Five plants were randomly selected and exposed to radiant heat of 20 °C, 60 °C, 80 °C, 100°C or 120°C for either 3, 6 or 9 minutes. The plants were then re-potted and monitored for a further two weeks under glasshouse conditions and signs of wilting or recovery were visually observed.

Data analysis

Effect of radiant heat on seed germinability

A three-way ANOVA was conducted using the statistical analysis program SPSS (IBM®) to determine the effect of the year the seeds were collected, temperature, duration of exposure, and all possible interactions of these factors.

In addition to total germination (%), Mean Germination Time (MGT), Germination Index (GI) and Time to 50% Germination (T_{50}), were calculated using the following formulae:

(i) Mean germination time was calculated using the equation:

Mean germination time = ΣDn/ Σn

Where n is the number of seeds, which were germinated on day Dn, and n is the number of days counted from the beginning of germination (Sadeghi *et al*. 2011).

(ii) The germination index was calculated as described in the Association of Official Seed Analysis (AOSA 1993) by following formula:

No. of germinated seeds/day of first count + … + No. of germinated seeds/day of final count

(iii) The time to reach 50% germination was calculated using the formula as described by Kader (2005):

Time taken to reach 50% germination = $ti \frac{\left(\frac{n}{2}-n_i\right)(t_j-t_i)}{n_j-n_i}$

Where N is the final number of germinated seeds, and n_j and n_i are the cumulative number of seeds germinated by adjacent counts at times t_j (day) and t_i, (day), respectively, when $n_i < N/2 < n_j$.

Effect of moisture content and radiant heat on seed germinability

A three-way ANOVA was conducted using the statistical analysis program SPSS (IBM®) to determine the effect of hydration level, temperature, duration of exposure and all possible interactions of these factors. The mean germination (%) was determined for each treatment and Microsoft Excel.

Effect of radiant heat on established plants

A damage score of (i) 0=no damage, (ii) 1=some dryness observed on only the tips of the leaves, or (iii) 2=extensive drying observed to the leaves, was used to assess the condition of the plants. The mean damage score for each treatment was calculated and presented in Table 4.

Results

Climate conditions for each collection year

Variations in the seed mass was observed between the different years the seeds were collected with the highest seed mass recorded for the seeds collected in 2016 (Table 1). In 2016, the average spring maximum monthly temperature was lower than the subsequent years, and the total rainfall was much higher than that observed in the subsequent years, and these factors may have contributed to the higher seed mass compared to the seeds collected in 2017-2019. The higher average spring temperature may have been a leading cause to the reduced seed mass for those collected in 2017, while the low total spring rainfall may have contributed to the low seed mass observed in the seeds collected in 2019.

Table 1: The total rainfall and average maximum temperatures observed for the spring months (September-November) for each year the seeds were collected in the field; 2016-2019 (Data was sourced from Mt Rothwell weather station located approximately 10km from the seed collection site) (Bureau of Meteorology, 2021). The seed mass recorded for each collection year is also presented in this table.

Collection Year	Spring Rainfall (mm)	Average Spring Temperature (^{O}C)	Seed Mass (grams)
2016	178.8	19	0.0709
2017	95.2	21.7	0.0429
2018	76	20.9	0.0641
2019	43.7	20.4	0.0481

Effect of radiant heat on seed germinability

The effect of temperature (p<0.001), duration of exposure (p<0.001), and seed collection year (p<0.001) had a significant impact on *N. trichotoma's* total seed germination (%), as did the interactions of these factors. The control experiment (no heat) demonstrated that seeds collected in 2016 had significantly higher total germination (%) than the seeds collected in more recent years, and the germinability of the seeds appeared to increase with the age of collection, with the total germination (%) increasing with the age of the seeds (Table 2).

Total germination (%) and GI decreased as the temperature gradient increased, while the effect of the duration of the heat exposure had a lesser effect on these factors (Table 2). A similar observation was made for MGT and time to 50% germination, with these parameters increasing when exposed to the higher temperature gradients. The older two seed collections; 2016 and 2017, reached 50% germination sooner than the more recently collected seeds; 2018 and 2019. In most cases, the heat treatments increased *N. trichotoma* germination (%) compared to the germination (%) observed in the control treatment. For all the seed collection times, the 140^{O}C temperature was significant at reducing total seed germination (%), with the exception of only 0.64% of the seeds germinating in the 6- and 9-minute treatments for the seeds collected in 2018. The seeds collected in 2019 observed a significant decline in viability when exposed to 120^{O}C for nine minutes, however this decline was not observed for the other treatments. There are variations in total germination (%) observed for the 80^{O}C, 100^{O}C and 120^{O}C treatments for all the collected seeds, with 2016 having the most consistency.

Table 2: Germination (%) (Ger %), germination index (GI), mean germination time (MGT), and time to 50% germination (T_{50}) after increased exposure to radiant heat for four populations of *N. trichotoma* seeds. The 140^{O}C treatment was not included as germination was insufficient to conduct analysis.

