DETERMINING THE TRAJECTORY OF GRAMINOID INVASIONS IN SOUTHERN AFRICA'S MOUNTAINS: THE CASE OF *NASSELLA*

ANTHONY MAPAURA

2018553921

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy: Botany (BOTA9100)

in the Department of Plant Sciences, Faculty of Natural and Agricultural Sciences, University of the Free State, Qwaqwa

JUNE 2022

SUPERVISOR: Dr. S-L. Steenhuisen

CO-SUPERVISORS: Dr. K. Canavan, Dr. V.R. Clark, Prof. D.M. Richardson

DECLARATION

I, **Anthony Mapaura** declare that the doctoral thesis that I herewith submit at the University of the Free State, is my independent work and that I have not previously submitted it for qualification at another institution of higher education.

Anthony Mapaura, May 2022

I, **Anthony Mapaura**, declare that I am aware that the copyright is vested in the University of the Free State.

Anthony Mapaura, May 2022

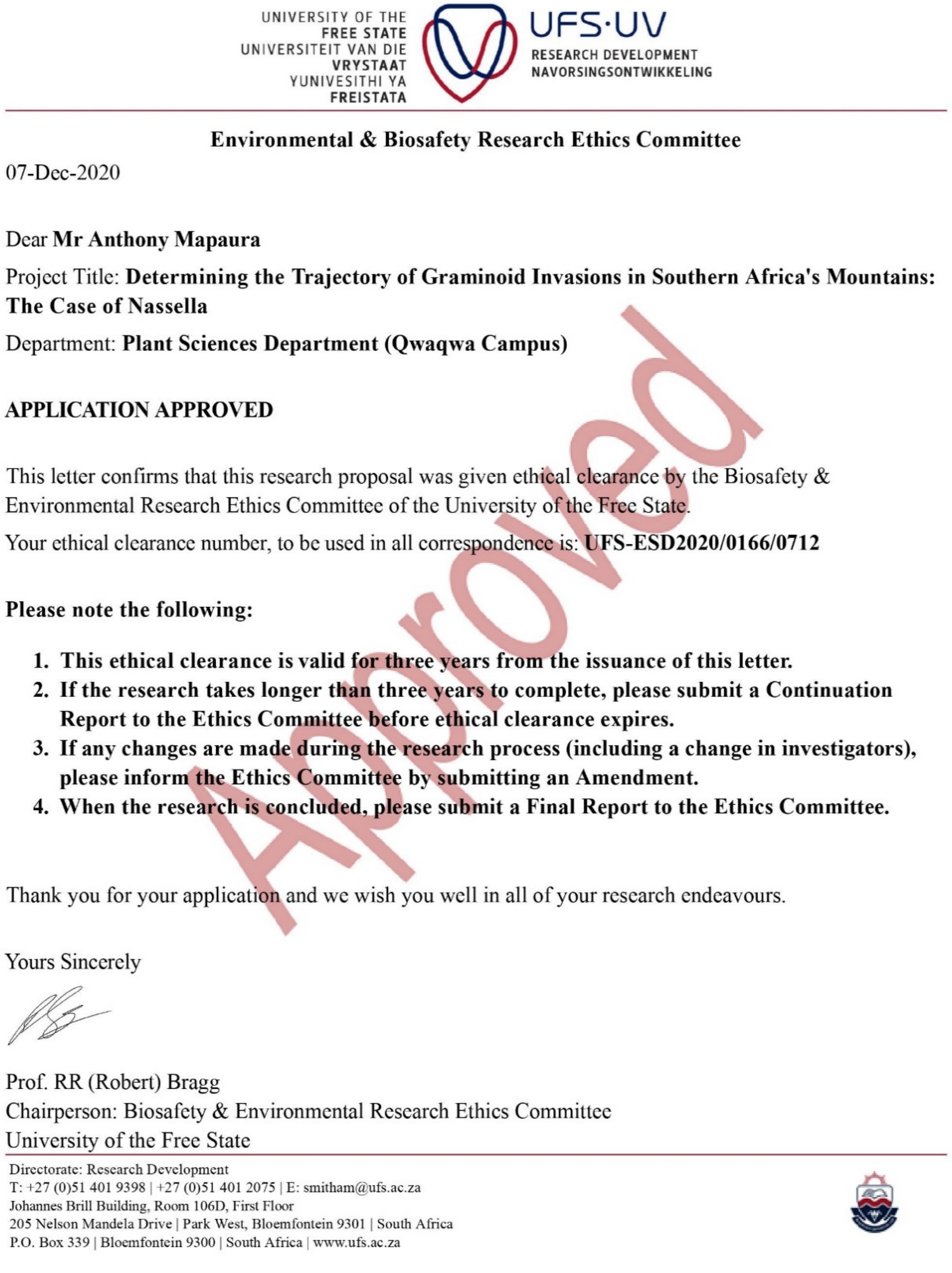
I, **Anthony Mapaura**, declare that all royalties as regards to intellectual property that was developed during the course of and/or in connection with the study at the University of the Free State, will accrue to the University.

Anthony Mapaura, May 2022

I, **Anthony Mapaura**, declare that I am aware that the research may only be published with the Dean’s approval.

Anthony Mapaura, May 2022

ETHICAL CLEARANCE



UNIVERSITY OF THE
FREE STATE
UNIVERSITEIT VAN DIE
VRYSTAAT
YUNIVESITHI YA
FREISTATA

UFS·UV
RESEARCH DEVELOPMENT
NAVORSINGSONTWIKKELING

Environmental & Biosafety Research Ethics Committee

07-Dec-2020

Dear **Mr Anthony Mapaura**

Project Title: **Determining the Trajectory of Graminoid Invasions in Southern Africa's Mountains: The Case of Nassella**

Department: **Plant Sciences Department (Qwaqwa Campus)**

APPLICATION APPROVED

This letter confirms that this research proposal was given ethical clearance by the Biosafety & Environmental Research Ethics Committee of the University of the Free State.

Your ethical clearance number, to be used in all correspondence is: **UFS-ESD2020/0166/0712**

Please note the following:

1. **This ethical clearance is valid for three years from the issuance of this letter.**
2. **If the research takes longer than three years to complete, please submit a Continuation Report to the Ethics Committee before ethical clearance expires.**
3. **If any changes are made during the research process (including a change in investigators), please inform the Ethics Committee by submitting an Amendment.**
4. **When the research is concluded, please submit a Final Report to the Ethics Committee.**

Thank you for your application and we wish you well in all of your research endeavours.

Yours Sincerely

Prof. RR (Robert) Bragg
Chairperson: Biosafety & Environmental Research Ethics Committee
University of the Free State

Directorate: Research Development
T: +27 (0)51 401 9398 | +27 (0)51 401 2075 | E: smitham@ufs.ac.za
Johannes Brill Building, Room 106D, First Floor
205 Nelson Mandela Drive | Park West, Bloemfontein 9301 | South Africa
P.O. Box 339 | Bloemfontein 9300 | South Africa | www.ufs.ac.za

Approved

ACKNOWLEDGEMENTS

I am deeply grateful to Dr. Sandy-Lynn Steenhuisen, Dr. Kim Canavan, Dr. Ralph Clark, and Prof. Dave M. Richardson, who were excellent supervisors and believed in me even when I felt things were not going well. They freely shared their enormous wealth of knowledge with me and made resources easily available to me when I needed them. They welcomed me into their homes and were readily available when I needed them, even for a casual chat.

Hearty thanks to Steven and Mitsy Sylvester and Rob Soreng for generously sharing their Maloti-Drakensberg data and flagging an undetected, large population of *N. neesiana* in Harrismith and for including me on their fieldwork. Their mentorship during the fieldwork was very valuable. I would like to thank David Currie, Dave Davenport, Danie and Phillip Erasmus, Gideon Gravenstein and Kitty and Richard Viljoen for providing valuable insights into the *Nassella* problems in the Eastern Cape province and allowing access to their properties. Rohan Kruger (Game Works Helicopters) shared valuable insights on the spread and aerial control of *Nassella trichotoma*. Maryke Stern facilitated contact with farmers with invaded farms in the Eastern Cape. Sincere thanks are due to Lyn Fish and Aluoneswi C. Mashau for their assistance with aspects of identification and providing literature. Nicholas Le Maitre provided helpful insights on the potential use of DNA techniques for segregating *Nassella* species and for assistance with DNA extraction. João de Deus Vidal Jr provided guidance on Species Distribution modelling. Guy Sutton's assistance with mixed effect modelling is greatly appreciated. Many thanks also to Dr Thabiso Mokotjomela for the many discussions we had and the literature he provided. Dr Grant Martin is thanked for his help at Rhodes University with networks and greenhouse assistance. I would also like to thank the internal journal club for the numerous critical discussions we had. They helped me develop critical thinking. Many thanks to Kalabo Moloi, Donald L. Adams and Evans V. Mauda for their assistance in the field.

I would also like to thank my prayerful wife Caroline R. for her support, encouragement and believing in me. She looked after the family alone during an economically challenging three years while I was away. Your strength and resilience during the Covid19-induced lockdowns was amazing.

This research was financially supported by the Afromontane Research Unit (ARU), University of the Free State (UFS), through the ARU Grant Programme and the UFS-QQ Risk & Vulnerability Science Centre (RVSC), funded by the South African Department of Science &

Innovation and the National Research Foundation (DSI–NRF, Grant Number 118601) award to the ARU; the Centre for Invasion Biology (CIB), University of Stellenbosch (DSI–NRF, Grant Number 85417), the Centre for Biological Control (CBC), Rhodes University; the Oppenheimer Memorial Trust (Grant Number 18576/03); and Rohan Kruger (Game Works Helicopters).

TABLE OF CONTENTS

LISTS OF FIGURES

LIST OF TABLES

SUMMARY

Nassella neesiana (Trin. & Rupr.) Barkworth, *N. tenuissima* (Trin.) Barkworth and *N. trichotoma* (Nees) Hack. & Arechav are three alien grass species that have become naturalised in South African montane grassland – mostly known historically from remote mountainous areas in the Eastern Cape. Currently, only *N. trichotoma* and *N. tenuissima* are recognised as serious invasive species in category 1b of the National Environmental Management: Biodiversity Act (NEM:BA, 10/2004): Alien and Invasive Species (AIS) Regulations of 1 October 2014. However, *N. neesiana*, which is currently not listed in NEM:BA, is a prolific invader in Australia and New Zealand, where it is causing serious damage to both natural and planted areas, and costing thousands of Australian dollars in control measures and lost land potential. In South Africa, some research and central government-facilitated management measures were undertaken from the late 1970s to the year 2000. Since then, very little data have been generated about the status of any of these species in South Africa. Literature pointed out, *inter alia*, the difficulty of identifying the species in the field, especially when plants were not fertile; this may be a reason for sparse distribution records including limited records from citizen science projects that record invasive species occurrences. Chapters 1 and 2 provide a synthesis of global knowledge on *Nassella* available at the start of this project.

The principal aims of the research were to (i) establish the current invasive status of the three *Nassella* species in South Africa, (ii) determine the impacts of *N. trichotoma* and *N. neesiana* on native flora, and (iii) to predict how their invasive status might change in the future under projected climate change scenarios. A combination of fieldwork, laboratory analysis, and modelling were used to answer these questions.

The impact of *N. trichotoma* and *N. neesiana* on co-occurring plant species and chemical properties of the soil was investigated using field surveys and modelling techniques (Chapter 3 and 4). The research focused on vascular plant biodiversity differences between dense patches of *Nassella* and matched nearby uninvaded areas – space-for-time substitution. *Nassella trichotoma* was found to reduce the diversity of native species in montane rangelands while increasing the concentrations of minerals in topsoil. *Nassella neesiana*, on the other hand, has little impact on native plants in a disturbed urban setting. However, the presence of both *Nassella* species is associated with increased concentrations of minerals in topsoils and an increase in ruderal vascular plant species. This study is the first known research on *N. neesiana* in South Africa.

The possibility of using DNA markers to distinguish the three *Nassella* species and separate them from co-occurring morphologically similar species was investigated (Chapter 5). Two candidate gene loci, *petL–psaJ* (*petL*) and ETS, were evaluated to determine their ability to discriminate the three *Nassella* species from each other and from three co-occurring native grass species (*Aristida diffusa* Trin*.*, *Festuca caprina* Nees and *Koeleria capensis* (Steud.) Nees), and the exotic species *Jarava plumosa* (Spreng.) S.W.L. Jacobs & J. Everett in South Africa. The three native grass species have been confused with *Nassella* species before. *Jarava plumosa* and the three *Nassella* species used to be in the same genus, *Stipa*, before the recent taxonomic revisions. The other minor objective was to find out if this method can be used as a viable option for field practitioners. The results indicate that the *petL* locus has no power to discriminate the three species, but can discriminate the different genera; in contrast, *ETS* has sufficient power to discriminate all the tested species, and can be outsourced at a reasonable cost, making it a viable identification method even for people without technical knowledge.

The current and future (2081 to 2100 period) potential distribution of the three *Nassella* species was investigated (Chapter 6). The bioclimatic variables were downloaded from the WorldClim database (http://www.worldclim.org), at a spatial resolution of 2.5 arc minutes. The distribution of all three species is mainly driven by temperature-based bioclimatic variables, especially Isothermality. The research found that – under current climatic conditions – all three species can potentially occupy more area than they currently do. Compared to currently suitable envelopes, future projected envelopes will be much smaller for the three species. The Maloti-Drakensberg mountains (including Lesotho) will remain at high risk of invasion across all future climate-change scenarios, and it should be a priority for keeping *N. trichotoma* out of the Maloti-Drakensberg, along with managing *N. tenuissima* and *N. neesiana* invasions in the Maloti-Drakensberg.

The overall finding of this research is that the three *Nassella* species remain very high-risk species for continued invasion of montane grasslands in South Africa, particularly in the most important regional water tower: the Maloti-Drakensberg. *Nassella neesiana* should be formally placed on an invasive "watch-list", for potential addition to the AIS Regulations (Category 1b), as it appears to have a much wider distribution and be much more widespread in the landscape than records show. Considering their impact on vascular plant diversity, rangeland quality and difficulty of management, they should be cause for major concern by landowners and government. This

research provides a platform for resurrecting a research and management focus on *Nassella* in South Africa (and Lesotho), with the aims of securing montane rangeland-based livelihoods, ensure conservation of endemic mountain biodiversity, and safeguard mountain catchment potential under climate change.

Key terms: invasion, cryptic invader, climate change, DNA markers, sdm R package

CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND

Invasive alien species can lead to major alteration of the environment, ecosystem services and lead to serious challenges to human health and livelihoods and hence has been the subject of much research over the years (Kumar Rai and Singh, 2020; Pyšek and Richardson, 2010). Invasive alien plant species (IAPS) present serious negative impacts to the environment and the socio-economic status of communities, costing nations millions of American dollars in control and potential revenue (Epanchin-Niell et al., 2021; Kumar Rai and Singh, 2020). The individual IAPS differ markedly in the drivers of invasiveness and their impact on the environment and socio-economic status of invaded areas making it impossible to develop a general management program for all IAPS. This means research targeted at specific species remains the best way to deal with the different IAPS in different environments.

Three species of the C_3 grass genus *Nassella* (Trin.) E. Desv., originating from South America (Barkworth and Torres, 2001), are among the numerous IAPS invading montane grassland in South Africa (Wells, 1977; Wells and De Beer, 1987), and are the dominant problematic montane Poaceae species in the region. These three species — *N. neesiana* (Trin. & Rupr.) Barkworth, *N. tenuissima* (Trin.) Barkworth and *N. trichotoma* (Nees) Hack. & Arechav — are thought to have been accidentally introduced to South Africa as biological contaminants in consignments of hay sometime between 1899 and 1902 (i.e. during the Second Anglo-Boer War) and naturalised in the country (Fish et al., 2015; Henderson, 2018; Ranwashe, 2019; Visser et al., 2017; Wells, 1978). The three species are also invasive in Australia, Europe, New Zealand, and the USA. In South Africa, only *N. trichotoma* and *N. tenuissima* are currently listed as invasive species in category 1b of the National Environmental Management: Biodiversity Act (NEM:BA, 10/2004): Alien and Invasive Species (AIS) Regulations of 18 September 2020. Species in category 1b of NEM:BA are prohibited from trade or planting in any form and should be subject to a systematic management programme. However, *N. neesiana*, which is currently not listed in NEM:BA and whose status is poorly known in the country, is considered a serious invader in

Australia and New Zealand, where it is a major pest in both natural and transformed land areas (Faithfull, 2012; Henderson, 2018; Howell and Sawyer, 2006; Ranwashe, 2019).

In South Africa, *Nassella* infestations have been primarily recorded in the montane grasslands of the Western and Eastern Cape provinces (>1600 m elevation), with additional records (historical and more recent) from low-lying but temperate areas around Cape Town. *Nassella trichotoma* is predominantly found in the Cape Midlands Escarpment area (Sneeuberg, Great Winterberg–Amatholes, and Stormberg), *N. tenuissima* in the Barkley East area of the Eastern Cape Drakensberg, and *N. neesiana* has a scattered distribution from the eastern Free State province, through the Eastern Cape (also Eastern Cape Drakensberg and Cape Midlands Escarpment) to the Western Cape. Research conducted in the 1970s and 1980s showed that *N. trichotoma* and *N. tenuissima* formed heavy infestations, particularly in disturbed and overgrazed areas, where they outcompete resident species (Wells, 1978, 1977; Wells and De Beer, 1987). This research led to vigorous government-funded awareness and control campaigns, which ended in 2000 when the herbicide with the active ingredient flupropanate, was no longer available on the market (Henderson, 2018). The only company that was manufacturing it in Japan ceased production. Formulations of it are being manufactured in Australia and is being sold under names like Taskforce®, and Flupropanate Granular Herbicide (or just Flupropanate). It is still not available on the market in South Africa, but it is being imported from Australia by individual companies and property owners when needed.