Population	Temp (^{O}C)	Time (min)	Ger (%)	GI	MGT	T_{50}
2016	Control	0	91.33	2.74	9.18	7.62
	80	1	100	3.50	7.36	6.52
		3	94.67	3.20	7.61	6.60
		6	74.67	2.38	9.82	6.76
		9	98.67	3.26	8.06	6.62
	100	1	100	3.08	8.80	7.58
		3	98.67	2.33	11.86	11.19
		6	99.33	2.61	10.74	10.03
		9	99.33	2.32	11.57	11.43
	120	1	84.00	1.33	17.06	16.29
		3	84.00	1.32	18.48	17.19
		6	86.00	1.48	15.14	13.63
		9	86.67	1.28	18.47	14.75
2017	Control	0	78.67	2.05	11.84	7.78
	80	1	78.67	2.32	9.31	7.91
		3	78.00	1.15	12.85	10.64
		6	46.00	2.31	10.28	9.16
		9	85.33	1.91	10.76	8.01
	100	1	68.00	2.06	9.94	8.89
		3	68.00	1.20	9.47	7.79
		6	39.33	0.86	16.16	9.52
		9	31.33	0.76	16.16	14.08
	120	1	34.00	1.23	17.78	16.33
		3	82.00	0.92	18.75	17.49
		6	66.67	1.28	16.80	16.13
		9	80.67	0.77	19.63	17.71
2018	Control	0	75.33	1.28	17.03	13.37
	80	1	59.33	1.15	14.62	13.88
		3	68.00	1.49	13.38	9.78
		6	50.67	0.88	16.84	13.97
		9	72.00	1.55	13.99	12.49
	100	1	68.67	1.32	15.35	14.48
		3	80.67	1.68	13.88	13.48
		6	66.00	1.39	15.15	12.52
		9	75.33	1.35	16.66	13.65
	120	1	74.00	1.12	18.19	16.64
		3	30.67	0.38	20.86	19.50
		6	40.00	0.44	22.27	21.86
		9	58.67	0.62	24.00	22.19
2019	Control	0	53.33	1.09	14.97	13.01
	80	1	62.67	1.23	13.79	11.64
		3	42.67	0.58	19.40	17.24
		6	16.67	0.21	22.14	20.55
		9	65.33	1.22	14.66	12.65
	100	1	44.00	0.48	25.05	22.68
		3	62.00	0.99	19.55	17.94
		6	64.00	1.28	14.42	11.85
		9	70.00	1.40	16.40	13.01
	120	1	68.00	0.85	21.12	18.42
		3	34.67	0.35	27.79	27.63
		6	38.00	0.45	25.20	21.69
		9	8.00	0.07	0	34.50

Effect of moisture content and radiant heat on seed germinability

As the seeds collected in 2016 provided the highest total germination (%) under controlled testing, as well as providing the most uniformed germination (%) across all temperatures, these seeds was selected for the subsequent tests.

The seed moisture content (p<0.001), temperature (p=0.002), and their interaction (p<0.001) were significant factors effecting *N. trichotoma* total seed germination (%). Seeds hydrated to 15% had no significant difference in germination (%) when exposed to 80^{O}C, 100^{O}C or 120^{O}C, and the first two temperatures increased germination (%) when compared to the control (Table 3). For the seeds hydrated to 50%, a significant decline in total germination (%) was observed when the seeds were exposed to 120^{O}C for nine minutes (p=0.002). The exposure to heat was lethal to seeds with moisture contents of 90%, with only 4.6% of the seeds germinating after one minute of exposure to 80^{O}C. No further germination was observed for seeds hydrated to 90%.

Table 3: The germination response of hydrated *N. trichotoma* seeds to radiant heat. The seeds were hydrated to either 15, 50, or 95% and then exposed to one of three temperatures; 80^{O}C, 100^{O}C, and 120^{O}C, for 1, 3 6, or 9 minutes. The control treatment of no heat or hydration pre-treatment and recorded 91.33% total germination.

Hydration	Temperature (^{O}C)	Duration (min)			
		1	3	6	9
15%	80°C	90.66	96	96	94
	100°C	99.33	100	98.66	97.33
	120°C	97.33	90.66	90.66	90.66
50%	80°C	100	96	90.66	98
	100°C	99.33	94.66	96	98.66
	120°C	88	88.66	88.66	81.33
95%	80°C	4.6	0	0	0
	100°C	0	0	0	0
	120°C	0	0	0	0

Effect of radiant heat on seedlings

All the plants exposed to the temperatures 20^{O}C and 60^{O}C were undamaged, and the leaves remained a vibrant green from the base to the tip. In the higher temperatures tested; 80^{O}C, 100^{O}C, and 120^{O}C, notable drying of the leaves was observed, but the base of the plant remained undamaged. Four plants exposed to the 120^{O}C treatment (two exposed for six minutes, and two exposed for nine minutes) suffered extensive drying, but these plants were observed to be re-sprouting from the base. Figure 2 shows the visual differences between the damaged scores of 1 (undamaged), 2 (leaf drying) and 3 (extensive leaf drying and re-sprouting). No plants were completely killed from the trialled heat treatments.