There appears to have been no research on *N. neesiana* in South Africa historically. Despite this more than 20-year hiatus, *Nassella* remains a serious threat in southern Africa. Research into its current status in South Africa is urgently required especially when its invasive impacts in Australia and New Zealand are considered (Henderson, 2018; Wells, 1978). Since the end of the central government-facilitated management program, individual property owners have been carrying out uncoordinated management on their properties. However, farmers with heavy infestations are finding it difficult to successfully manage *Nassella*, partly due to the costs involved, and also management attempts (such as chemical spraying, burning and uprooting) paradoxically encourage further invasion, forming dense monocultures (Henderson, 2018; McLaren et al., 1998) (Fig. 1.1). In addition, it is likely that *Nassella* will become more prevalent with climate change due to favourable conditions for C_3 grasses.

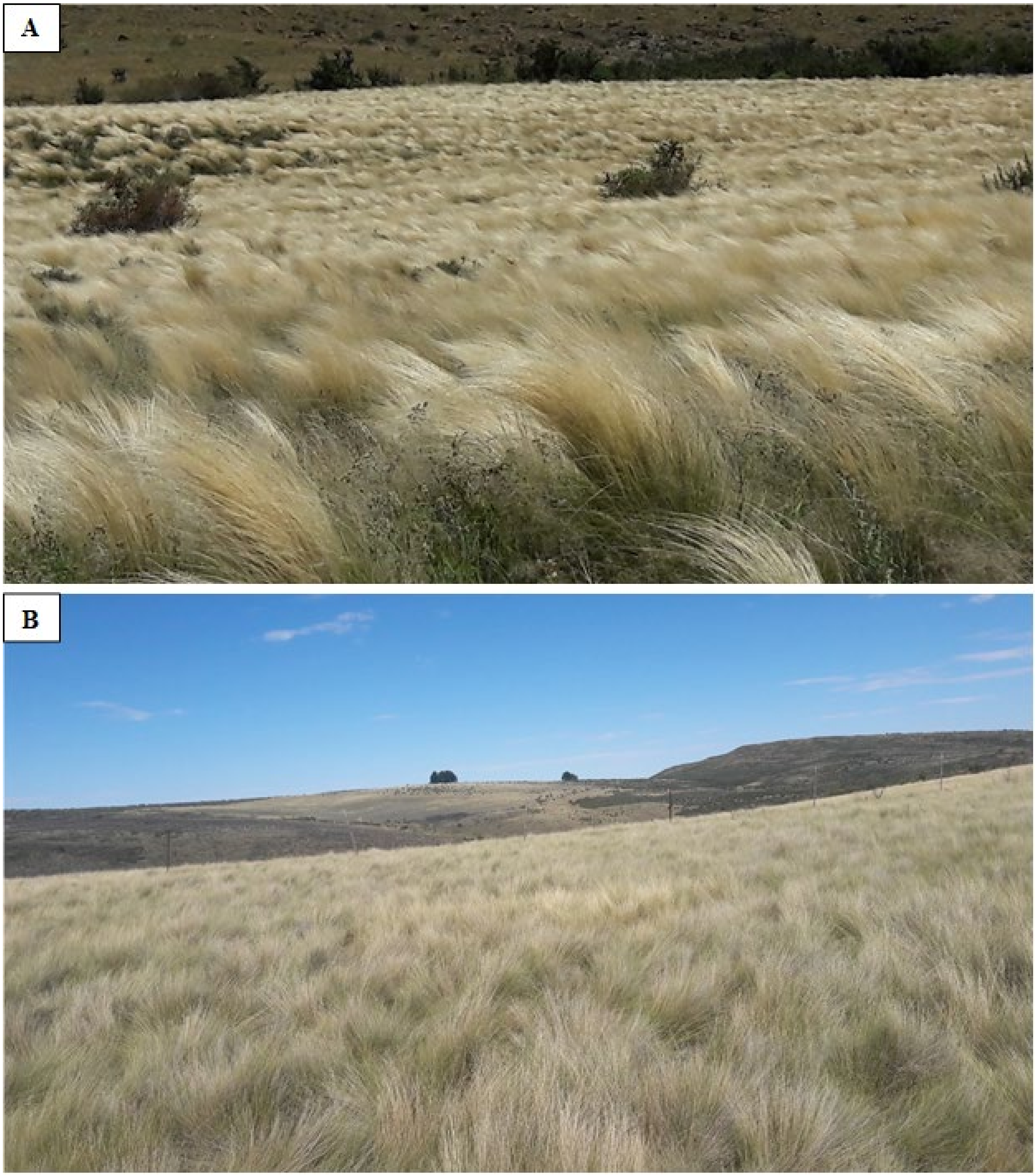


Fig. 1.1. *Nassella* invasions in the Eastern Cape province, South Africa: (A) a *Nassella tenuissima*-invaded paddock near Barkly East (Eastern Cape Drakensberg); (B) *N. trichotoma* invasion on the Boschberg, Somerset East (Cape Midlands Escarpment, Eastern Cape). Note the complete exclusion of native grasses by the *Nassella* tussock monocultures. Photos: A. Mapaura.

AIMS AND STRUCTURE OF THE THESIS

Research on *Nassella* invasion and control in South Africa effectively stopped in the late 1980s to the early 1990s. In addition, coordinated management was halted around 2000 when the then approved herbicide – Flupropanate – was no longer available on the market. This hiatus led to very little to no scientific information being generated on *Nassella*. Also, very few records are available in herbaria to confidently construct distribution maps, and the three *Nassella* species are not easily distinguishable morphologically from each other and from certain native tussock grasses (notably high elevation *Festuca*). This makes them poor candidates for citizen science platforms to identify populations. These factors all contribute to the species spreading without being detected. Considering the foregoing, the main question of this thesis is: what is the status of *Nassella* invasions in South Africa, and how is this going to change under predicted future climate-change scenarios? This research will contribute towards improved understanding of the species' invasions and management options. I hypothesise that *Nassella* invasions are more widespread and extensive than currently known and are likely to continue to spread regardless of future climate change scenarios. Although rising temperatures are expected to negatively impact *Nassella* species, the carbon fertilisation that will occur may allow the plants to overcome these impacts and persist under these conditions. I also hypothesise that *Nassella* expansions are mediated by changes in soil chemistry, since they re-invade much faster and more vigorously from areas they have been cleared from previously. To test the foregoing hypotheses and guide the research, the following objectives were developed:

Objectives

1. To understand the impact of *Nassella* on biodiversity and the environment. The key question is what changes to community composition and soil properties occur post-invasion. The hypotheses for this objective are:
   a) *Nassella* invasions reduce plant species diversity in invaded areas.
   b) *Nassella* invasions alter soil chemical properties to facilitate their reinvasion and reduce the success of resident species.
2. To investigate the possibility of using DNA markers to distinguish the three *Nassella* species and separate them from morphologically similar species. Traditional plant identification methods require flowering/fruiting material to be sure of the identity of a species. This can delay identification and inception of management interventions. The

hypothesis is that it is possible to identify these three species to species level and separate them from morphologically similar species using DNA barcoding.

3. To predict the possible distributional responses of the three *Nassella* species to climate change in South Africa and Lesotho. The hypotheses for this objective are that:
   a) *Nassella* invasions have not yet saturated the current envelopes for each species.
   b) Under climate change *Nassella* invasions will become more widespread, especially in high elevation mountains.

To achieve these objectives, I employed comparative approaches, and computer modelling. In Chapter 2, I examine the literature on the distribution, ecology, reproduction, dispersal and germination, uses, impacts, management, and knowledge gaps.

In Chapters 3 and 4, I investigate the impact of *N. neesiana* and *N. trichotoma* on co-occurring plant species and chemical properties of the soil. The research focused on vascular plant biodiversity differences between dense patches of *Nassella* and matched nearby uninvaded areas – space-for-time substitution.

In Chapter 5, I investigate the possibility of using previously established DNA markers for *Nassella* to distinguish the three *Nassella* species and separate them from morphologically similar species in South Africa.

In Chapter 6, I use species distribution modelling to predict the potential distribution of the three *Nassella* species and project their future distribution under climate change.

In Chapter 7, I present a general discussion of the results reported in this thesis, and consider how they contribute to answering the main question – what is the status of *Nassella* invasions in South Africa, and how is this going to change under predicted future climate-change scenarios? Recommendations on the way forward are provided.

CHAPTER 2

A synthesis of available information about *Nassella* invasions in South Africa and identifies research gaps. See Appendix A for the pdf version.

A published paper:

Mapaura A, Canavan K, Richardson DM, Clark VR, Steenhuisen SL. (2020). The invasive grass genus *Nassella* in South Africa: A synthesis. *South African Journal of Botany* 135, pp. 336-348. 8. https://doi.org/10.1016/j.sajb.2020.08.031

THE INVASIVE GRASS GENUS *NASSELLA* IN SOUTH AFRICA: A SYNTHESIS

Anthony Mapaura[1], Kim Canavan[2], David M. Richardson[3], Vincent R. Clark[4], Sandy-Lynn Steenhuisen[1]

*Corresponding author: A. Mapaura (amapaura@gmail.com) +27625177430

[1]Afromontane Research Unit & Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, 9866, South Africa

[2]Centre for Biological Control, Department of Entomology and Zoology, Rhodes University, Makhanda, 6140, South Africa

[3]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, 7602, South Africa

[4]Afromontane Research Unit & Department of Geography, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, 9866, South Africa

ABSTRACT

Three species of *Nassella* have naturalised in South Africa. *Nassella trichotoma* and *N. tenuissima* are declared weeds under category 1b of the National Environmental Management: Biodiversity Act (NEM:BA) and occur mainly in the montane grasslands of the Western and Eastern Cape provinces. *Nassella neesiana* is not listed in NEM:BA but is naturalised in the Eastern Cape and Free State provinces. Research conducted in the 1970s and 1980s led to vigorous government-funded awareness and control campaigns which ended in 2000. No research on *Nassella* distribution or control has been undertaken since then. Despite this hiatus, *Nassella* remains a dangerous genus in southern Africa, given the serious impacts of these species in similar social-ecological systems in Australia and New Zealand. This paper presents a synthesis of available information about *Nassella* invasions in South Africa and identifies research gaps. It specifically addresses these questions: What identification issues exist? What is the current spatial distribution of *Nassella*? What is the autecology of the genus? What are the social-ecological impacts of *Nassella*? What control measures are currently applied and what are their strength and limitations? What do we know about *Nassella* distribution and its response to climate change? This paper highlights many knowledge gaps about *Nassella,* such as the species' current distribution range, field identification and detection difficulties, and the uncoordinated control efforts that require urgent research to inform an effective management response.

Keywords: Biological invasions, invasive grasses, *Nassella neesiana*, *Nassella tenuissima*, *Nassella trichotoma*, Poaceae

INTRODUCTION

The genus *Nassella* (Trin.) E. Desv. belongs to the grass family, Poaceae (Gramineae), subfamily Pooideae in the tribe Stipeae (spear grasses). *Nassella* comprises at least 116 species (Barkworth et al., 2008; Romaschenko et al., 2012; Soreng et al., 2009). Species currently placed in this genus were originally included in the genus *Stipa* s.l. and for many years researchers held mixed views regarding the segregation of these genera. However, with strong support from molecular studies, their separation appears to be largely settled, save for a few species (Cialdella et al., 2014; Romaschenko et al., 2012). The name 'Nassella' comes from the Latin noun *nassa* which means "a fish basket" (Quattrocchi, 2000), probably because these grasses were used for making fishing baskets.

According to Barkworth and Torres (2001), with confirmation from researchers such as Romaschenko et al. (2012) and Soreng et al. (2009), most *Nassella* species are native to South America, while six are also found in the United States and Canada. The species are mostly native to Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Paraguay, Peru, Uruguay, and Venezuela. The highest species diversity of *Nassella* occurs in north-western Argentina which is home to 72 species (Barkworth and Torres, 2001).

Eleven *Nassella* species have been recorded as growing outside their natural ranges (Barkworth and Torres, 2001). Of these, *N. neesiana, N. tenuissima* and *N. trichotoma* have naturalised (*sensu* Pyšek et al., 2004) and become invasive in Australia, Europe, New Zealand, South Africa and the USA (Henderson, 2018; Howell and Sawyer, 2006; Ranwashe, 2019). The other species which have naturalised outside their natural ranges are not invasive (Barkworth and Torres, 2001). *Nassella neesiana, N. tenuissima* and *N. trichotoma* invade disturbed areas, particularly overgrazed pastures, and also indigenous grasslands, including those dominated by *Themeda triandra* Forrsk in temperate mountains (Faithfull, 2012; Taylor et al., 2016). *Nassella trichotoma* is regarded as a major invader in Australia, New Zealand and South Africa (Wells and De Beer, 1987).

There are no indigenous species of *Nassella* in South Africa. Three species are known to have naturalised in the country: *N. neesiana, N. tenuissima* and *N. trichotoma* (Fish et al., 2015; Ranwashe, 2019; Visser et al., 2017). All three species are thought to have been accidentally introduced to South Africa by the British Army during the Anglo-Boer War (1899-1902), through the ports of East London and Port Elizabeth, possibly as seed in hay from Argentina for horse fodder (Henderson, 2018; Wells, 1978). Only *N. trichotoma* and *N. tenuissima* are currently listed as invasive species in category 1b of the National Environmental Management: Biodiversity Act (NEM:BA, 10/2004): Alien and Invasive Species (AIS) Regulations of 18 September 2020[1]. These regulations stipulate that they cannot be traded or planted in any form and should be removed and destroyed wherever possible.

Research conducted on *Nassella* in South Africa in the 1970s and 1980s, especially by M.J. Wells, led to widespread awareness and many control campaigns. Effective enforcement of control of *Nassella* species ended in 2000 when the government subsidy stopped (Henderson, 2018). The termination of these subsidies and the coordinated control efforts also appears to have ended research efforts, the gathering of new information and formal reporting on *Nassella* invasions.

This paper collates all available information about *Nassella* invasions in South Africa and identifies research gaps. It specifically seeks to shed light on these questions:

What identification issues exist? What is the current spatial distribution of *Nassella*? What is the autecology of the genus? What are the social-ecological impacts of *Nassella*? What control measures are currently applied and what are their strength and limitations? What do we know about *Nassella* distribution and its response to climate change?

MATERIALS AND METHODS

The taxonomic delimitations used in this review of *Nassella* follow Barkworth and Torres (2001), supplemented with insights from Fish et al. (2015). Data were gathered from published and grey literature. Several methods were pursued in order to unearth as much literature as possible. These methods included searching repositories for the three species, following-up on literature cited in references, searching for articles by authors whose works were frequently referenced, and by consulting relevant research institutes in the country. Landowners, practitioners, and rangeland scientists also gave their own perceptions, thoughts and experiences during reconnaissance visits and informal interpersonal and email communications. These were also included, together with the observations and experiences of the authors when no published data could be found. *Nassella* research in South Africa effectively stopped in the 1980s and no recent scientific research could be found in the literature. However, Australia and New Zealand

---

[1] Updated from 1 October 2014

have similar socio-ecological systems to South Africa (Pyšek et al., 2020), and much research on the invasion ecology of the genus has been undertaken in these countries. This research was extensively consulted for this review, and the implications of this work were cautiously interpreted with reference to the South African context.

Distributional data were gathered from databases such as the Southern African Plant Invaders Atlas (SAPIA) (Henderson and Wilson, 2017), the Global Biodiversity Information Facility (GBIF) (Occdownload Gbif.Org, 2019), Botanical Dataset of Southern Africa (BODATSA) (Ranwashe, 2019) and iNaturalist records marked as Research Grade. In addition to these databases, the Qwaqwa (QWA) Herbarium and the Selmar Schonland Herbarium (GRA) were consulted, and no records were found at QWA while GRA had 13 records. All the GRA records were in the SAPIA database. These data were used to produce current distribution maps of the three *Nassella* species. Unverified records, such as those given by word of mouth were not included. The distributional data were converted to degree decimals and plotted on a map using the Free and Open Source QGIS software.

DESCRIPTION

Nassella are best distinguished from other members of the tribe Stipeae by the strongly convolute lemma and short, glabrous palea (Barkworth and Torres, 2001). They are perennial tussock grasses with membranous ligules which are sometimes pubescent or ciliate. The inflorescences are panicles. The *Nassella* species themselves are similar and difficult to distinguish from each other, particularly outside the flowering season; this is especially problematic for *N. trichotoma* and *N. tenuissima* which have very similar ecologies and growth habits (Jacobs et al., 1998) (Table 2.1).

Table 2.1. Description of the defining features of three naturalised *Nassella* species in South Africa (Barkworth and Torres, 2001; Fish et al., 2015).