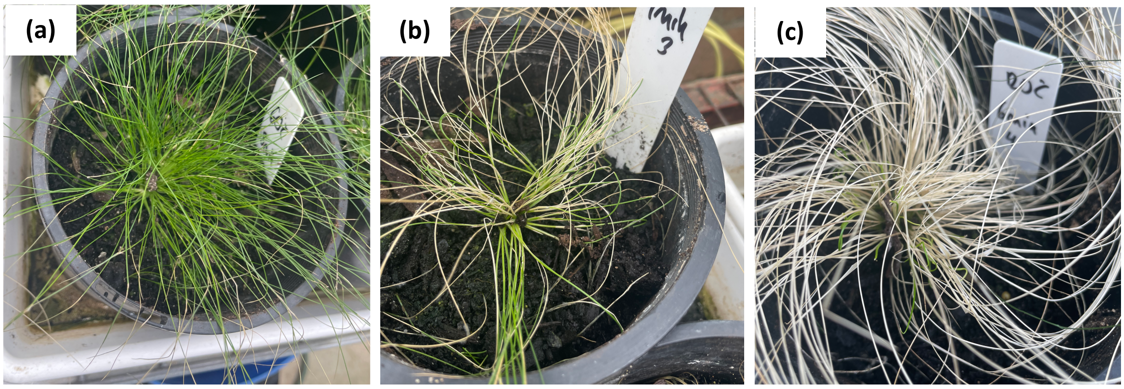

Figure 2: Photos demonstrating the effect of heat on seedlings. Photo (a) shows the leaves remaining completely green after exposure to 60^{O}C, photo (b) shows some drying to the leaves after exposure to 100^{O}C, and photo (c) shows re-sprouting at the base after exposure to 120^{O}C.

Discussion

Effect of climate parameters on seed development and germinability

The seeds collected in 2016 had greater seed mass than those collected in the subsequent years. Increased seed mass can improve total germination (%), germination time, and improve the success of the seedling's survival (Bonfil 1998; Cordazzo 2002). The results of this study support that greater seed mass increases total gemination (%) and germination time compared to seeds with a reduced mass. The various environmental factors, such as temperature and rainfall, that a plant experiences during its seed development stage can directly influence the progeny seeds dormancy and germination potential (Penfield and McGregor 2017; Souza *et al*. 2019). Plants exposed to higher rainfall and soil nutrients will often allocate more resources to reproduction, resulting in higher seed output and seeds of greater mass and size (Salisbury 1943; Lazaro-Nogal *et al*. 2015; Souza *et al*. 2019). The substantially higher spring rainfall experienced in 2016 compared to that observed in the following years, may therefore, account for the higher seed mass observed in these seeds.

Studies into *N. tricotoma's* seed longevity under field conditions suggest that the seeds will germinate within the first year of seed set, with only small proportion (<10%) demonstrating short-term persistence of up to three years (Ruttledge *et al*. 2020; Humphries and Florentine 2022). Under stored conditions, however, 20-year-old seeds of this species have been observed to germinate successfully (Taylor 1987). This suggests that it is unlikely that the age of the seeds influenced their germinability, rather the differences in seed mass across the different seed collection years better explains these variations. This is supported by the literature that often reports the inverse, whereby seed viability has been shown to wither as the seeds age under controlled storage conditions (Franzese and Ghermandi 2010; Purbey *et al*. 2018).

Another factor to consider that may have influenced the variation in germinability across each collection year is different degrees of dormancy. It is known that *N. trichotoma* experiences a primary dormancy period that extends for approximately three months, which prevents the seeds germinating in the summer, water-limited months. This species has also demonstrated the capacity to undergo secondary dormancy, which is induced when the seeds are exposed to prolonged unfavourable conditions, even after the primary dormancy period has ended (Buijs 2020).

Effect of radiant heat on seed age

Nassella trichotoma seeds demonstrated a high level of resistance to the tested radiant heat treatments, with seeds collected from three of the four years maintaining high total germination (%) at 120^{O}C. The only exception was seen in the seeds collected in 2019, where the seeds were significantly reduced (p=0.002) by nine minutes of exposure to 120^{O}C treatment. For the seeds collected in 2016, exposure to 80 and 100^{O}C increased total germination (%) compared to the control. The seeds collected in 2017-2019 also observed an increased total germination (%) compared to the control.

Mean germination time indicates the lag period between seed imbibition to radicle protrusion where the seed undergoes repairs from damages caused by the environment or seed aging (Mavi *et al*. 2010). In the present study, *N. trichotoma* seeds decreased MGT after exposure to the increasing temperature gradient, particularly when the exposure time was short. Longer duration of exposure to the temperature gradient resulted in an increase in MGT, indicating these seeds required time to repair prior to germination.

The ability for a species to germinate with greater speed and uniformity increases its competitiveness following fire (Hodges *et al.* 2021). Mean germination time can be used as an indicator in determining field emergence, with increased MGT being correlated to lower and less vigorous field emergence (Matthews and Khajeh-Hosseini 2006). Whilst this could indicate that high intensity fire that heats the soil to 120^{O}C or above could damage the seeds enough to reduce competitive emergence in field, this would, however, be subjective to the fire response of the native species in the corresponding seedbank (Hodges *et al*. 2021). Time to 50% germination followed the same pattern as observed for MGT, whereby germination rate slowed as the temperature gradients and duration of exposure increased.