Character		*Nassella neesiana*	*Nassella tenuissima*	*Nassella trichotoma*
Synonyms		*Stipa neesiana* Trin. & Rupr	*Stipa tenuissima* Trin.	*Stipa trichotoma* Nees
Common names		Chilean needle grass	Mexican feather grass, white tussock, witpolgras	Nassella tussock; serrated tussock, Nassella-polgras
General		A perennial tussock forming grass, up to 1 m high.	A perennial tussock forming grass, up to 1 m high. Tussock distinctly white in winter. Culms slender and wiry.	A perennial tussock forming grass, up to 0.65 m high. Mature tussocks droop
Leaves		Flat, 300 mm long and 1-5 mm wide, strongly ribbed on adaxial surface with rough margins	Very tightly rolled inward, and up to 700 mm long and 0.5 mm wide, appear as thin wiry filaments which are rough to touch.	Tightly rolled, 80-500 mm long and 0.5 mm wide, hairless with very fine serrations. Rough when stroked downwards.
	Ligules	Truncate with a few hairs	Acute	Obtuse
Inflorescence		Open panicle	Slender, compact panicle rarely extending above leaves.	Open, much-branched panicle, detaching at maturity, leaving the plants free of inflorescences for most of the year.
	Spikelet	15-17 mm long (excluding awn)	4-5 mm long (excluding awn)	6-8.5 mm long (excluding awn)
	Glume	Longer than lemma		
	Lemma		Elliptic	Obovate
	Awns	50-120 mm long, geniculate	45-90 mm long, unnoticeably geniculate, centrally placed on lemma	15-35 mm long, off centre of lemma
	Time of flowering	November to March	January	August to March

SIMILAR SPECIES IN THE FIELD

Nassella highlights the challenges presented by cryptic invaders that are not readily distinguishable from native grasses (Henderson and Wilson, 2017). They are very similar to native *Stipa* species (Connor et al., 1993; Global Invasive Species Database (GISD), 2019) of which South Africa has four indigenous species, including the endemic *S. dregeana* Steud. var. *dregeana* (Ranwashe, 2019). As highlighted by Henderson (2018), *N. trichotoma* looks superficially like *Festuca caprina* and *Tenaxia stricta* and has been confused with these species even by experienced researchers. Henderson (2018) also reports that *N. trichotoma* often shares its habitat with *T. stricta* in rocky mountainous areas. The short, white, hairless ligule with a rounded apex found on *N. trichotoma*, differentiates it from *T. stricta* and similar tussock species which either lack the ligule or possess a ring of hairs around the top of the ligule (Henderson, 2018). As mentioned above, the three *Nassella* species are also difficult to distinguish from one another. This difficulty in species identification explains why few records are submitted through citizen science such as SAPIA (Henderson and Wilson, 2017). As of 9 April 2022, iNaturalist had 18 records of *Nassella/Stipa* species in South Africa: seven records of the native species *Stipa dregeana* Steud. (*Stipa capensis*) (1), seven for *N. neesiana* (3) and four for *N. trichotoma* (1) (numbers of "Research Grade" observations are in brackets)[2].

DISTRIBUTION AND ECOLOGY

Nassella species are C_3 grasses and are therefore generally restricted to temperate regions of the world where they inhabit a wide ecological range (Milton, 2004; Romaschenko et al., 2008). The species occur mainly in degraded pastures but also in natural grasslands and open woodlands (Henderson, 2001; Taylor et al., 2016). Temperature tolerance ranges from as low as -5°C to about 25°C and they can survive short periods of frost and ice cover (Bourdôt et al., 2012; Healy, 1945). They favour areas with annual rainfall between 300 and 800 mm (Healy, 1945; Henderson, 2001; Wells and De Beer, 1987). They are therefore better at tolerating droughts than most pasture species in areas where they occur (Grech et al., 2012; Miller, 1998; Wells, 1977). *Nassella* species have been shown to grow in a variety of soil types and fertility ranging from poor well-drained acidic soils (Campbell, 1998) to fertile soils (Badgery et al., 2005; Wells and De Beer, 1987). The

---

[2] This is an update, for the original see the publication (Appendix A)

distribution of the species is influenced by soil disturbance and reduction in native perennial grasses (Badgery et al., 2005; Healy, 1945). In South Africa, *Nassella* is restricted to the montane grasslands of the Eastern and Western Cape mountains (Fig. 2.1).

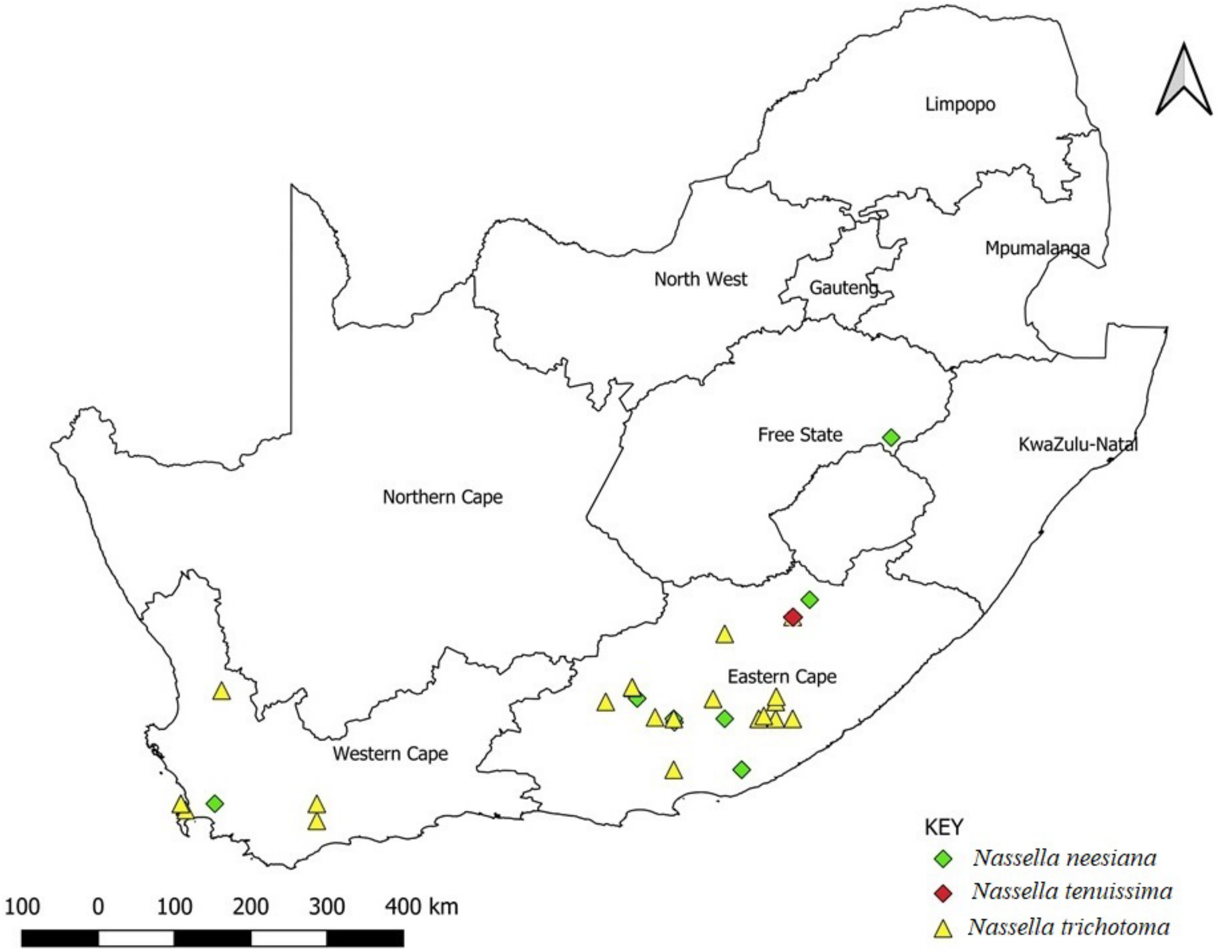


Fig. 2.1. The known distribution of *Nassella neesiana, N. tenuissima and N. trichotoma* in South Africa based on records from BODATSA, (Ranwashe, 2019), SAPIA, (Fish et al. 2015), GBIF (Barker et al., 2020) and Research Grade iNaturalist observations.

Nassella neesiana is native to Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Peru, and Uruguay (Barkworth and Torres, 2001; Soreng et al., 2009). It has naturalised in Australia, Europe, New Zealand, and South Africa (Barkworth and Torres, 2001; Fish et al., 2015; Henderson, 2018; Howell and Sawyer, 2006; Verloove, 2005). While it is considered a serious invader in Australia and New Zealand (Henderson, 2018; Howell and Sawyer, 2006; Ranwashe, 2019), it is not currently listed in the NEM:BA regulation although it could be a threat in South Africa judging from the distribution pattern shown on Fig 2.1. It is known to have naturalised in the Eastern Cape and the Free State provinces (Fig. 2.1) at altitudes between 600 m and 1700 m (Germishuizen and Meyer, 2003).

Nassella tenuissima is native to Argentina, Chile, Mexico, and the USA (Barkworth and Torres, 2001; Jacobs et al., 1998; Soreng et al., 2009). It has become naturalised in New Zealand, Australia, Europe, South Africa and the USA (outside the natural range) (Barkworth and Torres, 2001). *Nassella tenuissima* has been widely distributed mostly as a result of its value as a horticultural grass. In South Africa, the species has so far only been recorded from a single locality in the Eastern Cape Drakensberg Mountains (Fig. 2.1) (Fish et al., 2015; Henderson, 2001; Ranwashe, 2019). A search in February 2020, by a team of grass taxonomists and one of the authors could not locate the species. While in other parts of the world it has been recorded at altitudes ranging from near sea level to 2900 m (Jacobs et al., 1998; Occdownload Gbif.Org, 2019), the recorded populations in South Africa occur at altitudes between 2000 and 2600 m (Germishuizen and Meyer, 2003) and only in grassland (Henderson, 2007).

Nassella trichotoma is native to Argentina, Bolivia, Brazil, Chile, Peru and Uruguay (Barkworth and Torres, 2001; McLaren et al., 1998; Wells, 1977; Westbrooks and Cross, 1993). It is invasive in Australia, New Zealand and South Africa (Henderson, 2018; Howell and Sawyer, 2006; McLaren et al., 2004; Ranwashe, 2019), with some invasive populations in parts of Europe (Barkworth and Torres, 2001; Campbell, 1982). In South Africa, it has been recorded as being invasive in the Western, Eastern and Northern Cape provinces (Fig. 2.1), and there are unconfirmed records from Limpopo, Gauteng, Free State and Mpumalanga (Ranwashe, 2019). By 1985 it had invaded about 70000 ha with just over 9000 ha of this being moderately to heavily invaded (Wells and De Beer, 1987). Wells and De Beer (1987) projected that by the mid-2020s about 2 million ha of South Africa could be invaded. The possibility of this projection coming true is unknown as data on the extent of invasion are not available. It has been recorded at altitudes between 100 and 1600 m (Germishuizen and Meyer, 2003). It is commonly encountered in Savanna and Grassland but also occurs in Fynbos, Nama-Karoo and Succulent-Karoo Biomes (Henderson, 2007).

REPRODUCTION, DISPERSAL AND GERMINATION

Reproduction

Nassella species appear to rely exclusively on sexual reproduction via seeds (Campbell, 1982) which are produced in large quantities. For example, *N. trichotoma* has been shown to produce as many as 100000 seeds per plant per year (Campbell, 1982; Wells, 1978, 1977; Wells and De Beer, 1987), giving an estimated production of 900-3400 million seeds per hectare in heavily invaded areas (Healy, 1945).

Nassella neesiana and *N. tenuissima* produce chasmogenes ('normal seeds') and cleistogenes (seeds produced in closed stem flowers), and can also self-pollinate (Connor et al., 1993; Faithfull, 2012; Jacobs et al., 1998). The cleistogenes are reportedly more common when the production of chasmogenes is suppressed, such as during prolonged mowing and overgrazing (Kriticos et al., 2010). Cleistogamy ensures that the species persists and keeps the seed bank replenished during those periods when the production of inflorescences is subdued.

Dispersal

Seeds can be dispersed over long distances, especially in the case of *N. trichotoma* whose seeds are borne on inflorescences that detach readily when seeds mature and can be blown for great distances, as much as 16 to 30 km per day depending on wind speed and physical impediments (Campbell, 1982; Joubert, 1984; Wells, 1978). However, *N. neesiana* and *N. tenuissima* are usually only dispersed for short distances because their seeds remain attached to the plant. This could explain why *N. tenuissima* seems not to have spread from the single locality from where it has been reported in the Eastern Cape.

Seeds are also dispersed by many other agents such as agricultural machinery, floodwaters, in mud or soil, clothing, trains, and cars (Campbell, 1982; Cooperative Research Centres, 2003; Wells, 1978). Seeds can pass through the digestive system of cattle (Healy, 1945) and sheep (Cook, 1998) unharmed and thus can be spread in animal faeces. However, the consumption of *Nassella* by livestock is limited and mostly occurs by accident during the process of grazing more palatable forage (Bläß et al., 2010). Seeds can also be spread through silage and hay if proper care is not taken. This is particularly important in cases where fodder has to be moved large distances to feed livestock due to a lack of available forage (Weller et al., 2015, 2016). The preparation of silage and hay is a cut and carry process. The seeds of *Nassella* can be carried to other areas in the silage or hay (this is how it is believed to have been brought to South Africa from Argentina).

Germination and growth

Most of the seed bank for *Nassella* species is found in the top 2.5 cm of the soil; seeds close to the soil surface lose viability more rapidly than seeds buried deeper, and deep-buried seeds experience stronger dormancy (Bourdôt et al., 1992; Faithfull, 2012; Joubert, 1984). Once in the soil the seeds of *N. trichotoma* can lie dormant for up to 20 years (Wells, 1978, 1977; Wells and De Beer, 1987). However, the viability of seeds declines quickly. Taylor (1987), found that after 6 months viability declined to between 20 - 61% under field conditions in New Zealand. This is not the case with all *Nassella* species; for example, *N. neesiana* seeds lose viability at a rate of 38% per annum (Connor et al., 1993) and so may not last for more than a few years in the soil (Bourdôt and Hurrell, 1992). The soil seed bank can be immense, ranging from 1700 to more than 42000 seeds/m^2 in New Zealand (Healy, 1945) and up to 75000 seeds/m^2 for *N. trichotoma* in South Africa (Joubert, 1984) although they are normally found in much lower quantities with a high proportion showing damage from factors such as animal trampling and insect predation and hence probably not viable (Joubert, 1984). While *N. trichotoma* seeds are dispersed everywhere, seedlings do not take hold in healthy native grasslands, possibly due to shading (Campbell, 1982; Faithfull, 2012; Joubert, 1984). Germination or survival of seedling is probably prevented by shading, with evidence of higher germination under unshaded conditions in undamaged seed (Taylor, 1987). Most seeds that germinate do not survive the first summer due to competition for moisture from established plants (Badgery et al., 2008). These two mechanisms work together to ensure that *N. trichotoma* does not establish easily in dense native grasslands. Some stakeholders in the Eastern Cape have pointed out that *N. trichotoma* invasions in South Africa start from a point of initial establishment and expand outwards from that point as new plants establishing themselves close to already established plants (Fig. 2.2). This suggests that most of the seeds fall close to parent plants even though they have the potential to be dispersed further.

Fig. 2.2. A *Nassella trichotoma* patch (tufted grasses in background) expanding in an overgrazed paddock in the Boschberg near Somerset East, Eastern Cape, South Africa. Photo: A. Mapaura.

Seeds can germinate at any time of the year, but mostly in autumn and winter in South Africa (Joubert, 1984). Germination appears to be regulated by rainfall and temperature, with low temperatures and droughts limiting germination (Bourdôt and Hurrell, 1992). Seeds germinate over a wide range of conditions and over a long period (Healy, 1945), probably as a result of dormancy mediated by the permeability of testa (Campbell, 1998). The need for dormancy is demonstrated by the fact that fresh seeds show lower germination rates compared to seeds set in the previous years (Lamoureaux et al., 2006; Lamoureaux and Bourdôt, 2002). Removing the testa and cutting off the awn-end of seed increases germination rate in the seeds (Campbell, 1998).

Nassella trichotoma grows more slowly than desirable pasture grasses and also flowers several years after germination (Campbell, 1998). Research in New Zealand has shown that tussock expansion is much faster than previously believed but is rarely noticed until flowering starts (Bourdôt and Saville, 2019). The seeds of *N. trichotoma* are viable well before the panicles extend above the tussock canopy (Lamoureaux et al., 2006; Lamoureaux and Bourdôt, 2002). It produces a very strong rooting system, reaching depths of 20 cm, which makes uprooting difficult even for young plants (Wells, 1977; Wells and De Beer, 1987).

Nassella neesiana, on the other hand, grows so rapidly that it is often difficult to distinguish the seedling and juvenile stages (Faithfull, 2012). Seeds can germinate and plants mature and produce viable seeds within a single year (Bourdôt and Hurrell, 1992; Faithfull, 2012). On poor soils, however, flowering may be delayed until the third or fourth year due to poor vegetative growth (Campbell, 1998). The fast growth rate ensures that the plants outcompete all other grasses and occupy open spaces rapidly. Moretto and Distel (1998), reported that *N. tenuissima* is a poor root and shoot competitor, and so establishes and thrives in vegetation gaps where there is no competition from other grasses, and where seed dormancy is broken by fluctuating temperatures. They also noted that germination was concentrated during the rainy season since the seeds are highly susceptible to desiccation.

USES

Since their accidental introduction into South Africa, *Nassella* species have not been used for anything. Several uses have, however, been noted around the globe, especially in the native range of the taxa. All species are occasionally grazed by goats and cattle when they are still young and in the active vegetative growth stage (Campbell, 1982; Westbrooks and Cross, 1993). *Nassella tenuissima* is also used as an ornamental grass in some countries, but this use is illegal in many countries due to the possibility of it escaping and becoming invasive. In Argentina, *N. tenuissima* is reportedly used for thatching (Global Invasive Species Database (GISD), 2019).

IMPACT

Research to date suggests that invasive alien grasses generally have a greater socio-economic impact than environmental impact in South Africa (Nkuna et al., 2018; Visser et al., 2017). However, since the majority of recorded impacts are on crop and animal production there is a bias towards socio-economic data (Nkuna et al., 2018). The abundance of *Nassella* plants can increase rapidly, leading to them assuming dominance over whole pastures, completely displacing both planted and natural grass species (Fig. 2.3) (Faithfull 2012). Of the three *Nassella* species, *N. trichotoma* is the most devastating and widespread in South Africa and known to completely smother even planted pastures (Wells, 1977). Its feat as an invader led Wells (1977) to conclude that: '…this is almost the perfect weed, a beautifully adapted, highly efficient organism: a self-perpetuating factory of valueless fibres.'

Fig. 2.3. A paddock invaded by *Nassella trichotoma* on the Boschberg near Somerset East, Eastern Cape province, South Africa. Photo: A. Mapaura.