The 140^{O}C treatment proved to significantly devitalize the seeds, with the exception of one seed germinating in each; the six- and nine-minutes exposure treatments (0.64%) for the seeds collected in 2018. This temperature has been observed to be lethal in other awned grass seeds, such as *Piptochaetium napostaense* (Kin *et al*. 2016). Seed awns are an important adaptive trait for seed survival in fire prone ecosystems, and increased fire intensity has been observed to select for awns with increased length, as this allows for the seed to burrow further into the soil to escape lethal temperatures (Garnier and Dajoz 2001). The hygroscopic awn on *N. trichotoma* seeds allows it to burrow into the soil profile, with the majority of this species seedbank found buried at approximately 2.5cm depth (Joubert 1984). The effect of shallow seed burial has been observed to alleviate the severity of heat shock from fire events, and temperatures 2cm below the soil surface rarely exceed 80^{O}C (Penman and Towerton 2008). A controlled study demonstrated that surface temperatures that reach 500^{O}C are reduced to approximately 70^{O}C at 2cm depth (Girona-Garcia *et al*. 2015). While another study showed a significant reduction in temperature (approximately 60%) at a depth of 1cm, and increased soil moisture further reduced the transfer of heat into the soil profile (Valette *et al*. 1994). It is therefore, unlikely that the majority of *N. trichotoma* seeds that have burrowed into the soil will be exposed to temperatures that exceed its lethal temperature threshold.

Effect of radiant heat on hydrated seeds

The moisture levels of vegetation and the soil influence the intensity of prescribed fire, with the highest intensity fires observed under low moisture levels. Soils with high moisture content decreases the thermal energy of the fire (Valette *et al.* 1994), and this is due to (i) a reduced rate of fuel consumption (Marino *et al*. 2012), and (ii) water in the soil dissipating as steam (Stoof *et al*. 2011). The soil moisture levels strongly influence the moisture level of the seeds that reside within the subsequent soil seedbank, particularly those lacking physical dormancy structures (Dasberg 1971; Josiah *et al*. 1994). *Nassella trichtoma* seeds possess no such structures and can readily take up water when it is available in the soil (Campbell and Nicol 1999).

Germination (%) for the seeds hydrated to 15 and 50% experienced no significant changes in germination (%) compared to the control treatment. The seed hydrated to 95% experienced a significant decline in germination (%) at all tested temperatures. The literature suggests that seeds hydrated to 95% are killed by much lower temperatures than those with lower moisture contents (Ruprecht *et al.* 2016; Tangney *et al*. 2018), such as 15%, or even 50% as observed in the present study. As seed moisture content increases, so too does the seeds physiological activity (Walters *et al*. 2005). When the seeds are hydrated above 90%, there is high free water content within the seeds that can be heated to a temperature that damages cellular processes, and subsequently stopping the physiological processes that initiate the germination process (Tangney *et al*. 2018). Also, proteins associated with protecting the seed while it is in a dormant state, including the synthesis of small heat shock proteins, ceases to provide adequate protection at high seed moisture levels as they do when the seed is in a dry and dormant state (Wehmeyer *et al.* 1996).

Effect of radiant heat on seedlings

Prescribed burning has been implemented effectively for weed control programs and ecological restoration of grasslands (Assis *et al*. 2020; Mainardis *et al*. 2020). While it is known that adult *N. trichotoma* plants are able to resist high intensity fire, our results suggest that this resistance is also demonstrated in three-month-old seedlings. The *N. trichotoma* seedlings resisted all the temperatures and duration of exposure times tested in this study, and no plants were killed up to two weeks post heat exposure. Exposure to the 120^{O}C treatment caused extensive drying to the leaves, and in some cases, the plant was seen to re-sprout from the base. The ability to re-sprout rapidly following a fire is critical for maintaining competitive dominance, and ensuring survival of the population as the seedbank may have been depleted following a fire through either increased seed germination or devitalization (Tangney *et al*. 2020; Hodges et al. 2021).

This study demonstrated that *N. trichotoma* can withstand high temperatures related to fire. As this species produces unpalatable, sclerophyllous leaves, that allow it to contribute a large volume of dry biomass to the grasslands it invades (Distel 2020). This allows for this species to increases the intensity of fire regimes and successfully displace native plants. It would be recommended to use fire integrated with other control actions such as; broadcasting competing seeds, herbicide, or controlled grazing (Distel 2020) to improve its efficacy in grassland ecosystems.

Conclusion

In this paper we explored the response of *N. trchotoma* seeds and seedlings to fire through controlled radiant heat exposure trials. Whilst the seeds were able to survive temperatures of 120^{O}C, the majority of the seeds were killed when exposed to 140^{O}C. This suggests that only seeds at the soil surface, or within the top 1cm, could be killed by hot fire, as these temperatures are alleviated to favourable temperatures for spiking germination (approximately 80^{O}C) below this depth. The MGT and T_{50} increased under higher temperature gradients and duration of exposure, suggesting these treatments caused intracellular damage, while the delayed germination was due to the seeds requiring time to repair. The seed mass for each collection year appeared to be a key indicator of germinability, with higher seed mass producing the highest germination (%). Implementing fire with increased moisture levels may not be effective for managing this species, as no change in germination (%) was observed for seeds pre-treated to 50% hydration. Almost all the seeds were killed when hydrated to 95%; however, achieving effective heat penetration into the soil profile at this soil moisture level poses its own challenges. Lastly, whilst no seedlings were killed by the applied treatments, the 120^{O}C treatment did result in extensive drying to the leaves and re-sprouting was observed.

It has been observed that rapid seedling recruitment from the seedbank has been observed for *N. trichotoma* following fire events, the results of the present study suggest that a moderate heat fire will reduce time to 50% germination, which supports these in field observations. For fire to be used effectively to kill *N. trichotoma*, it should be implemented alongside other control actions.

Conflicts of Interest

The authors declare no conflicts of interest

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Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

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Chapter 7. Research synthesis, management implications, and recommendations for future research

Chapter 7. Research synthesis, management implications, and recommendations for future research

This final chapter summarises the major findings discussed in the previous research chapters. In this reflective work, the outcomes of the investigation have been synthesised, and findings which can be linked between the chapters are further discussed. To provide a concluding focus to the study, the key findings from each section have been used to propose a suite of suitable management recommendations for *Nassella trichotoma* control, and also to outline emerging opportunities for future research. In particular, implications for grassland restoration are proposed, with a focus on the state and transition of the study site (Little Raven North), from before and after this research. The findings must be interpreted with some caution, as the condensed time-frame that the studies were conducted under, do not qualify this work as being representative of long-term management outcomes. However, it is suggested that these outcomes confidently show the early stages of succession as a result of the application of systematically designed experimental treatments.

Implications for *Nassella trichotoma* management

The research presented in this thesis has made a significant contribution to knowledge regarding the seed ecology of *N. trichotoma*. Of particular interest, it was determined that the seeds are short-lived, with the majority of the seedbank persisting for only one year or less (Chapter 5). This suggests that preventing seed set for one year will significantly reduce the seedbank density of this weed, this outcome being observed in all the treatments implemented in Chapter 4. This validates the proposition that developing co-ordinated, landscape-scale management plan for this weed is so important. It is important to emphasise in this respect that this species has ecological traits that significantly enhance effective seed dispersal (Osmond et al. 2008), thus any untreated *N. trichotoma* patches can quickly migrate into treated areas (Campbell and Vere 1995), a phenomenon which needs to be carefully considered during weed management.

In terms of developing a suitable management plan, it is recognised that the seeds of *N. trichotoma* were observed to resist fire related temperatures of up to 120^{O}C (Chapter 6). It is surmised that as the seed-fall is able to burrow into the soil profile (Badgery et al. 2008), the majority of the seedbank will be protected from lethal temperatures due to the insulating ability for the soil to alleviate the thermal impact (Penman and Towerton 2008). Further, the effect of exposure to elevated temperatures did not significantly enhance germination (%) or uniformity (Chapter 6), and therefore it is unlikely that a fire event will trigger a germination response in this species, despite the literature observing high volume germination of this species post-fire (Joubert 1984; Hamilton 2012). In this latter respect, it is likely that increased germination under field conditions after fire is, instead, related to a decrease in competition (Moretto and Distel 1997). The strategy of attempting to implement fire as a control tactic against *N. trichotoma* thus should incorporate the spread of native seeds soon following the fire. This suggestion comes from the observation that a delay in germination (increased T_{50}) was observed for *N. trichotoma* seeds exposed to increasing temperatures and exposure durations (Chapter 6), which allows a brief window of opportunity for broadcast species to germinate and become established while the *N. trichotoma* seeds repair any intracellular heat-related damages (Mavi et al. 2010).

The results of Chapter 6 suggest that implementing fire in different seasons catalyses different seed mortality rates. It was observed that seeds exposed to 140^{O}C were killed after exposure of just one minute, and also seeds were killed by all the tested temperatures with in the presence of high seed moisture content (95%). Consequently, if land managers wish to use fire in the attempt to devitalise the seedbank, it is recommended that either; (i) the fire is implemented in Summer in order to generate a very hot burn, where surface soil temperatures an reach up to 140^{O}C or more, or alternatively, (ii) the fire could be implemented soon following a rainfall event, when the seeds are in a fully hydrated state. Notwithstanding this logic, achieving these outcomes, particularly burning in conditions with high soil moisture, may prove to be difficult in a practical setting (Valette et al. 1994). Another recommendation is to implement fire in Spring, in order to burn off emerging flower heads to prevent additional seed entering the seedbank. This is done rather than just implementing fire to purely target the existing seedbank. Indeed, as the seedbank was determined in Chapter 5 to be transient, it is suggested that this strategy is likely to produce a more effective management outcome.

The results of this thesis clearly indicated that competition from the native broadcast species proved to be the most significant factor for controlling the re-establishment of *N. trichotoma,* post fire treatment (Chapter 4). It has been well documented in the literature that *N. trichotoma* is not competitive, either in areas of high competition (Moretto and Distel 1997), or in shady conditions (Campbell 1998). The cover of invasive grasses (including *N. trichotoma*) was significantly less in the fire-treated plots where the native species were observed to shown to significantly increase when compared to the unburnt treatments (Chapter 4). The key findings and the associated implications for management, are further discussed in Table 7.1.

Table 1. This table describes the key research outcomes found in the body of work, and what management implications can be drawn from these findings in relation to *N. trichotoma* control.