Environmental impact

There is a general lack of data on the environmental impacts of *Nassella* species, especially in South Africa (Visser et al., 2017). In general, *Nassella* invasions are associated with reduced invertebrate diversity, altered fire regimes, and the suppression of co-occurring native grasses (Faithfull, 2012). *Nassella neesiana*, and possibly all *Nassella* species, has an increasing impact on native grass diversity as its residence time increases (Faithfull, 2012). It has also been reported that even though numerous native generalist insect species consume it in Australia (Faithfull, 2012), it significantly reduced invertebrate abundance, composition, and richness compared to native grass dominated areas (Faithfull, 2012). However, much of the loss of diversity in invaded areas probably precedes invasion caused by anthropogenic disturbances such as mowing, major soil disturbance, and previous control with herbicides that contain flupropanate or glyphosate, that also kill many of the native species (Faithfull, 2012).

Invasion of *N. trichotoma* has been reported to be associated with altered fire regimes, for example in the Geelong region of Victoria, Australia, invaded stands resulted in extending the fire season by as much as two months (McLaren et al., 2004). The altered fire regime has major impacts on the regeneration of native species through changing the soil carbon and nitrogen status and soil microbial structure and function (Dickens and Allen, 2014).

Soil seed banks in areas invaded by *Nassella* species include seeds of other species, both native and non-native, but many of these species are not represented in the above-ground plant assemblage (Faithfull, 2012; Joubert, 1984; Wells and De Beer, 1987). Joubert (1984) suggested that this could be due to allelopathic interactions, but no research has been done to confirm this type of interaction. However, it is not clear that all invasive populations of *Nassella* have such an effect; for example, *N. trichotoma* in natural montane grasslands in the Eastern Cape co-occurs with several species of native grasses (A. Mapaura, pers. obs.). The non-germination of indigenous grasses in densely invaded patches of *Nassella* could also be a result of the dense cover of *Nassella* which prevents light from reaching the soil to stimulate seed germination, and also competition for other resources, particularly water. The effect of canopy cover is more likely considering that after clearing the *Nassella* plants seeds of other native plant species are able to germinate and establish (Joubert, 1984). The mechanisms whereby *Nassella* impacts native species are not understood, although much research is currently underway, especially in Australia and New Zealand. Research is needed to determine the factors leading to the suppression of native grasses in *Nassella*-invaded patches.

Socio-economic impact

In pastures the more palatable grasses and plants are generally smothered by less palatable and poisonous species as the former are preferentially eaten by animals (Wells et al., 1983). This progressively reduces the quality of the pastures, impacting negatively on the pastures' carrying capacities (Nkuna et al., 2018; Richardson et al., 2011; Viljoen, 1999). As palatable pasture plants decrease, inedible and indigestible plants such as *Nassella* species increase, reducing native grass and forb richness by as much as 50% (Faithfull, 2012; Morgan, 2001). *Nassella* species have been reported to impact native species more than other non-native species present in an area where they invade (Faithfull, 2012).

Nassella species are not palatable and hence increase as the palatable grasses decrease in pastures, especially where there is bad pasture management (Wells, 1977; Wells and De Beer, 1987). *Nassella trichotoma* has a very high fibre content (as high as 86%) and very low nutritive value (protein content as low as 4%), making it non-nutritional hence seldom grazed by animals (Wells and De Beer, 1987). Furthermore, if animals are forced to graze it, the fibres, which are indigestible, can form boles in the rumen, causing rapid loss of health, which may lead to death (Campbell, 1998; Wells and De Beer, 1987). This reduction in pasture quality can be massive, leading to sub-economic carrying capacities (Anderson et al., 2010; Campbell, 1998; McLaren et al., 2004). In New Zealand, carrying capacity reductions of up to 90% were reported on some farms, leading to the abandonment of sheep farming (Healy, 1945). In worst-case scenarios in New South Wales, Australia, some farms were so heavily invaded that the cost of control was higher than the value of the farm, leading farmers to abandon farming or to sell their farms and seek alternative employment (Campbell, 1998). Some farmers in the Eastern Cape, South Africa, have lost the use of part of their farms to *Nassella* invasion (A. Mapaura pers. obs.).

In monetary terms, losses can be huge. Productivity losses and management costs were estimated to range from as little as \$ AUD7.00 ha^{-1} for light-invaded areas controlled by hand to \$ AUD122.00 ha^{-1} for heavy invasions in SE Australia in the early 1980s (Vere and Campbell, 1984). Most of the losses were suffered by the wool and lamb industries due to reduced carrying capacity on invaded farms (Vere and Campbell, 1984). Young animals, especially lambs, can have their skin or eyes damaged by the sharp awns, while the awns are difficult to remove from wool and reduce its quality (Anderson et al., 2010; Campbell, 1998).

MANAGEMENT

General

South Africa seems to be less susceptible to invasion by non-native grasses than many other parts of the world (Visser et al., 2016). Nonetheless, invasions of several species of non-native grasses are having major, and rapidly growing, impacts on human livelihoods and biodiversity. Several researchers have made urgent calls for more effective management of invasive grasses, including *Nassella*, in South Africa (Henderson, 2018; Henderson and Wilson, 2017; Milton, 2004; Nkuna et al., 2018; Visser et al., 2017; Wells et al., 1983). The spread of invasive alien species is facilitated by the increasing global movement of people and goods, while anthropogenic environmental changes facilitate their establishment and proliferation (Early et al., 2016). In light of limited resources and a multiplicity of competing needs, it is important to evaluate both monetary and non-monetary benefits of all available control methods before embarking on any action, or deciding to take no action (Bourdôt et al., 2015; Hanley and Roberts, 2019).

The ability to control any invasive species depends on the ability of managers to identify it correctly at every stage of its growth and their ability to notice it before it has become a problem (Wang et al., 2016; Wells, 1977). However, as discussed above, *Nassella* species are cryptic; their presence is often noticed after the species are well established and are causing dramatic impacts (McLaren et al., 2004; Smith and Lamoureaux, 2006; Wells, 1977). Late flowering in *N. trichotoma*, for example, makes early detection difficult since the species is easily distinguishable when in flower, meaning that by the time they are detected they will be already established and difficult to control (Campbell, 1998; Smith and Lamoureaux, 2006).

Following the pioneering work of M.J. Wells and others in the 1970s and 1980s, *N. trichotoma* and *N. tenuissima*, were listed as invasive species in South Africa, and were placed in category 1b in the NEM:BA regulations. Vigorous awareness and control campaigns were also launched by the Department of Agriculture in the 1980s and 1990s. The Government provided landowners with task teams and subsidised herbicides (Henderson, 2018). However, all progress made with tackling these invasions was lost when the subsidy was removed, and the herbicide Flupropanate was withdrawn from sale in 1997; government-driven control and control enforcement thus effectively stopped. Since then, there has been no centrally coordinated effort to control *Nassella* invasions in South Africa, and farmers have borne the burden of these invasions alone, often leading to individual farmers working independently of their neighbours (Henderson, 2018), or not at all. The ideal control measure will be one that reduces the soil seed bank, while at the same time minimising re-invasion from neighbouring areas (Bourdôt and Hurrell, 1992; Joubert, 1984). To prevent re-invasion, an area-wide control system is crucial, to ensure that neighbouring property owners and managers cooperate to deal with the invasion (Miller, 1998).

Cultural control

Nassella grasses are unpalatable - most animals will not ordinarily graze them (Anderson et al., 2006; Wells and De Beer, 1987); this greatly reduces the potential for using livestock to control *Nassella* invasions (Grech et al., 2012). Goats have, however, been reported to graze actively growing low *Nassella* weed population densities of less than 20% ground cover but due to low nutritive value grazing is avoided unless there are no other options (Campbell, 1982; Westbrooks and Cross, 1993). *Nassella neesiana* reportedly produce good feed in the early vegetative stage and so livestock can be used to reduce their productivity (Cooperative Research Centres, 2003; Miller, 1998; Westbrooks and Cross, 1993). However, *N. neesiana* has a very short juvenile stage which means that it is available for grazing for a very brief period (Faithfull, 2012). To reduce the potential of spreading the seeds through silage, the silage can be quarantined for at least 6 weeks to ensure that the seeds are not viable and also to diminish the sharpness of the seeds which can be injurious to the intestinal lining of animals (Weller et al., 2016).

Afforestation has been used in Australia and New Zealand to control *Nassella* especially in cases where agriculture was no longer possible due to heavy invasions (Campbell, 1982; Healy, 1945; Jones et al., 2000; Miller, 1998). However, it takes up to six years to shade-out the weed and prevent flowering, and another four years before the tussocks die (Campbell, 1982; Miller, 1998; Wells, 1977). It would take many more years for the seedbank to be depleted. To speed up the establishment of tree seedlings, Flupropanate can be sprayed to kill *Nassella* without affecting the tree seedlings (McLaren et al., 2008). As noted, before, *Nassella* species invade grassland ecosystems, and afforestation is neither always possible, practical nor desirable in these ecosystems. Tree species that could potentially be used for such purposes are mainly non-native species that themselves cause major problems as invasive species (Richardson et al., 2020). All these reasons make afforestation an unsuitable control option for South Africa.

Native species may lack or have transient seed banks which limits their recruitment and re-establishment, hence are not efficient at naturally colonising open spaces (Morgan, 2001). Experiments in Australia concluded that native grasses have low survival rate and natural densities making them poor competitors against *Nassella* spp. (Morgan, 2001). Seeding of pastures with native grass species after *Nassella* removal has, therefore been suggested as a possible solution to cover the bare ground left after *Nassella* removal (Cooperative Research Centres, 2003; Miller, 1998). However, seedlings have little to no effect in smothering *Nassella* since most of the native grasses grow much slower than *Nassella* (Faithfull, 2012; Grech et al., 2012). Unlike most C_3 grasses, *Nassella* spp. are more drought hardy and tolerate poor soils better than most good pasture grasses, hence for an effective seeding method to work a grass with similar or better traits has to be found to suppress *Nassella* both in the short and long term (Grech et al., 2012; Miller, 1998).

Burning the dead organic material on the soil surface may destroy between 18% to 20% of the *Nassella* seeds present in the topsoil (Faithfull, 2012; Joubert, 1984), suggesting that this treatment may be used to reduce the seed bank, bearing in mind that fire also stimulates seed germination. However, although *Nassella* grasses burn readily, they also recover quickly especially if the tuft is not burnt completely, while associated native species are often killed (Healy, 1945). Fire also reduces the seed bank of good pasture species in the soil leaving the ground bare and creating ideal conditions for reinvasion by *Nassella* species (Campbell, 1982; Joubert, 1984; Wells and De Beer, 1987). Burning as a control measure may, therefore, inadvertently increase rather than decrease *Nassella* populations. More research is required to ascertain how different regimes of burning affect *Nassella* invasion dynamics (Badgery, 2004). *Nassella* often invades fire adapted natural grassland ecosystems dominated by *T. triandra*, in both Australia and South Africa (Faithfull, 2012; Faithfull et al., 2012) which makes fire exclusion as a control method unviable. The hot fires that would eventually result after years of fire exclusion, are often not a problem for *Nassella*, which always has some seeds left unburnt due to high seed bank, and seeds can be reintroduced by wind from distant areas and take advantage of the reduced cover (Badgery, 2004; Faithfull, 2012; Healy, 1945).

Mechanical control

Small, low-density patches and isolated plants can be controlled by hand weeding or digging when equipment is cleaned meticulously and the clippings are destroyed (Cooperative Research Centres, 2003; Healy, 1945; Taylor et al., 2016; Wells, 1977). However, the potential exists for exacerbating the invasions (Faithfull 2012; Taylor 2016). These methods are of little use in natural grasslands where native grasses such as *T. triandra* have vulnerable growing points and lack the ability to grow horizontal tillers compared to *Nassella* which, although lacks horizontal tillers, produces large numbers of long-lived seeds which take-up any open spaces quickly (Faithfull et al., 2012). Grubbing as a management tool cannot eradicate *Nassella* but can reduce densities or keep population levels static (Bourdôt et al., 1992; Smith and Lamoureaux, 2006).

Grubbing was also found to be more effective against medium sized tufts as opposed to large and small plants, but more research is required to determine the best time to perform it based on *Nassella* population dynamics (Bourdôt and Saville, 2019; Smith and Lamoureaux, 2006).

Cultivating or ploughing has been suggested as a possible control mechanism for managing *Nassella* invasions (Wells, 1977). *Nassella* seeds are concentrated in the top 2.5 cm of the soil (Bourdôt and Hurrell, 1992; Faithfull, 2012; Joubert, 1984), so ploughing has the potential to bury them to depths they cannot emerge from (Campbell, 1982), but this has to be done with care since the seeds can attain a state of permanent dormancy (Bourdôt and Hurrell, 1992). However, ploughing may inadvertently exacerbate the problem by bringing deep-buried seeds to the surface where they can germinate (Wells, 1977; Westbrooks & Cross, 1993). For this method to be successful, ploughing must be done when the soil is dry and must be repeated every year for many consecutive years until the soil seed bank has been exhausted (Bourdôt and Hurrell, 1992; Wells, 1977). In Australia, cultivation has been used successfully in areas where the land is arable and soil fertility is reasonable. Foraging crops are planted for 2-3 years to reduce the soil seedbank, followed by sowing of competitive pasture species (Campbell, 1998). However, *Nassella* is often found in areas which are rocky and difficult to access, making ploughing futile (Grech et al., 2012). In natural rangelands, such as those being invaded by *Nassella* in South Africa, ploughing is undesirable as it would have major impacts on forb diversity (which is the richest component of grassland flora).

Chemical control

Several herbicides have been used to manage *Nassella* invasions in different parts of the world. Most of the herbicides are based on formulations that include glyphosate or flupropanate as active ingredients (Grech et al., 2012; Viljoen, 1987; Wells and De Beer, 1987). Flupropanate has been championed as the panacea for *Nassella* invasions for many years because it was shown to have over 90% efficiency in *Nassella* over a wide range of environmental conditions (Campbell, 1998; Conolly and Taylor, 2016; Viljoen, 1999, 1987; Wells, 1977). Although it can take more than a year to kill the plants completely, it is easy to use, is non-toxic to mammals and was once considered highly selective, especially when applied to young *Nassella* plants (Campbell and Ridings, 1988; Viljoen, 1987; Wells and De Beer, 1987). However, recent research shows that it is not as specific as previously thought as it impacts many native species (Campbell and Van de

Ven, 1996; Grech et al., 2014; Lusk et al., 2017). Glyphosate has also been used extensively and has been shown to be effective against seed-head production (Campbell, 1998), but it is a broad-spectrum herbicide that is indiscriminate in its impacts on non-target species (Grech et al., 2012b). While glyphosate and flupropanate are effective independently, their effectiveness will be reduced if they are mixed (Taskforceherbicide, 2020).

Viljoen (1987) found that flupropanate was effective at lower rates than recommended with less effect on non-target species, which also greatly reduced costs (Campbell, 1998; Campbell and Ridings, 1988; Viljoen, 1987; Wells and De Beer, 1987). Other researchers also reported that once applied, the chemical has a long residual action that prevents germination and establishment of seedlings for 3 to 5 years, (Campbell, 1998; Viljoen, 1999, 1987). However, recent studies seem to suggest that these results may not always hold true. For example, flupropanate has been shown to negatively impact beneficial pasture grasses and forbs even at the recommended dosage (Grech et al., 2014; Lusk et al., 2017). Furthermore, research in New Zealand showed that the residual effect was shorter than widely believed (Bourdôt et al., 2017).

Application of flupropanate was found to increase both bare ground and broad leaf weeds especially in grazed areas (Grech et al., 2014; Lusk et al., 2017; Wells and De Beer, 1987). This can lead to reinvasion by *Nassella* once the residual effect has diminished (Badgery et al., 2008; Lusk et al., 2017) and so follow-up spraying is required (Grech et al., 2012). However, follow-up spraying would have to be done over many years to deplete the seedbank, making re-spraying uneconomical (Viljoen, 1987). The increase in bare ground, (Fig. 2.4), and in broadleaved species reduces pasture palatability and quality (Grech et al., 2014); this could be addressed by seeding treated areas with productive pasture species.

Fig. 2.4. A patch of *Nassella trichotoma* treated with aerial spraying of flupropanate in the Boschberg near Somerset East, Eastern Cape province, South Africa showing the bare ground left as *Nassella* dies Photo: A. Mapaura.

An increasing number of *N. trichotoma* populations are reportedly becoming resistant to flupropanate in Australia due to continuous use of the herbicide over long periods (Grech et al., 2012; McLaren and Anderson, 2011a; Powells, 2018). Flupropanate resistance in South Africa has not yet been investigated.

Biological control

There has been a perception that the uniform and simplistic architecture of grasses and lack of or limited secondary metabolites in grasses makes polyphagy common, limiting the use of classical biological control (hereafter referred to as biocontrol) for grasses in general (Evans, 1991). A further deterrent has been the concern over potential non-target damage on important grass crops (Briese et al., 2000). However, a recent study by Sutton et al. (2019) has demonstrated that many grasses have suitably specific and damaging herbivores to warrant consideration for biocontrol.

A biocontrol programme was initiated on *Nassella* in South Africa and the first surveys for herbivores were done by Wells (1977) in the native range in Argentina looking for potential biocontrol agents to be released on *N. trichotoma*. This survey determined high levels of herbivory, and a follow up survey by Erb (1988) identified ten species of weevil (Coleoptera) and two moth species (Lepidoptera). However, none of these herbivores were found to be suitably host-specific and this programme was discontinued.

Since then, research into biocontrol on *Nassella,* specifically *N. neesiana* and *N. trichotoma*, has been undertaken in Australia and New Zealand. Surveys in the native range in Argentina since 1995 have found an additional 22 potential agents (Table 2.2). To date, only one of these agents, a fungus (*Uromyces pencanus*), has been approved for release after completion of host-specificity testing; this agent is due for release in New Zealand pending export permits (Anderson et al. 2017). Many of the potential agents on *Nassella* have been found to be host-specific to each species and therefore each *Nassella* species will likely require its own biocontrol agent.