Research Outcomes	Management Implications
Nassella trichotoma seeds persist in the seedbank only up to one year.	Preventing fresh annual seed set from *N. trichotoma* is critical for the reduction of the soil seedbank. If this can be achieved, recruitment from the seedbank can be significantly reduced. In Chapter 4, it was demonstrated that applying glyphosate to the adult grass in early-mid Spring prevented seed production, and this resulted in a significant decline in seedbank density in the two subsequent seedbank surveys.
Fire can kill *N. trichotoma* seeds if the intensity is great enough to heat the soil to 140^{O}C.	Land managers wanting to incorporate fire into sites with high densities of *N. trichotoma* must achieve significant heat intensity in order to kill the seedbank. The combination of herbicide and fire used in Chapter 4 observed a high intensity burn as (i) the herbicide applied in early Spring allowed for the dried, dead *N. trichotoma* biomass to ignite with ease, and (ii) the burn was conducted in the early Summer season where untreated vegetation was also beginning to dry. As the literature suggests that most of the seeds remain within the top 2cm of the soil profile, if temperatures of 140^{O}C can penetrate the soil to this depth, the seedbank should effectively be killed.
Fire related temperatures below the lethal 140^{O}C did not significantly improve germination rate, uniformity or percentage.	Fire has long been excluded from *N. trichotoma* management programs as it has been reported to spike a mass germination event from the seedbank. The results in Chapter 6 did not reflect this, rather the effect of fire (in terms of radiant heat exposure), below that of the lethal heat, slowed germination time (T_{50}). This suggests that there was a delay in germination while the seeds repaired damages caused by the elevated temperatures. While other factors could be related to the spike in field germination after a fire event, such as reduced competition, or a germination response to smoke and char, the effect of fire temperature alone did not enhance germination uniformity. This could indicate that broadcasting seeds immediately following a burn could allow for these seeds to germinate under low competition and subsequently establish with higher success.
High seed moisture significantly increased sensitivity to fire related temperatures.	The findings of Chapter 6 also indicated that *N. trichotoma* seeds hydrated to 95% were highly sensitive to fire related temperatures. All temperatures trialled effectively killed the seeds that were subjected to this re-hydration pre-treatment. In this regard, fire implemented soon following a rainfall event could provide effective control of the seedbank, even if the burn is at a lower intensity. The challenge associated with this is moist soil can alleviate the heat effect of fire,

	and this could prevent the soil reaching a temperature that would prove lethal.
Established *N. trichotoma* seedlings were not effectively killed by fire related temperatures.	Chapter 6 demonstrated that established *N. trichotoma* seedlings demonstrated high tolerance to fire-related temperatures. Another factor as to why fire has not widely been used in *N. trichotoma* management programs is due the ability of this weed to re-shoot rapidly from its base following a fire event. In the field study, this was observed first hand, with *N. trichotoma* plants located outside of the plots regenerating within two weeks of the fire treatment. Due to this factor, management programs that include fire in *N. trichotoma* dominated landscapes must pre-treat all the *N. trichotoma* plants with a herbicide treatment in order to prevent their regrowth.
Broadcasting native seeds after treating *N. trichotoma* with herbicide and fire reduce the re-establishment of the weed.	The combined treatment of herbicide and fire applied directly to *N. trichotoma* allowed for the significant increased rate of establishment of the native broadcast species. In the unburnt plots, these grasses failed to establish in competitive densities. The establishment of the broadcast species appeared to reduce the re-establishment of *N. trichotoma* after four years post treatment.

Implications for grasslands management

In order to select suitable management strategies for grassland restoration programs, it is imperative to determine the processes that are facilitating the weed dominant degraded state. In the case of the study site and many similar grasslands in south-east Australia, the impacts of over-grazing, urban expansion and invasive weed species, as demonstrated in Figure 7.1, are some the most prevalent factors that have pushed these important grasslands into a low-functioning and degraded state (Marshall 2021; Victorian National Parks Association 2022). These describe disturbances which directly impact on the competitive cover of perennial native grasses, which are then displaced by the invaders (van Klinken and Friedel 2018). Land managers should be cautious when continuing grazing during the flowering season of keystone native perennial grasses, as the emerging flower heads can be removed (Agriculture Victoria 2022). This leads to a decline in native seedbank density, which not only reduces the resilience of the native species, it reduces the competition for emerging invasive grass seedlings (van Vreeswyk et al. 2004). Additionally, grazing from livestock has been observed to alter the soil chemistry and composition, often increasing soil compaction, and again, these factors tend to favour the establishment of invasive species (Alves et al. 2019; Jordon 2020).

This process facilitates a negative feedback loop where the *N. trichotoma* is undisturbed by grazing due to low palatability, and is therefore more able to produce greater biomass and contribute higher densities of seeds to the seedbank, which ultimately displace the native grasses (Distel et al. 2007). As a consequence of urban expansion into the Greater Western Grasslands, the natural landscape is fragmented to a degree whereby native plant propagules can no longer naturally migrate into areas where the seedbank has been depleted (Standish et al. 2009). At the study site, the depletion of native grass cover forced the cessation of grazing practices and consequent land abandonment. This study site was later acquired by the local government for conservation purposes, where minimal management action has been implemented to return the depleted ecosystem services.

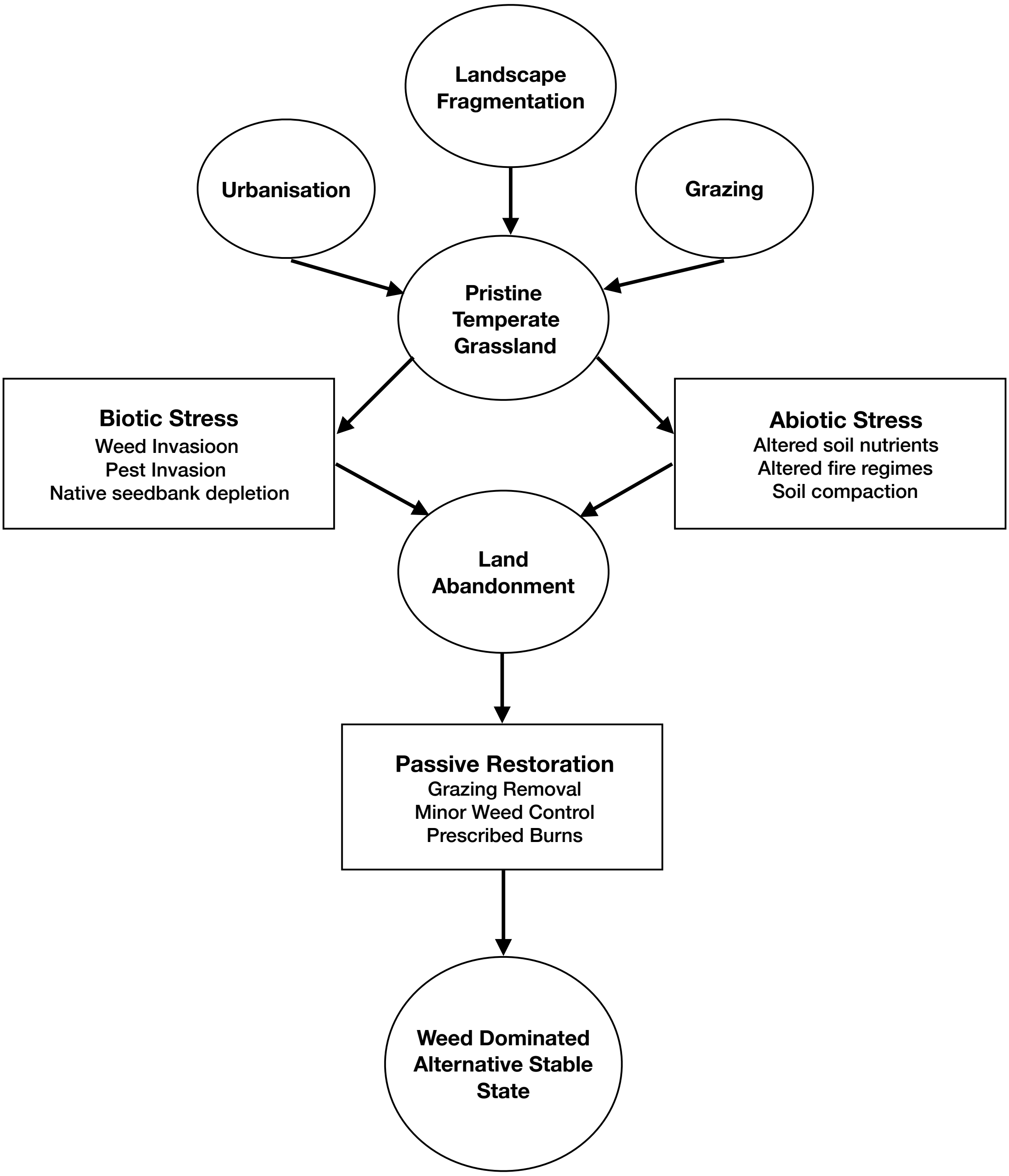

Figure 1. The conceptual model describes the pathway of degradation for Little Raven North.

The findings of this thesis, particularly those indicated in Chapter 4, suggest that the combined treatment of herbicide sprayed directly onto *N. trichotoma*, followed by burning the dried biomass with a hot, summer burn and then broadcasting native grass seeds, could improve the habitat value at the study site (Figure 7.2). After the implementation of this treatment, native grass cover improved from 7.3% to 60%, while the untreated plots were observed to have less than 1% of native grass cover. Whilst it cannot be determined after only four years if these plots treated with herbicide, fire and broadcast seeds will achieve a restored state, the trajectory of the community, however, appears to be moving in the right direction.

The results obtained from the studies conducted in Chapter 4 demonstrated that the inclusion of fire was critical to improve the establishment rates of the broadcast species. This is likely due to decreased above-ground cover and less competition from the soil seedbank, as it was determined in Chapter 6 that heat associated with high fire intensity, where soil temperatures reach >140^{O}C, effectively kill *N. trichotoma* seeds. Whilst the two C_3 grass species used for this study were effective at providing competition against *N. trichotoma*, as only two species were utilised, the question of biodiversity remained low for native grasses. In total, only nine native grass species were surveyed over all of the sampling periods. It would be clearly beneficial to include a wider diversity of native seeds into the broadcast seed-mix, including C_4 perennial grasses to provide competition in summer growing seasons, as well as a mixture of locally sourced forbs and herbs. Broadcasting a greater diversity of natives can provide year-round competition of invasive species, as different growing seasons can be accounted for (Foster et al. 2007; Waller et al. 2013). It also diversifies the habitat, which can attract pollinators and other higher order species (Orford et al. 2016; Mizsei et al. 2020). The herbicide, fire and broadcasting seeds treatment resulted in a significant improvement in terms of habitat value compared to the untreated plots. It would be recommended that this treatment combination be trialled at a larger scale, together with the monitoring of the effects of this treatment beyond four years, in order to make more definitive management recommendations.