Table 2.2. Potential and tested biocontrol agents against *Nassella* weeds.

Biocontrol agent	Type of agent	Target *Nassella* species	Notes and References
Alternaria sp. (Pleosporaceae)	Fungus	*N. trichotoma*	Not tested (McLaren and Cowan, 2012)
Ascochyta leptospira var. *variispora* (Didymellaceae)	Bacteria	*N. trichotoma*	Potential (Briese and Evans, 1998; Hussaini et al., 2000)
Corticiaceae sp.	Fungus	*N. trichotoma*	Potential (Anderson et al., 2002; Briese and Evans, 1998; McLaren and Cowan, 2012; McLaren and Anderson, 2011b)
Dinemasporium sp. (Chaetosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Hussaini et al., 2000)

Biocontrol agent	Type of agent	Target *Nassella* species	Notes and References
Epicoccum sp. (Didymellaceae)	Fungus	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Fusarium oxysporum (Nectriaceae)	Fungus	*N. trichotoma*	Not effective (Hussaini et al., 2000; McLaren and Cowan, 2012)
Hendersonula sp. (Botryosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Mucor sp. (Mucoraceae)	Fungus	*N. trichotoma*	Not tested (McLaren and Cowan, 2012)
Paratrichodorus sp. (Trichodoridae)	Nematode	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Phytophthora cryptogea (Pythiaceae)	Fungus	*N. trichotoma*	(McLaren and Cowan, 2012)
Puccinia graminella (Pucciniaceae)	Fungus	*N. neesiana*	Not effective (Anderson et al., 2011, 2006)
Puccinia nassellae (Pucciniaceae)	Fungus	*N. neesiana* *N. trichotoma*	Not host specific and not sufficiently pathogenic (Anderson et al., 2002; Anderson et al., 2011, 2006; McLaren and Cowan, 2012; McLaren and Anderson, 2011b)
Puccinia saltensis var. saltensis (Pucciniaceae)	Fungus	*N. neesiana* *N. tenuissima*	Potential (Briese and Evans, 1998)
Rhizoctonia sp. (Ceratobasidiaceae)	Fungus	*N. trichotoma*	Not tested (Briese and Evans, 1998; McLaren and Cowan, 2012)

Biocontrol agent	Type of agent	Target *Nassella* species	Notes and References
Rotylenchus sp. (Hoplolaimidae)	Nematode	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Septoria sp. (Mycosphaerellaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Stagonospora sp. (Phaeosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Tranzscheliella spp. (Ustilaginaceae)	Fungus	*N. trichotoma* *N. neesiana*	Not sufficiently pathogenic (McLaren and Cowan, 2012; McLaren and Anderson, 2011b)
Uredo sp. (incertae familiae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Uromyces pencanus (Pucciniaceae)	Fungus	*N. neesiana*	Approved for release in New Zealand (on 22 June 2011) pending export permit. Australia requires more host range tests before introduction and release permit can be given (Anderson et al., 2006, 2010, 2011, 2017; Briese and Evans, 1998; Flemmer et al., 2010)
Ustilago hypodytes (Ustilaginaceae)	Fungus	*N. neesiana* *N. trichotoma*	Not specific (Briese and Evans, 1998)
Ustilago sp. (Ustilaginaceae)	Fungus	*N. trichotoma*	More research needed (Anderson et al., 2002; Briese and Evans, 1998)

Integrated control

Integrated control of invasive plants involves a combination of at least two primary control methods *viz*. cultural, mechanical, chemical, and biological. For many weed species, the best control is achieved when a combination of control measures is used (Cooperative Research Centres, 2003).

Wells (1978), working in South Africa, detailed an integrated strategy based on prevention of seeding by removing and burning seed heads, killing mature tussocks by chipping, ploughing, or spot-spraying and prevention of seed dispersal by planting windbreaks, keeping stock away at seeding time, maintaining a good plant cover, particularly on the veld, by not burning. This strategy is essentially the same as that advocated for Australia (Campbell, 1998; Miller, 1998), where it has been concluded that a plan to limit seed reinvasion must be in place to support other control measures, such as using appropriate growing regimes, fertilisers, and spot-spraying. Underpinning this integrated control tactic is the realisation that a competitive environment needs to be maintained to prevent (re)invasion (Campbell, 1998; Wells, 1978).

Because of the difficulty and poor performance of most conventional control methods, research into management systems that utilises *Nassella* but minimises its spread needs to be pursued (Grech et al., 2012b). To counter and reduce incidences of chemical resistance, integrated approaches which utilise different chemicals for short periods coupled with mechanical and cultural methods would be desirable (Powells, 2018; Wells, 1977). A multi-faceted, multi-disciplinary *Nassella* control program involving biological, ecological, economic, social and legal issues will probably be the most effective (Anderson et al., 2003; Early et al., 2016).

CLIMATE CHANGE AND PROJECTED FUTURE DISTRIBUTION

The frequent droughts and extreme weather events such as cyclones and extreme temperatures being witnessed in southern Africa are the manifestation of climate change (Chersich and Wright, 2019). These weather events provide invasive plants with opportunities for dispersal and growth as native species can be negatively impacted (Masters and Norgrove, 2010). With frequent droughts, grazing pressure will remove good pasture species at a rate higher than recruitment which could favour non-palatable invasive species like *Nassella* (Faithfull, 2012; Grech et al., 2012; Morgan, 2001).

South Africa is in a unique situation in that the effects of climate change are taking place concurrently with socio-economic transformation coupled with rapid change in land use brought by majority rule (Richardson et al., 2000). The effects of climate change maybe more rapid and pronounced in such a situation than would otherwise be the case. This will have serious implications on species distribution and is of particular interest in the future control of invasive species such as *Nassella*. Global distribution models for *N. neesiana* and *N. trichotoma* have been done using CLIMEX models but not for *N. tenuissima*.

Nassella neesiana

Bourdôt at al. (2012), produced a CLIMEX model of the projected global distribution of *N. neesiana*. This model showed that the distribution will vary between countries and continents. In the Northern Hemisphere, suitable areas will generally expand with some current suitable areas becoming less suitable while in the Southern Hemisphere there is a general decline in suitable areas. In South Africa, suitable areas are expected to shrink marginally with the majority of the area remaining suitable. More importantly, *N. neesiana* has not yet saturated its current suitable area, and apparently currently occupies a small portion of it.

Nassella trichotoma

A model of suitable geographic range for *N. trichotoma* under current climate conditions indicates that there is great potential for expansion (Watt et al., 2011). An estimate of the global potential distribution under projected future climate-change scenarios to 2080 using CLIMEX showed a general increase in Europe and a decrease in the Southern Hemisphere. For South Africa, the projections show the suitable area marginally decreasing and Lesotho remaining optimal (Watt et al., 2011). The trend is that the lower reaches of the mountains will become unsuitable while areas higher up will remain suitable.

DISCUSSION

Our review has highlighted the invasive nature, ecology, and major impacts that *Nassella* invasions cause in terms of biodiversity, soil productivity, and the economy based on both local and foreign literature. Dense invasions of *Nassella* could lead to huge losses and costs to the animal husbandry industry, tourism, and severe impacts on biodiversity in the temperate montane grassland regions of South Africa. Globally, mountain areas are very important and contribute immensely to biodiversity and are already under pressure from population expansion and so cannot afford the threat from these invasive species (Rahbek et al., 2019). In South Africa, these mountains are home to many endemic, range-restricted and threatened species, and the continued expansion of these invasive grass species will likely contribute to their decline. The need to understand the status of these species in South Africa and to institute control measures is important and urgent.

A major problem is that *Nassella* species are difficult to differentiate from several native tussock species and from each other. It is very likely that some of the literature on *N. trichotoma* could actually be mistakenly referring to other *Nassella* species as very little information was found for the other two species. The available identification keys rely heavily on the presence of inflorescences. The problem is that when the inflorescence appears the seeds are mature, and it is too late to prevent propagation. Easy-to-use keys that do not rely heavily on inflorescences and which make it possible to identify the species before they flower are central prerequisites to the implementation of a successful control program (Taylor et al., 2016; Wang et al., 2016). The development of such a key, its distribution in areas susceptible to *Nassella* invasions, and training of stakeholders are crucial requirements.

The use of DNA techniques to distinguish similar species is rapidly becoming a reality. In South Africa, these techniques could be useful for separating the three *Nassella* species from any morphologically similar species. Sufficient phylogenetic distance exists among the three *Nassella* species (Cialdella et al., 2014) and it was demonstrated in a pilot study in Australia that *N. neesiana* and *N. trichotoma* could be separated using the *petL* plastid gene (Wang et al., 2014). This gene needs to be sequenced in *N. tenuissima* as well. This would make it feasible to identify the species using these DNA markers.

The true extent of the current distribution and abundance of all three *Nassella* species in South Africa is not known. However, information gathered from stakeholders indicates that *Nassella* invasions have been increasing since the last published distribution records. The situation may be worse than currently thought considering that *Nassella* is highly mobile, especially the more invasive *N. trichotoma*. There is therefore urgent need to determine the current distribution and densities of *Nassella* in South Africa.

Chemical control has been the most widely used method and is considered the most effective way to keep *Nassella* invasions in check. The herbicides currently being used lack specificity, causing damage to desirable species in grassland and thus inadvertently giving *Nassella* species a competitive advantage. Also, the repeated use of flupropanate has led to chemical resistance in Australia. This development means research that integrates flupropanate into an integrated control strategy or reduces its use to a bare minimum might be required. The need for alternate integrated methods becomes even more compelling when it is considered that the chemicals on the market are not as target specific as initially thought even at the minimum recommended concentrations. This calls for more research into the best way to use these chemicals. For example, how often can spraying be repeated before it increases the possibility of resistance developing? What is the effect of environmental conditions such as rainfall, slope and wind on the efficacy of chemicals applied aerially? The possibility of incorporating adjuvants such as Citowett, Frigate and Reverseal 9 into herbicides, as suggested by Viljoen (1999), needs to be investigated.

Biological control research has highlighted a number of potential agents for invasive *Nassella* populations. Biocontrol offers a long-term and sustainable management option and can be used in concert with other control efforts. *Uromyces pencanus*, a rust fungus that has been approved in New Zealand for *N. neesiana,* can be tested using the standard procedures to verify its suitability for South Africa. Native range surveys also identified a number of agents that are specific to *N. tenuissima* and *N. trichotoma* that could be tested in South Africa.

With the predictions of climate change becoming a reality, information on the physiological responses of these invasive species to changing conditions is urgently needed. Models of currently suitable geographic areas show that *Nassella* species are far from saturating suitable areas. Most of these suitable areas are high mountainous areas, suggesting that the invasions could become a serious problem if no action is taken. Projecting into the future, existing models predict a decline in the suitable areas for *Nassella* in South Africa. The decline would likely be in the western fringes of the montane areas in the Eastern and Western Cape and the Free State provinces. To be able to effectively take action against *Nassella* invasion in South Africa, fine-scale predictions that take into account local variables are required. Such models could provide useful inputs to implementable coping strategies.

It is also important to understand the response of invasive species in relation to their co-occurring species especially as it pertains to their photosynthetic pathways. *Nassella spp.* (C_3) have been recorded invading natural montane grasslands dominated by *T. triandra* (C_4) and *Merxmuellera* (C_3) (Clark and Jr., 2019; Faithfull, 2012). Under the projected increase in temperature and atmospheric CO_2, C_3 plants are expected to become more vigorous while C_4 plants will have marginal responses since they are already operating at near maximum rate but the net gain of C_3 will be offset by the frequent droughts (Kriticos et al., 2010). The response of each specific species is affected by factors such as intra-specific and inter-species competition, herbivory pressure and management (Chen et al., 1996; Kriticos et al., 2010). These traits need to be included in species distribution models that seek to estimate responses of invasive species to climate change and hope to result in effective control programs (Chen et al., 1996).

CONCLUSIONS

The three *Nassella* species discussed in this paper are difficult to distinguish from each other and from other tussock grasses, especially when not flowering. This has had a significant effect on invasion detection and there is an urgent need for a new approach to enable the collection of more accurate distribution data. Identification guides that are accessible to non-scientists are required to encourage citizens to contribute data through platforms like iNaturalist. Current control efforts are uncoordinated and depend on the interest and the ability of individual property owners to fund control measures. As a result, efforts are failing to reduce existing invasions or prevent future spread. Invasive grasses have not generally been considered good targets for biological control however the prospects for *Nassella* spp. are promising and should be explored for South Africa. With climate change already a reality, models to predict future distribution ranges are needed to guide the prioritization of regions for management intervention.

ACKNOWLEDGEMENTS

This research was financially supported by the Department of Science and Innovation-National Research Foundation (DSI-NRF) Centre of Excellence for Invasion Biology (CIB), the Centre for Biological Control (Rhodes University), as well as the Afromontane Research Unit (ARU, University of the Free State). DMR specifically thanks the NRF (grant 85417) and the Oppenheimer Memorial Trust (grant 18576/03) for financial support to support this project through the CIB; AM thanks Rohan Kruger (Game Works Helicopters) for financial support for the project.

We thank David Currie, Dave Davenport, Danie & Phillip Erasmus, Gideon Gravenstein and Kitty and Richard Viljoen for providing valuable insights into the Nassella problems in the Eastern Cape province and allowing access to their properties. Rohan Kruger (Game Works

Helicopters) shared valuable insights on the spread and aerial control of *Nassella trichotoma*. Maryke Stern facilitated contact with farmers with invaded farms in the Eastern Cape. Sincere thanks are due to Lyn Fish and Aluoneswi Caroline Mashau for their assistance with aspects of identification and providing literature. Nicholas Le Maitre provided helpful insights on the potential use of DNA techniques for separating *Nassella* species.

REFERENCES

Anderson F, Pettit W, Briese DT, McLaren DA. 2002. Biological control of serrated tussock and Chilean needlegrass. *Plant Protection Quarterly* **17**: 104–111.

Anderson FE, Barton J, McLaren D. 2010. Studies to assess the suitability of *Uromyces pencanus* as a biological control agent for *Nassella neesiana* (Poaceae) in Australia and New Zealand. *Australasian Plant Pathology* **39**: 69–78. doi: 10.1071/AP09057.

Anderson FE, Díaz ML, Barton J, Flemmer AC, Hansen PV, McLaren DA. 2011. Exploring the life cycles of three South American rusts that have potential as biocontrol agents of the stipoid grass *Nassella neesiana* in Australasia. *Fungal Biology* **115**(4–5): 370–380. doi: 10.1016/j.funbio.2011.01.008.

Anderson FE, Díaz ML, McLaren DA. 2006. Current status of research on potential biological control agents for *Nassella neesiana* and *Nassella trichotoma* (Poaceae) in Australia. Preston C, Watts JH, Crossman ND, Editors. Proceedings of the 15th Australian Weeds Conference: 591–594. Adelaide, South Australia: Weed Management Society of South Australia.

Anderson FE, Gallego L, Sánchez RM, Flemmer AC, Hansen PV, McLaren D, Barton J. 2017. Plant/pathogen interactions observed during host range testing of the rust fungus *Uromyces pencanus*, a classical biological control agent for Chilean needle grass (*Nassella neesiana*) in Australia and New Zealand. *Biocontrol Science and Technology* **27**(9): 1096–1117. doi: 10.1080/09583157.2017.1384795.

Anderson GL, Delfosse ES, Spencer NR, Prosser CW, Richard RD. 2003. Lessons in developing successful invasive weed control programs. *Rangeland Ecology & Management / Journal of Range Management Archives* **56**(1): 2–12.

Badgery WB. 2004. Managing competition between *Nassella trichotoma* (serrated tussock) and native grasses. PhD thesis, The University of Sydney.

Badgery W, Kemp DK, MIchalk DL, King WMCG. 2005. Competition for Nitrogen between Australian Native Grasses and the Introduced Weed *Nassella trichotoma*. *Annals of Botany* **96**: 799–809. doi: 10.1093/aob/mci230.

Badgery, WB, Kemp, DR, Michalk, DL, King, WM (2008) Studies of competition between *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock) and native pastures. 2. Seedling responses. Australian Journal of Agricultural Research 59, 237-246.

Barker, N., Clark, V.R., Dold, T., 2020. F FBIP: Great Escarpment Biodiversity Research Programme data mobilisation. South African National Biodiversity Institute. Occurrence dataset https://doi.org/10.15468/qwpfu2 (accessed via GBIF.org on 19.05.2022).

Barkworth M, Arriaga M, Smith J, Jacobs S, Valdés-Reyna J, Bushman B. 2008. Molecules and morphology in South American Stipeae (Poaceae). *Systematic Botany* **33**(4): 719–731. doi: 10.1600/036364408786500235.

Barkworth M, Torres M. 2001. Distribution and diagnostic characters of *Nassella* (Poaceae: Stipeae). *Taxon* **50**(2 part 4): 439–68. doi: 10.2307/1223891.

Bläß C, Ronnenberg K, Tackenberg O, Hensen I, Wesche K. 2010. The relative importance of different seed dispersal modes in dry Mongolian rangelands. *Journal of Arid Environments* **74**: 991–997. doi:10.1016/j.jaridenv.2009.12.002.

Bourdôt G, Basse B, Kriticos D, Dodd M. 2015. Cost-benefit analysis blueprint for regional weed management: *Nassella neesiana* (Chilean needle grass) as a case study. *New Zealand Journal of Agricultural Research* **58**(3): 325–338. doi: 10.1080/00288233.2015.1037460.

Bourdôt GW, Hurrell GA. 1992. Aspects of the ecology of *Stipa neesiana* Trin. & Rupr. seeds. *New Zealand Journal of Agricultural Research* **35**(1): 101–8. doi: 10.1080/00288233.1992.10417707.

Bourdôt GW, Hurrell GA, Saville DJ. 1992. Eradication of Nassella tussock (*Nassella trichotoma*), an unlikely outcome of grubbing. *New Zealand Journal of Agricultural Research* **35**(3): 245–252. doi: 10.1080/00288233.1992.10427501.

Bourdôt GW, Jackman S, Saville DJ. 2017. Plant mortality and seedling recruitment responses to flupropanate in grassland populations of *Nassella trichotoma*. *New Zealand Plant Protection* **70**: 160–164. doi: 10.30843/nzpp.2017.70.42.

Bourdôt GW, Lamoureaux SL, Watt MS, Manning LK, Kriticos DJ. 2012. The potential global distribution of the invasive weed *Nassella neesiana* under current and future climates. *Biol Invasions* **14**(8): 1545–1556. doi: 10.1007/s10530-010-9905-6.

Bourdôt GW, Saville DJ. 2019. *Nassella trichotoma* – plant growth rates and effects of timing of grubbing on populations in North Canterbury grassland. *New Zealand Journal of Agricultural Research* **62**(2): 224–245. doi: 10.1080/00288233.2018.1483954.

Briese DT, Evans HC. 1998. Biological control of serrated tussock (*Nassella trichotoma*): is it worth pursuing? *Plant Protection Quarterly* **13**(2): 94–97.

Briese DT, McLaren DA, Pettit WJ, Zapater M, Anderson F, Delhey R, Distel R. 2000. New biological control initiatives against weeds of South American origin in Australia: *Nassella* tussock grasses and blue heliotrope. Spencer NR, Editor. Proceedings of the X International Symposium on Biological Control of Weeds: 215–223.

Campbell MH. 1982. The biology of Australian weeds. 9: *Nassella trichotoma* (Nees) Arech. *Journal of the Australian Institute of Agricultural Science* **48**(2): 76–84.

Campbell MH. 1997. Effect of low rates of flupropanate on selective removal of serrated tussock (*Nassella trichotoma* (Nees) Arech.) seedlings from a young improved pasture. *Plant Protection Quarterly* **12**: 175-176.

Campbell MH. 1998. Biological and ecological impact of serrated tussock (*Nassella trichotoma* (Nees) Arech.) on pastures in Australia. Australia: Plant Protection. Plant Protection Quarterly Report No.: 13 (2).

Campbell MH, Ridings HI. 1988. Tolerance of grazed and ungrazed *Phalaris aquatica* to glyphosate, tetrapion and 2,2-DPA. *Australian Journal of Experimental Agriculture* **28**: 747–751.

Campbell MH, Van de Van R. 1996. Tolerance of native grasses to Frenock® and Round up®. Proceedings of the Eleventh Annual Conference of the Grassland Society of NSW, 120-121.

Chersich MF, Wright CY. 2019. Climate change adaptation in South Africa: a case study on the role of the health sector. *Global Health* **15**: 22. https://doi.org/10.1186/s12992-019-0466-x

Chen D, Hunt HW, Morgan JA. 1996. Responses of a C 3 and C4 perennial grass to CO 2 enrichment and climate change: Comparison between model predictions and experimental data. *Ecological Modelling* **87**: 11–27.

Cialdella AM, Sede SM, Romaschenko K, Peterson PM, Soreng RJ, Zuloaga FO, Morrone O. 2014. Phylogeny of *Nassella* (Stipeae, Pooideae, Poaceae) Based on Analyses of Chloroplast and Nuclear Ribosomal DNA and Morphology. *Systematic Botany* **39**(3): 1–15. doi: 10.1600/036364414X681419.

Clark VR, Vidal JD. 2019. The vegetation of Asante Sana Private Game Reserve and surrounds, Sneeuberg Centre of Floristic Endemism (Eastern Cape Province, South Africa). *Esperiana Memoir* 8: 25-38.

Connor HE, Edgar E, Bourdôt GW. 1993. Ecology and distribution of naturalised species of *Stipa* in New Zealand. *New Zealand Journal of Agricultural Research* **36**(3): 301–307.

Conolly J, Taylor S. 2016. Mexican feather grass (*Nassella tenuissima*) control – the ACT experience. Randall R, Lloyd S, Borger C, Editors. 20th Australasian Weeds Conference: 228–231. Perth: Weeds Society of Western Australia.

Cook C. 1998. The transmission of serrated tussock (*Nassella trichotoma*) seeds through the sheep rumen and their viability after ingestion. Report No.: 13 (2).

Cooperative Research Centres, 2003. Alert list for environmental weed. Weed Management Guide: Chilean Needle Grass - *Nassella neesiana*. Australian Pamplet. Weed Management and the Commonwealth Department of the Environment and Heritage.

Dickens SJM, Allen EB. 2014. Exotic plant invasion alters chaparral ecosystem resistance and resilience pre- and post-wildfire. *Biological Invasions* **16**(5): 1119–1130. doi: 10.1007/s10530-013-0566-0.

Early R, Bradley BA, Dukes JS, Lawler JJ, Olden JD, Blumenthal DM, Gonzalez P, Grosholz ED, Ibanez I, Miller LP, et al. 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications* **7**(12485): 1–9.

Erb HE. 1988. *Stipa trichotoma*: some considerations on the species, its habitat and natural distribution in Argentina. Cape Town, South Africa.

Evans, H., 1991. Biological control of tropical grassy weeds, in: Baker, F.W.G., Terry, P.J. (Eds.), Tropical Grassy Weeds. CAB International, Wallingford, U.K., pp. 52–72.

Faithfull IG. 2012. Biodiversity impacts of Chilean needle grass, *Nassella neesiana* to Australia's indigenous grasslands [PhD]. [Australia]: Victoria University of Technology.

Faithfull IG, Hocking C, McLaren DA. 2012. Managing native grasslands to minimise invasion by Chilean needle grass (*Nassella neesiana*). Eldershaw V, Editor. Melbourne, Victoria, Australia: Weed Society of Victoria Inc. Available at http://www.caws.org.au/awc/2012/awc201210591.pdf (Accessed 08.10.2012).

Fish L, Mashau AC, Moeaha MJ, Nembudani MT. 2015. Identification Guide to Southern African Grasses: An Identification Manual with Keys, Descriptions, and Distributions. Pretoria: SANBI. (Strelitzia; Vol. 36).

Flemmer AC, Anderson FE, Hansen PV, McLaren DA. 2010. Microscopic observations of a compatible host/pathogen interaction between a potential biocontrol agent (*Uromyces pencanus*) and its target weed (*Nassella neesiana*). *Mycoscience* **51**(5): 396–400. doi: 10.1007/S10267-010-0051-X.

Germishuizen G, Meyer NL, NBI. 2003. *Plants of Southern Africa: An Annotated Checklist*. Pretoria, South Africa: National Botanical Institute.

Global Invasive Species Database (GISD). 2019 Apr 18. Species profile *Nassella tenuissima*. Available at http://www.iucngisd.org/gisd/species.php?sc=463 (Accessed 08.10.2019).

Grech C, Threlfall T, Fridman M, McLaren D. 2012a. Alternative herbicides for flupropanate resistant serrated tussock. Eldershaw V, Editor. Developing solutions to evolving weed problems; Melbourne, Victoria, Australia.

Grech CJ, McLaren DA, Lowien J, McWhirter L, Butler KL, Sindel BM. 2012b. Assessment of management options for Chilean needle grass in sheep-grazing systems. *Grass and Forage Science* **69**: 119–128.

Grech CJ, McLaren DA, Lowien J, McWhirter L, Butler KL, Sindel BM. 2014. Effects of flupropanate application on bare ground and broadleaf weeds when used to control Chilean needle grass in introduced pastures. *New Zealand Journal of Agricultural Research* **57**(2): 100–109. doi: 10.1080/00288233.2014.886597.

Hanley N, Roberts M. 2019. The economic benefits of invasive species management. *People and Nature* **1**(2): 124–137. doi: 10.1002/pan3.31.

Healy AJ. 1945. *Nassella* tussock (*Nassella trichotoma* (Nees.) Hack.). Field Studies and their Agricultural Significance. *Department of Science and Industrial Research* **Bulletin No. 91**(22): 1–90.

Henderson L. 2001. *Alien Weeds and Invasive Plants: A Complete Guide to Declared Weeds and Invaders in South Africa*. Pretoria, South Africa: Agricultural Research Council. (Plant Protection Research Institute Handbook no. 12).

Henderson L. 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia* **37**(2): 215–248.

Henderson L. 2018. Shocking *Nassella* invasion threatens South Africa's mountain grasslands. Pretoria: ARC –Plant Protection Research Institute. SAPIA News Report No.: 48.

Henderson L, Wilson JRU. 2017. Changes in the composition and distribution of alien plants in South Africa : an update from the Southern African Plant Invaders Atlas. *Bothalia - African Biodiversity and Conservation* **47**(2): 1–26. doi: 10.4102/abc.v47i2.2172.

Howell CJ, Sawyer JWD. 2006. *New Zealand Naturalised Vascular Plant Checklist*. Wellington: New Zealand Plant Conservation Network.

Hussaini IP, Lawrie AC, McLaren DA. 2000. Pathogens on and variation in *Nassella trichotoma* (Poales: Poaceae) in Australia. Spencer NR, Editor. Proceedings of the X International Symposium on Biological Control of Weeds: 269–280. Montana, USA.

Jacobs SWL, Everett J, Torres MA. 1998. *Nassella tenuissima* (Gramineae) recorded from Australia, a potential new weed related to serrated tussock. *Telopea* **8**(1): 41–46.

Jones RE, Vere DT, Campbell MH. 2000. The external costs of pasture weed spread: an economic assessment of serrated tussock control. *Agricultural Economics* **22**(1): 91–103.

Joubert DC. 1984. The soil seed bank under *Nassella* tussock infestations at Boschberg. *South African Journal of Plant and Soil* **1**(1): 1–3.

Kriticos DJ, Crossman ND, Ota N, Scott JK. 2010. Climate change and invasive plants in South Australia. Canberra, Australia: CSIRO Climate Adaptation Flagship.

Kriticos DJ, Lamoureaux S, Bourdot GW, Pettit W. 2004. *Nassella* tussock: current and potential distributions in New Zealand. *New Zealand Plant Protection* **57**: 81–88.

Lamoureaux S, Bourdôt GW. 2002. Fruit viability on grubbed *Nassella trichotoma* (Nees) tussocks. *Australian Journal of Experimental Agriculture* **42**: 1057–61. doi: 10.1071/ea02032.

Lamoureaux S, Bourdôt GW, Verkaaik M. 2006. Outsmarting *Nassella* tussock – answers to often-asked questions based on recent research findings. Preston C, Watts JH, Crossman ND, Editors. Proceedings of the 15th Australian Weeds Conference; Adelaide, Australia. Weed Management Society of South Australia.

Lusk CS, Hurrell GA, Saville DJ, Bourdôt GW. 2017. Changes in plant species composition after flupropanate application for *Nassella* tussock control, in Canterbury hill-country pastures. *New Zealand Journal of Agricultural Research* **60**(3): 263–276. doi: 10.1080/00288233.2017.1321556.

Masters G, Norgrove L. 2010. Climate change and invasive alien species. *CABI Working Paper*: 1–30.

McLaren D, Anderson FE. 2011. Protecting Australia's Agricultural Grasslands from Herbicide-Resistant Serrated Tussock. RIRDC Publication. Report No.: Pub. No. 11/070. Available at https://www.agrifutures.com.au/product/protecting-australia-s-agricultural-grasslands-from-herbicide-resistant-serrated-tussock (Accessed 08.10.2019).

McLaren D, Cowan R. 2012. Biological control of serrated tussock. North Sydney: Meat and Livestock Australia Limited. Report No.: Final Report.

McLaren DA, Anderson FE. 2011a. Protecting Australia's Agricultural Grasslands from Herbicide-Resistant Serrated Tussock. RIRDC Publication. Report No.: Pub. No. 11/070. Available at https://www.agrifutures.com.au/product/protecting-australia-s-agricultural-grasslands-from-herbicide-resistant-serrated-tussock (Accessed 08.10.2019).

McLaren DA, Anderson FE. 2011b. Biological control of serrated tussock and chilean needle grass. Rural Industries Research and Development Corporation, Canberra, Australia. Report No.: Pub. No. 11/040.

McLaren DA, Snell K, Butler KL. 2008. An assessment of native tree susceptibility to the simulated aerial application of the herbicide flupropanate, for management of exotic unpalatable grasses. van Klinken RD, Osten VA, Panetta FD, Scanlan JC, Editors. Hot Topics in the Tropics; Queensland, Australia. Queensland Weeds Society.

McLaren DA, Stajsic V, Gardener MR. 1998. The distribution and impact of South/North American stipoid grasses (Poaceae: Stipeae) in Australia. 1998.

McLaren DA, Stajsic V, Iaconis L. 2004. The distribution impacts and identification of exotic stipoid grasses in Australia. *Plant Protection Quarterly* **19**(2): 59–66.

Miller L. 1998. Management of serrated tussock in farming areas. *Plant Protection Quarterly* **13**(2): 91–93.

Milton SJ. 2004. Grasses as invasive alien plants in South Africa. *South African Journal of Science* **100**: 69–75.

Moretto AS and Distel RA. 1998. Requirement of vegetation gaps for seedling establishment of two unpalatable grasses in a native grassland of central Argentina. *Australian Journal of Ecology* **23**: 419-423.

Morgan JW. 2001. Seedling recruitment patterns over 4 years in an Australian perennial grassland community with different fire histories. *Journal of Ecology* **89**: 908–919. doi:10.1111/j.1365-2745.2001.00617.x.

Nkuna KV, Visser V, Wilson JRU, Kumschick S. 2018. Global environmental and socio-economic impacts of selected alien grasses as a basis for ranking threats to South Africa. *NB* **41**: 19–65. doi: 10.3897/neobiota.41.26599.

Occdownload Gbif.Org. 2019. GBIF Occurrence Download- *Nassella tenuissima*. The Global Biodiversity Information Facility. doi: 10.15468/DL.YPBDSK.

Powells J. 2018. Flupropanate resistant serrated tussock (*Nassella trichotoma*) identified at multiple locations in the Monaro, New South Wales. Johnson S, Weston L, Wu H, Auld B, Editors. 21st Australasian Weeds Conference: 397. The Weed Society of New South Wales Inc.

Pyšek P, Pergl J, van Kleunen M et al. 2020. South Africa as a donor of naturalized and invasive plants to other parts of the world. In: van Wilgen BW, Measey J, Richardson DM, Wilson JR, Zengeya TA. Editors. *Biological invasions in South Africa*. Springer, Berlin, pp 755–782. doi.org/10.1007/978-3-030-32394-3_26.

Pyšek P, Richardson DM, Rejmánek M, Webster GL, Williamson M, Kirschner. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon* 53: 131–143. doi:10.2307/4135498.

Quattrocchi U. 2000. CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. Vol. 3: M - Q. Boca Raton, FL: CRC Press.

Rahbek C, Borregaard MK, Colwell RK, Dalsgaard B, Holt BG, Morueta-Holme N, Nogues-Bravo D, Whittaker RJ, Fjeldså J. 2019. Humboldt's enigma: What causes global patterns of mountain biodiversity? *Science* **365**(6458): 1108–1113. doi: 10.1126/science.aax0149.

Randall RP. 2017. *A Global Compendium of Weeds*. Third. Western Australia: R.P. Randall.

Ranwashe P. 2019. BODATSA: Botanical Collections. Available at http://ipt.sanbi.org.za/iptsanbi/resource?r=brahms_online&v=1.4 (Accessed 27.07.2019).

Richardson DM, Bond WJ, Dean WRJ, Higgins AI, Midgley G, Milton SJ. 2000. Invasive alien species and global change: A South African perspective. In: Mooney HA, Hobbs RJ, editors. *Invasive Species in a Changing World*. Washington, DC: Island Press. p. 303–350.

Richardson DM, Foxcroft LC, Latombe G, Le Maitre DC, Rouget M, Wilson JRU. 2020. The biogeography of South African terrestrial plant invasions. In: Van Wilgen BW, Measey J, Richardson DM, Wilson JRU& GJ, Zengeya T, Editors. *Biological Invasions in South Africa*. Springer, Berlin p. 67-96.

Richardson DM, Wilson JRU, Weyl OLF, Griffiths CL. 2011. South Africa: Invasions. In: Simberloff D, Rejmánek M, Editors. *Encyclopedia of Biological Invasions*. Berkeley: University of California Press. p. 643–651.

Romaschenko K, Peterson P, Soreng R, Garcia-Jacas N, Futorna O, Susanna A. 2012. Systematics and evolution of the needle grasses (Poaceae: Pooideae: Stipeae) based on analysis of multiple chloroplast loci, ITS, and lemma micromorphology. *Taxon* 61: 18–44. doi:10.1002/tax.611002.

Romaschenko K, Peterson PM, Soreng RJ, Garcia-Jacas N, Futorna O, Susanna A. 2008. Molecular phylogenetic analysis of the American Stipeae (Poaceae) resolves *Jarava* sensu lato polyphyletic: evidence for a new genus, *Pappostipa*. *Journal of the Botanical Research Institute of Texas* **2**(1): 165–192.

Smith LP, Lamoureaux SL. 2006. When science meets policy – *Nassella* tussock in New Zealand as a case study. 15th Australian Weeds Conference proceedings: 450–453. Weed Management Society of SA.

Soreng R, Peterson P, Davidse G, Judziewicz E, Zuloaga F, Filgueiras TS, Morrone O. 2009. Tropicos - Name Search. Available at http://www.tropicos.org/NameSearch.aspx (Accessed 08.05.2019).

Sutton GF, Canavan K, Day MD, den Breeyen A, Goolsby JA, Cristofaro M, McConnachie A, Paterson ID. 2019. Grasses as suitable targets for classical weed biological control. *BioControl* 64: 605–622. doi: 10.1007/s10526-019-09968-8.

Taylor NJ. 1987. Ecological aspects of *Nassella* tussock (*Stipa trichotoma*). Botany Division, DSR, Lincoln, New Zealand.