This state and transition model in Figure 7.2 shows the impact of spraying herbicide on *N. trichotoma*, followed by the implementation of a controlled burn and the broadcasting of native seeds on the structure and function (habitat value) of this study site (Chapter 4). In State 1, the site had a very low abundance of native cover and a very high cover of invasive grasses. The implementation of herbicide and fire moved the treated plots into an intermediate state, where both native and invasive grass cover was low. In State 3, after the addition of native seeds, invasive grass cover had reduced from 44.4% to 33.5%, while the native grass cover occupied over half (60%) of the plots. This demonstrates a state shift from low to moderate habitat value.

State 1 (before treatment)

Species	Treatment	Abundance	Habitat Value
Native grass	NT	4.8%	Low
	HFS	7.3%	Low
Invasive grass	NT	56.4%	Low
	HFS	44.4%	Low

+ Herbicide

+ Fire

State 2 (after fire)

Species	Treatment	Abundance	Habitat Value
Native grass	NT	2.25%	Low
	HFS	14.6%	Low
Invasive grass	NT	64.3%	Low
	HFS	7.7%	Moderate

+ Broadcast seeds

State 3 (final)

Species	Treatment	Abundance	Habitat Value
Native grass	NT	0.1%	Low
	HFS	60%	Moderate
Invasive grass	NT	81.7%	Low
	HFS	33.5%	Moderate

Figure 2. State and transition model for herbicide, burn and seed broadcasting treatment

Recommendations for further research

This research has exposed important knowledge gaps in our understanding of the biology of *N. trichotoma.* It would therefore seem that if further directed research is undertaken, results could improve the management of *N. trichotoma* and grassland communities where this weed is present. These elements of directed research could include:

1. Further investigations into if the use of a more diverse seed mix, in order to provide more effective, year-round competition against *N. trichotoma* as well as other common grassland weed species. Trialling seeds mixes that account for Summer and Winter-growing native perennial grass species, as well as the inclusion of locally sourced herbs and forbs, may also diversify habitat structure. This study identified that native seed diversity is very low in degraded areas of the Greater Western Grasslands, therefore, studying the effects of incorporating higher diversity in the seed mix could have important implications in improving restoration outcomes.
2. Our findings indicated that the herbicide, fire and seed broadcasting treatment was successful for achieving a reduction in *N. trichotoma* cover, while increasing the rate of establishment for the native species at a plot scale. However, future research on the effect of this treatment combination at a large paddock or at a landscape scale, would provide insight into the real-world implications of this approach.
3. Whilst this research was focused on grassland restoration, further research into the adaption of these methods to an agricultural system could broaden the implications of this management strategy. This would include the use of pasture grass species instead of relying only on native species. In this type of system, fencing may be a significant management tool. Also, the comparative cost for implementing various treatments, such as the cost of tillage versus the cost of fire in an agricultural system, is likely to differ. Therefore, further research is required in order to establish suitable management implications for these systems.
4. Research trials that explore the effect of the explored treatments at sites interstate and internationally could be beneficial, as it is suspected that variations in climate and species (including biotypes) may influence the management approaches.
5. The seedbank for this species was observed to persist for one year or less. This indicates that re-emergence of *N. trichotoma* in a treated site after one year is likely a result of the seeds migrating to a site through anemochory dispersal, contaminated cars or machinery, or water runoff. Research into technologies that could limit the movement of *N. trichotoma* seeds, such as the effect of various fencing types, would assist in keeping treated areas free of reinvasion.

6. It may prove beneficial to test the impact of high heat under different soil moisture conditions, as our research identified seeds with high moisture content to be highly sensitive to fire-related temperatures.
7. It is known that invasive species, such as *N. trichotoma*, are fast to adapt to a new environment, and take advantage of disturbance events. Under climate change parameters, environmental disturbances are likely to increase, which may favour *N. trichotoma*. Further, the growth and reproduction rate of invasive C_3 grass species has been observed to increase under projected elevated CO_2 concentrations. Understanding how *N. trichotoma* is likely to respond to these various climate change parameters, could assist in long-term management programs for targeting this species and for identifying areas at risk at a local, national and global scale.
8. Research into community engagement through marketing and various communication platforms is required to improve the implementation of weed management programs. It is likely that, due to the ecology of *N. trichotoma*, control methods should be conducted in parallel by the whole local community in order to prevent reinvasion.

Conclusion

This thesis began by asking the question: *Can long-term restoration of a degraded temperate grassland be achieved by targeting the above and below ground density of the dominant weed?* As a result of this investigations, the thesis findings suggest that targeting a dominant weed, such as *N. trichotoma* in a degraded grassland area can, at least, start the recovery process. It was further observed that the reduction in *N. trichotoma* and other invasive grass species cover was correlated to the rate of establishment of native broadcast grass species. As a consequence, the key messages to land managers attempting to restore a *N. trichotoma* dominated grassland are to; (i) prevent seed set every year, and (ii) integrate herbicide and fire strategies to establish competition through the introduction of native plant propagules.

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