Taylor S, Conolly J, Gruber A. 2016. Chilean needle grass (*Nassella neesiana*) control – the ACT experience. Randall R, Lloyd S, Borger C, Editors. 20st Australasian Weeds Conference: 223–227. Perth: Weeds Society of Western Australia.

Vere DT, Campbell MH. 1984. Economics of Controlling Serrated Tussock in the Southeastern Australian Rangelands. *Journal of Range Management* **37**(1): 87–93.

Verloove F. 2005. A synopsis of *Jarava* Ruiz & Pav. and *Nassella* E. Desv. (*Stipa* L. s.l.) (Poaceae: Stipeae) in southwestern Europe. *Candollea* **60**(1): 97–117.

Viljoen BD. 1987. Effect of rate and time of application of tetrapion on nassella tussock (*Stipa trichotoma* Nees) in South Africa. *South African Journal of Plant and Soil* **4**(2): 79–81.

Viljoen BD. 1999. Influence of adjuvants on tetrapion efficacy for nassella tussock (*Stipa trichotoma* Nees) control and subsequent seedling establishment: a preliminary study. *South African Journal of Plant and Soil* **16**(2): 102–105. : doi: 10.1080/02571862.1999.10634855.

Visser V, Wilson JRU, Canavan K, Canavan S, Fish L, Le Maitre D, Nänni I, Mashau C, O'Connor T, Ivey P, et al. 2017. Grasses as invasive plants in South Africa revisited: Patterns, pathways and management. *Bothalia* **47**(2): 29 pages. doi: 10.4102/abc.v47i2.2169.

Visser V, Wilson JRU, Fish L, Brown C, Cook GD, Richardson DM. 2016. Much more give than take: South Africa as a major donor but infrequent recipient of invasive non-native grasses. *Global Ecology and Biogeography* **25**: 679–692. doi: 10.1111/geb.12445.

Wang A, Gopurenko D, Wu H, Stanton R, Lepschi BJ. 2014. DNA barcoding for identification of exotic grass species present in eastern Australia. Baker M, Editor. 19th Australasian Weeds Conference (2014): 444–447. Tasmanian Weed Society.

Wang A, Wu H, Gopurenko D. 2016. Chloroplast genome of serrated tussock (*Nassella trichotoma*): structure and evolution. Randall R, Lloyd S, Borger C, Editors. 20st Australasian Weeds Conference: 152–156. Perth: Weeds Society of Western Australia.

Watt MS, Kriticos DJ, Lamoureaux SL, Bourdôt GW. 2011. Climate change and the potential global distribution potential of serrated tussock (*Nassella trichotoma*). *Weed Science* **59**(4): 538–545. doi: 10.1614/WS-D-11-00032.1.

Weller SL, Florentine S, Sillitoe JF, Grech C, McLaren D, Chauhan BS. 2016a. Detecting the seeds of *Nassella neesiana* in large round hay bales, by means of non-destructive core sampling. *PLoS One* **10**(9): 1–15. doi: 10.1371/journal.pone.0137343.

Weller SL, Florentine SK, Sillitoe JF, Grech CJ, McLaren DA. 2016b. An investigation of the effects of stage of ensilage on *Nassella neesiana* seeds, for reducing seed viability and injury to livestock. *Scientific Report* **6**(22345): 1–7. doi: 10.1038/srep22345.

Wells MJ. 1977. Progress with research on *Nassella* tussock. Proceedings of the Second National Weeds Conference of South Africa: 47–55. Stellenbosch, Cape Town.

Wells MJ. 1978. *Nassella* tussock. In: Stirton CH, Editor. *Plant Invaders: Beautiful but Dangerous*. Cape Town, South Africa: Department of Nature and Environmental Conservation of the Cape Provincial Administration. p. 140–143.

Wells MJ, De Beer H. 1987. *Nassella* Tussock. *Agricultural Research Council* Weeds A.21/1987.

Wells MJ, Engelbrecht VM, Balsinhas AA, Stirton CH. 1983. Weed flora of South Africa 2: power shifts in the veld. *Bothalia* **14**(3/4): 961–965.

Westbrooks RG, Cross G. 1993. Serrated Tussock (*Nassella trichotoma*) in the United States. *Weed Technology* **7**(2): 525–528.

CHAPTER 3

THE IMPACT OF *NASSELLA TRICHOTOMA* (NEES) HACK. EX ARECHAV. ON PLANT DIVERSITY, RICHNESS AND SOIL PROPERTIES IN SOUTH AFRICA

ABSTRACT

Nassella trichotoma (Nees) Arech. is a highly invasive South American grass that is invading montane grasslands in the Eastern and Western Cape provinces of South Africa. Although *N. trichotoma* has been a recognised major problem in these mountains for decades, the primary focus and concern has been on impacts on rangeland productivity and management, while the impacts of invasions on plant biodiversity have not been considered. As a beginning to understanding *N. trichotoma* impact on local plant biodiversity, 81 pairs of plots (total 162 plots) were laid out in part of the Eastern Great Escarpment grasslands in the Eastern Cape, in March 2020 and March 2021 at altitudes from 1334 m to 1850 m. Paired plots were chosen so that they had similar environmental conditions (aspect, elevation, rockiness, etc.), with one plot being in a *N. trichotoma* invasion and the other not. Paired plots were located no further than 5 m of each other. A total of 20 *N. trichotoma* patches were sampled with two to four plot pairs per site. In each plot, all vascular plant species were recorded, and the cover abundance of each species per plot was visually estimated. The ANOVA function in the R-package, 'mvabund', was used to infer the impact of *N. trichotoma* on other species. Generalised mixed effect models and linear mixed effect models were used to infer the effect of invasion on diversity and soil variables. Species diversity was higher in uninvaded plots than invaded plots ($p<0.05$). The native *Tenaxia disticha* ($p < 0.01$), *Pentaschistis airoides* ($p < 0.01$), and *Themeda triandra* ($p < 0.001$), were significantly less abundant in invaded plots. Uninvaded areas had more species diversity and fewer exotic species than invaded areas. Topsoil minerals were significantly higher in invaded plots than in uninvaded plots. The results indicate that *N. trichotoma* is a powerful driver of biodiversity erosion in these montane grasslands and should be a cause for major concern by landowners and government.

INTRODUCTION

Invasive alien plant species (IAPS) are a major concern globally because they drive biodiversity loss, adversely alter ecosystems, and can negatively impact economies in areas they have invaded (Afreen et al., 2018; Aguilera et al., 2010; Kiełtyk and Delimat, 2019; Kumar Rai and Singh, 2020; López Rosas et al., 2005; Power and Sánchez Vilas, 2020). The type and severity of impacts are perceived differently by different stakeholders and according to ecosystems. There are also case studies where invasions have resulted in positive impacts to ecosystems and communities (Dong et al., 2015; Ehrenfeld, 2003; Kumar Rai and Singh, 2020). Some IAPS act as important food sources for fauna (especially during times of drought), are used as building material, or harvested for their medicinal properties (particularly by rural communities).

Understanding the impact of invasive species is important when deciding which species to target for management considering the limited resources available (Kumschick et al., 2015). Often IAPS attract different research attention depending on how morphologically distinctive they are, with some very damaging species facing very little attention. For example, trees or shrubs invading a grassland are more likely to attract notice compared to a grass species invading a grassland. The recently developed Environmental Impact Classification for Alien Taxa (EICAT) scheme, which relies on scientific information, may assist in reducing this bias through standardising impact assessments across species (Kumschick et al., 2017; Volery et al., 2020).

The impact of IAPS on native vegetation has been shown to vary from one invasive species to another (Hejda et al., 2017, 2009; Richardson and Pyšek, 2006) with the greatest impact most often being exerted by those species which create monocultures and have higher densities than native species (Hejda et al., 2009; Hejda and Pyšek, 2006). Plant invasions can reduce native plant and animal species diversity, richness and evenness to varying degrees (Hejda et al., 2009; Kiełtyk and Delimat, 2019; López Rosas et al., 2005) as well as alter soil physiochemical status (Afreen et al., 2018; Stefanowicz et al., 2018; Vanderhoeven et al., 2005; Vila et al., 2006).

Soil is an important component of an ecosystem as it offers mechanical support to life, a source of water and nutrients, and well as heat which is important in the rooting zone of plants (Onwuka, 2018). Invasive alien plants can alter the amount and form of soil nutrients that are available to plants on invaded sites (Stefanowicz et al., 2018). This can lead to a novel ecosystem which can lead to extinction of some species from the invaded sites. As demonstrated by Dassonville et al. (2008), the degree to which an IAP alters soil depends on the levels of nutrients in the soil before invasion, with nutrient-rich soils being impacted less than soils with poor initial nutrient levels.

Invasive alien plant impact studies are either experimental, observational, or modelling based (Stricker et al., 2015). Experimental studies usually require a long timeframe with considerable number of resources to perform. Often these two factors are limiting, and most field research has been observational. Observational research in invasion biology generally uses a space-for-time substitution approach in which invaded and uninvaded areas are compared (Thomaz et al., 2012). A major challenge in understanding long-term processes by looking at contemporary patterns involves separating cause from effect (Walker and Smith, 1997). Recent research has tried to reduce this challenge by comparing sites that are very close together and whose history before invasion is known. Notwithstanding the uncertainties that may exist, observational research provides a rapid and reliable way of understanding invasion impacts with fewer resources and is often the first step before experimental methods. Modelling approaches are newer compared to the other two but are rapidly gaining momentum. However, they are currently riddled with many uncertainties and hence require further development before they become mainstream (Corrales et al., 2020). Another challenge of modelling approaches is that they require quality field collected data which is often not available or difficult to acquire due to funding challenges (Corrales et al., 2020). This makes observational research the “go to” method at the moment.

One of the major invaders in South African high elevation montane grasslands in the ‘Cape Midlands’ area is *Nassella trichotoma*. *Nassella trichotoma* has been recognised as a major problem since the 1970s. The primary focus and concern of its invasion has been on impacts on rangeland productivity and management, while the impacts of invasions on the environment have not been considered. An active government-facilitated control programme was implemented in the 1980s but came to a stop when funding for the program ended in 2000 (Henderson, 2018). Since then, uncoordinated management by individual farmers has been going on. However, farmers with heavy infestations are finding it difficult to manage partly due to the high costs of management and because the invader comes back much more vigorously after it has been cleared, forming thick monocultures (Henderson, 2018).

Nassella trichotoma invasions take over entire natural and planted pastures (Badgery et al., 2008; Joubert, 1984; Wells, 1977). *Nassella trichotoma* is known to reduce native plant diversity in areas that it invades in Australia (Badgery et al., 2008). Most of what is known about *N. trichotoma* invasion impact on native species is based on research conducted in Australia and New Zealand, countries which have areas that are climatically similar to South Africa. However, it is important to also conduct local research to ensure that evidence used is scientifically based and relevant to conditions in South Arica. Interventions should be research-based, because some control methods may unintentionally exacerbate invasions rather than reduce them (Hejda and Pyšek, 2006; Torres et al., 2018).

During preliminary field surveys, the establishment and expansion of *N. trichotoma* invasion was noted to start from an area of initial invasion and expand outwards systematically creating an invasion front. This pattern of spread is generally found in plants that reproduce vegetatively. *Nassella trichotoma*, however, produces seeds that are dispersed by several agents, including animals and wind, and it has also been demonstrated that the seeds are present in adjacent grasslands where plants are absent (Joubert, 1984). The reason for that mode of invasion in *N. trichotoma* is not known but it suggests that most of the seeds fall close to the parent plants. Faithfull (2012) postulated that, for *N. neesiana* in Australia, the cause could be due to its ability to deplete soil moisture in spring, which kills native grasses thus eradicating competition in its immediate vicinity. It has been noted that once it has established in an area, it is difficult to eradicate as it quickly re-establishes on previously invaded areas (Wells and De Beer, 1987). It is suspected that the ability of *N. trichotoma* to quickly recolonise areas it has been cleared from may be due to altered soil chemical properties. A better understanding of any soil alteration by *Nassella* requires further studies on more soil parameters than the usual nitrogen, carbon and water cycles (Badgery et al., 2008; Ehrenfeld, 2003; Stricker et al., 2015). This research looked at 18 soil variables which are important to plant health. Considering the foregoing, the hypotheses of this study include that *N. trichotoma* invasion is (a) causing a reduction in native plant species richness and diversity, and (b) changing soil nutrient pools in invaded areas.

MATERIALS AND METHODS

Study site

This research was carried out in the high elevation grasslands of the Sneeuberg, Cape Midlands Escarpment, in the Eastern Cape province of South Africa (Fig. 3.1). The main activity in the study area is natural rangeland-based livestock and game farming, with cattle and sheep being the main livestock (Boardman et al., 2003). Historically, these high elevation grasslands have been overgrazed (Clark et al., 2009) which has led to localised extinction of some plant species (Palmer, 1990). The Sneeuberg is floristically rich, having high local plant endemism and was designated as a Centre of Floristic Endemism by Clark et al. (2009).

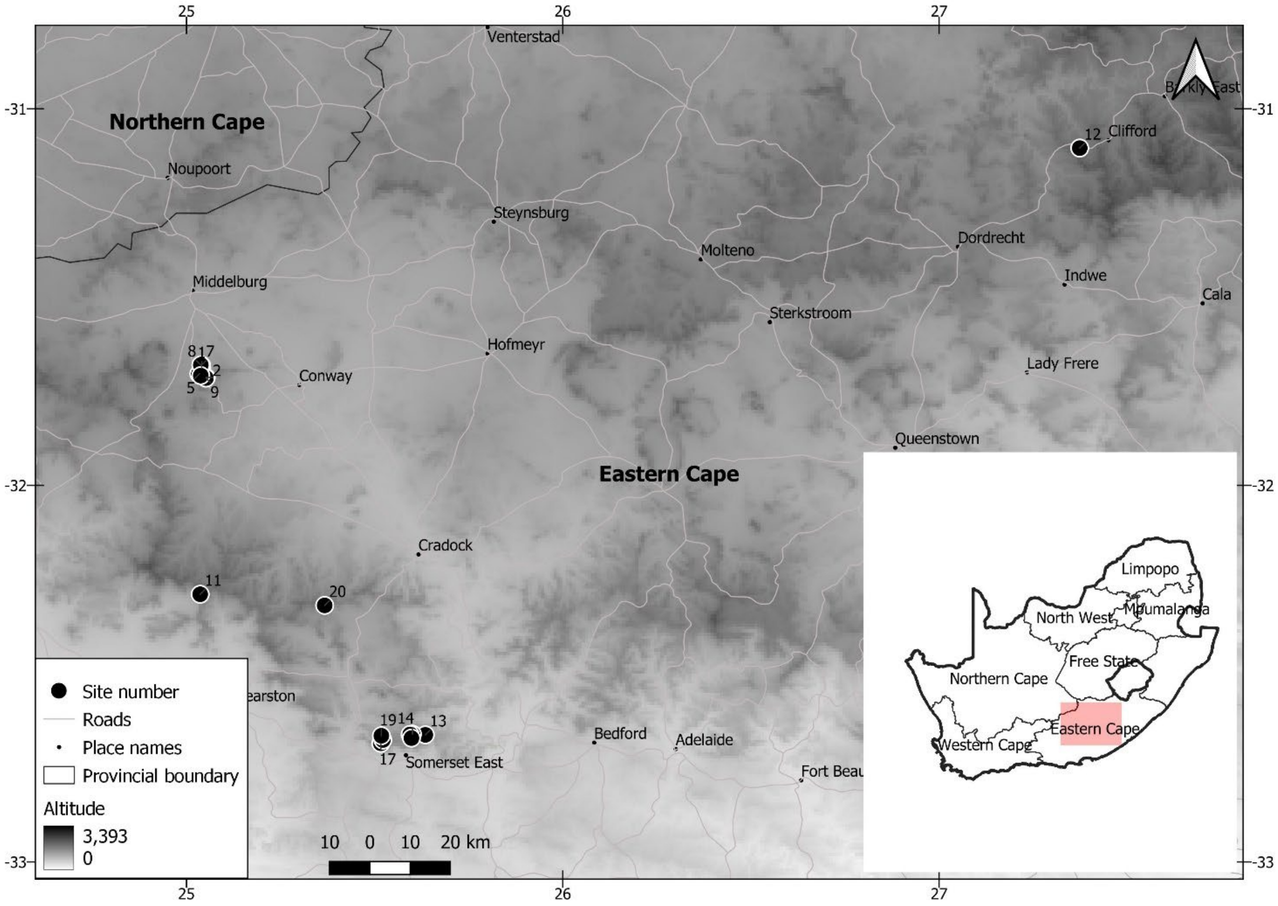


Fig. 3.1. Locations of the study sites in the Sneeuberg (1-11, 13-20) and Eastern Cape Drakensberg (12), Eastern Cape, South Africa. The numbers refer to site numbers (Table 3.1).

Twenty study sites were carefully selected within the study area based on the following criteria: (a) their land-use history for at least the past 10 years is known; (b) no herbicides have been used as a form of control on the sites; (c) 'natural' i.e., no cultivation, major soil disturbance, or planting or enrichments have been undertaken, (d) and the sites are all used for rangeland based activities (game or livestock farming). The sites are shared between the Grassland (12 sites, 60%) and Nama-Karoo Biomes (eight sites, 40%). All eight sites of the Nama-Karoo Biome were in the NKu4-Eastern Upper Karoo vegetation type; seven (35%) of the Grassland Biome sites were in Gd1-Amathole Montane Grassland vegetation type, two (10%) each were in Gh1-Karoo Escarpment Grassland vegetation type and Gs17-Tarkastad Montane Shrubland vegetation type, and one (5%) was in Gd4-Southern Drakensberg Highland Grassland vegetation type (Table 3.1). *Nassella trichotoma* in the study area grows in dense patches with clear boundaries (or invasion fronts). The studied patches were of different sizes ranging from about 11 m^2 to just over a hectare. The very big patches in the study area had been receiving aerial spray in recent years so did not meet the criteria.

Table 3.1. A list of the 20 sites selected for the study of *Nassella trichotoma* and their characteristics. The vegetation cover is a mean of the plots done at the site. (The codes given to the vegetation types by Mucina and Rutherford, 2006, are in brackets)

Site	Altitude (m.a.s.l.)	Latitude	Longitude	Vegetation cover (%)		Biome, Vegetation Type
				Invaded	Uninvaded	
1	1733	-31.688833	25.040650	80	69	Nama Karroo Biome, Eastern Upper Karroo (NKu4).
2	1850	-31.713700	25.052483	87	96	Grassland Biome, Tarkastad Montane Shrubland (Gs17).
3	1515	-31.706167	25.036533	84	64	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
4	1526	-31.705517	25.035700	99	59	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
5	1544	-31.703767	25.034500	89	51	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
6	1549	-31.703667	25.032933	93	50	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
7	1588	-31.680567	25.040017	95	92	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
8	1549	-31.680283	25.038083	93	75	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
9	1830	-31.715167	25.051867	99	96	Grassland Biome, Tarkastad Montane Shrubland (Gs17).
10	1527	-31.709583	25.039050	100	80	Nama Karroo Biome, Eastern Upper Karroo (NKu_4).
11	1530	-32.289170	25.036470	88	41	Grassland Biome, Karoo Escarpment Grassland (Gh1).
12	1857	-31.103767	27.373433	100	100	Grassland Biome, Southern Drakensberg Highland Grassland (Gd4).
13	1354	-32.662439	25.634686	95	96	Grassland Biome, Amathole Montane Grassland (Gd1).
14	1399	-32.660029	25.600387	96	89	Grassland Biome, Amathole Montane Grassland (Gd1).
15	1425	-32.661297	25.594274	93	82	Grassland Biome, Amathole Montane Grassland (Gd1).
16	1429	-32.671606	25.600071	95	95	Grassland Biome, Amathole Montane Grassland (Gd1).
17	1399	-32.684884	25.516373	93	93	Grassland Biome, Amathole Montane Grassland (Gd1).
18	1364	-32.675863	25.522770	91	89	Grassland Biome, Amathole Montane Grassland (Gd1).
19	1334	-32.664815	25.517823	91	86	Grassland Biome, Amathole Montane Grassland (Gd1).
20	1733	-32.720133	25.578261	87	91	Grassland Biome, Karoo Escarpment Grassland (Gh1).

Sampling design

Floristic sampling was conducted in invaded and non-invaded areas using data collecting sheets (Appendix B). Sampling was done in March 2020 and March 2021 when most plants were in flower and easy to identify. Soil samples from the March 2020 survey were analysed in September 2020 (the delay was due to the Covid-19 induced national lockdown measures). Despite this delay, no anomalies were detected in the soil results compared to the 2021 samples.

Sites were sampled with two to five plots per invasion patch depending on the size of the patch. Where the patch size allowed it and it was possible to find enough pairs, five plots per invasion patch were done. This gave an average of 4 plots per site. Paired plots were chosen so that one plot of the pair was in an area where *N. trichotoma* was present (the presence of a single tuft of *N. trichotoma* was considered invaded) (invaded plot), with the other plot in a nearby area where *N. trichotoma* had no cover (uninvaded plot) (Fig. 3.2 and Fig. 3.3). Both plots were placed so that they had similar environmental conditions and site history (aspect, elevation, rockiness, land use, slope, etc.) (Hejda et al., 2009; Thomaz et al., 2012; Vanderhoeven et al., 2005). Each pair was located no further than 5 m apart and often within 3 m of each other. Where possible, plots were placed as close as possible to the ecotone. A plot size of 2 m x 2 m was used for this research as this has been used in similar research (Koteen et al., 2011; Vila et al., 2006). Although the Global Invader Impact Network (GIIN) (Barney et al., 2015) recommends a plot size of 1m x 1m for the grasses and forbs, this was considered inadequate for this research considering that *Nassella* can have a crown size of up to 60 cm (Wells and De Beer, 1987). A total of eighty-one (81) paired plots (total 162 plots) were sampled in the austral summer in March 2020 (39 pairs) and March 2021 (42 pairs). One of the major criticisms of paired-plot comparisons is that the differences between them may merely reflect pre-invasion conditions. Keeping both pairs within the same site and as close together as possible reduces this challenge (Maurel et al., 2010). However, to avoid ecotonal effect, recommendations from the GIIN were used (Barney et al., 2015). Using these recommendations, the minimum allowable distance is 32.5 cm from the edge since *N. trichotoma* has no stolons and its maximum height is 65 cm. In each plot, all vascular plant species encountered were recorded, and the cover abundance of each species visually estimated and written as a percentage cover of the whole plot. Plant species were identified in the field; where this was not possible specimens were collected and identified using the Selmar Schonland Herbarium (GRA) in Makhanda (formerly Grahamstown), the University of the Free

State’s Qwaqwa Herbarium (QWA) and a virtual herbarium (Barker et al., 2020). The status of each species was recorded as belonging to one or more of the following categories - native/alien forbs, native/alien grasses, endemic species, naturalised species, and naturalised invasive species. The status of each species was determined following the Botanical Dataset of Southern Africa, (BODATSA) (Ranwashe, 2019). Other parameters measured per plot were the percentage bare ground (percentage of plot), slope (subjectively - gentle, moderate, steep), aspect, rock cover (percentage of plot), disturbance level (subjectively - low, medium, high) and previous land use (descriptive). These parameters were recorded so that the assumption that they are not different between paired plots can be verified. Significant differences between paired plots will make it difficult to attribute any differences between them to just the invader (Thomaz et al., 2012; Walker and Smith, 1997).

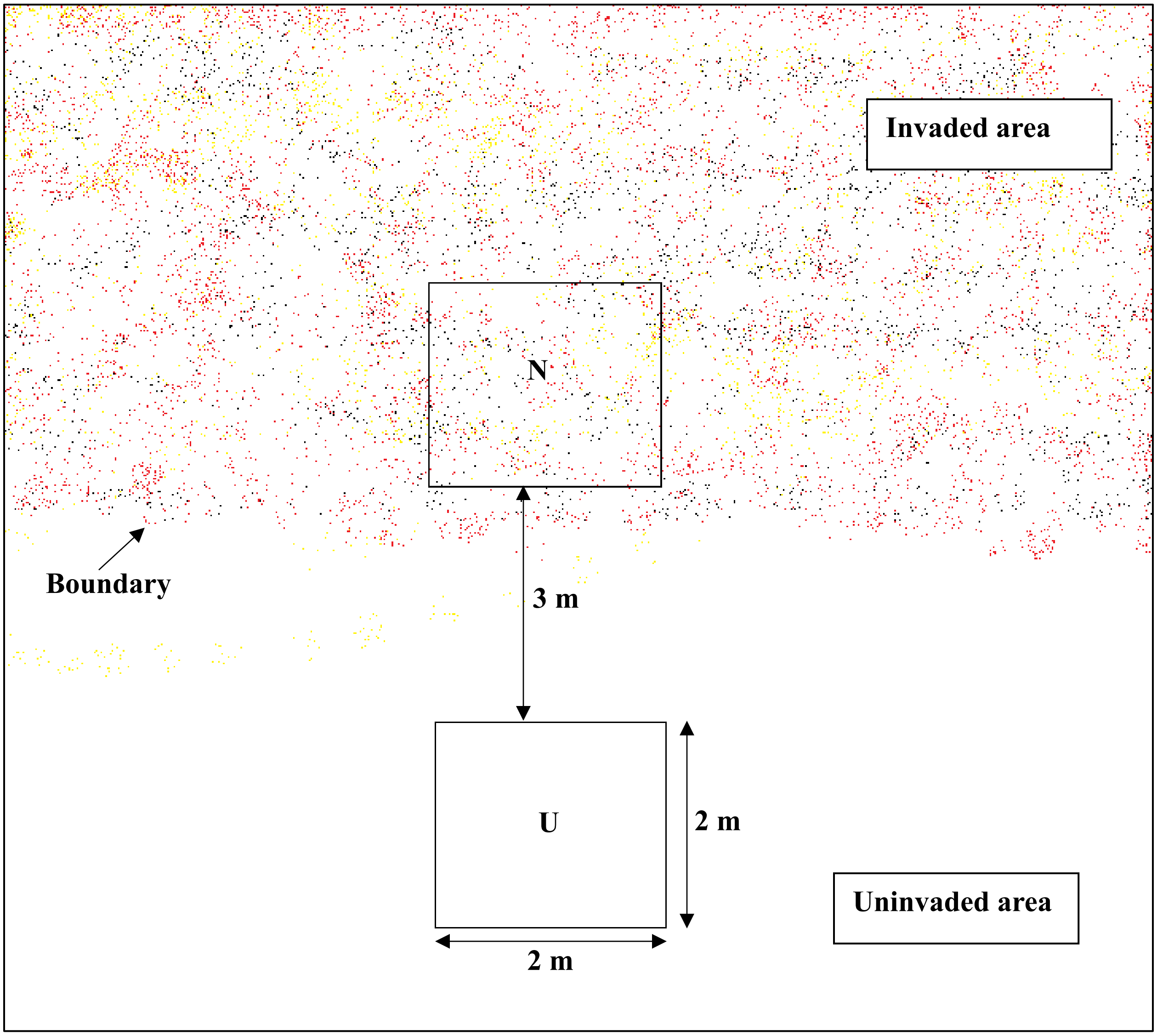


Fig. 3.2. A schematic diagram of how plots were laid out at a site.

Fig. 3.3. One of the sites invaded by *Nassella trichotoma*, in the Boschberg (Somerset East, Eastern Cape), showing approximately where the plots were placed within the site. The number refers to the pair number and the letter refers to the invaded status – N = invaded, U = uninvaded. Photo: A. Mapaura

Soil sampling

Soil sampling generally followed Vanderhoeven et al. (2005). For every plot surveyed a soil core (20 cm deep and 5 cm diameter) was collected from near the corners of each plot and at the centre. The soil samples were then combined so that there was one cumulative soil sample for the invaded and uninvaded plots in each site (n = 40). The litter was removed and discarded before soil sampling. Each composite soil sample was thoroughly mixed and a sub-sample weighing about 0.5 kg taken for soil testing. This gave a total of 20 samples from the invaded sites and 20 samples for the uninvaded sites. The soil samples were collected in plastic zipper bags and kept refrigerated (4°C) until they were sent off for testing at the Soil, Water and Plant Diagnostics Laboratory, Elsenburg, Western Cape. Soil parameters tested at the lab were boron (B), calcium (Ca), carbon (C), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nitrogen (N), P_citric acid, pH, phosphorus (P), potassium (K), sodium (Na), total cations, zinc (Zn), and particle size (5 fraction). These parameters are required for natural plant growth in all plants and an oversupply or undersupply in natural situations can lead to altered floristic assemblages (Vanderhoeven et al., 2005). In cultivated pastures and agriculture these can be controlled to sustain specific species. These parameters are important for plant growth and their quantities in the soil affects how different plant species will succeed or fail to establish Soil testing was carried out at the Soil, Water and Plant Diagnostics Laboratory, Elsenburg, Western Cape, following the handbook by the Non-Affiliated Soil Analysis Work Committee & Soil Science Society of South Africa, (1990).

DATA ANALYSIS

All statistical analyses were performed in R-Statistical software (R Core Team, 2021). For diversity calculations, *N. trichotoma* was not included in order to assess its impact on the remaining species (Kiełtyk and Delimat, 2019; Vila et al., 2006; Wearne and Morgan, 2004).

Floristic composition

The differences in species and environmental variables between invaded and uninvaded areas were analysed using the species-abundance data analysis R-package, mvabund (Wang et al., 2012). The mvabund package is a model-based analysis of multivariate abundance data such as the species abundance data collected in this research. Like other multivariate data analysis approaches, it is able to take into account confounding factors such as site differences. The model was fitted with a negative binomial error distribution to test the hypotheses that *N. trichotoma* influences species abundance and that the effect of *N. trichotoma* also differed by site. The ANOVA function was used to infer the impact of *N. trichotoma* and the interaction of *N. trichotoma,* using the Wald statistic and 999 resamples. Univariate analyses, using the Wald statistic and 999 resamples, were run on the model to find out which species were impacted less by *N. trichotoma* invasion and which ones were not. To determine which environmental variables were strongly associated with species assemblages, a manyglm model with a negative binomial error was fitted. Of all the environmental variables that were measured per plot, only bare ground and litter cover were influential, since the other variables like rockiness, elevation, and distance from nearest road/path were specifically selected to be similar between paired plots.

Species richness (S) (which is the total number of species encountered), Shannon index (H), Simpson's index (D), Inverse Simpson (D1), and Pielou's evenness (J) were calculated for every plot using the formulars below:

Shannon index (H) = $-\sum p_i(\ln p_i)$

p_i = the proportion of individuals that belong to species i, which is obtained from the number of individuals in species i divided by the total number of individuals in the sample and $\mathrm{Ln}p_i$ = natural log of pi.

$$\text{Simpson's index (D)} = \frac{\Sigma(\mathrm{n}(\mathrm{n}-1)}{\mathrm{N}\,(\mathrm{N}-1)}$$

Where: n = number of individuals of each species and N = total number of individuals of all species

$$D1 = \frac{1}{D}$$

$$\text{Pielou's evenness (J)} = \frac{H}{\ln(\mathrm{S})}$$

Where: H is Shannon index diversity index and S is the total number of species in a sample

Generalised mixed effect models and linear mixed effect models, from the R-package LME4, (Bates et al., 2015), were used to infer the effect of invasion. To analyse the differences in the grass species between invaded and uninvaded plots, the generalised additive model was used.

Soil variables

To determine which soil physical and chemical parameters differed significantly between invaded and uninvaded plots across the 20 investigated sites, linear mixed effects models (LMM) were used for each variable separately. Mixed effect models were used here because they are able to account for the effect of soils which may arise due to the different sites having different underlying geology. Plot status (invaded/uninvaded) was fitted as a fixed effect, and site was fitted as a random effect variable. The analyses were done using type 2 Wald's Chi-Square test.

RESULTS

Floristic composition and diversity

No significant differences were found between invaded and uninvaded plots with respect to distance from nearest road/path, elevation, rockiness, and slope. For this reason, any differences between invaded and uninvaded plots are most likely due to the presence of *N. trichotoma*. A total of 87 vascular plant species were recorded (Appendix C), comprising of 73 genera in 12 families, with 66 genera and 23 families in uninvaded plots, while 58 genera in 19 families were in invaded plots. Nine of the species recorded in the uninvaded plots were endemic to South Africa, with two (*Clutia impedita* Prain and *Hermannia violacea* (Burch. ex DC.) K.Schum.) being Cape Midlands Escarpment endemics. Five of the species recorded in the invaded plots were endemic to South Africa, with *Alchemilla bicarpellata* Rothm. restricted to the Eastern Cape.

Both invaded and uninvaded plots had more forbs than grasses (Table 3.2). About 73% of the species recorded in the uninvaded areas were forbs, while 71% of the species in the invaded areas were forbs. Slightly over 89% of the grasses recorded in the invaded areas were native while 95% of the grasses recorded in the uninvaded areas were native. *Bromus catharticus* Vahl was the only other non-native grass recorded in both invaded and uninvaded plots. *Chenopodium album* L., and *Datura stramonium* L. were some of the most common exotic forbs.

The investigated sites were significantly different in terms of species composition ($p < 0.05$). After accounting for the effect of the different sites, species abundance was significantly different between *N. trichotoma* invaded plots and non-invaded plots. Univariate tests showed that *Tenaxia disticha* ($p < 0.01$), *Pentaschistis airoides* ($p < 0.01$), and *Themeda triandra* ($p < 0.001$), differed significantly in abundance between invaded and uninvaded areas ($p < 0.05$) (Appendix D). *Themeda triandra* – a highly palatable native rangeland grass — was recorded in 17 (21%) of the invaded plots despite being present in 51 (63%) uninvaded plots.

Invaded plots had a vegetation cover ranging from 80% to 100% (average 92.40%) while uninvaded plots had 41% to 100% (average 79.70%) (Table 3.1). Uninvaded plots had a significantly greater percentage of bare ground (d.f.= 160, $p < 0.05$), while invaded areas had significantly higher litter cover (d.f. = 160, $p < 0.05$). Uninvaded areas had significantly more native grasses ($p < 0.001$) and native forbs ($p = 0.006$), than invaded areas. Endemic plants were significantly more abundant ($p < 0.05$) in uninvaded plots than in invaded plots. Alien plants (both grasses and forbs) were significantly more abundant in invaded areas than uninvaded areas. Shannon index, Simpson and Inverse Simpson, were significantly higher in uninvaded areas while Pielou's evenness was significantly higher ($p < 0.05$) in invaded areas (Table 3.2).

Table 3.2. Comparative data between invaded and uninvaded plots. Overall numbers are given in brackets.

Parameter	Mean values per plot		Chi-Sq	d.f.	p-value
	Invaded	Uninvaded			
Endemic species	0.220 (5)	0.530 (9)	9.622	1	< 0.001
Native grasses	2.155 (17)	3.099 (17)	23.486		< 0.001
Alien grasses	1.074 (2)	1.000 (1)	32.882	1	< 0.001
Native forbs	2.487 (40)	3.351 (48)	7.538	1	0.006
Alien forbs	1.179 (7)	1.188 (2)	3.675	1	0.055
Shannon index (H)	0.970	1.070	2.9771	1	< 0.001
Simpson (D)	0.450	0.360	8.008	1	< 0.001
Pielou's evenness (J)	0.520	0.430	12.236	1	< 0.001
			F		
Grass species	2.960 (19)	3.140 (21)	1.347	1	0.248
Naturalised species	0.110 (2)	0.000	14.14	1	< 0.001
Naturalised invasive	1.330 (8)	0.270 (5)	200.9	1	< 0.001
Inverse Simpson (D1)	2.200	2.710	9.055	1	< 0.001

Soil variables

All sampled areas, both invaded and uninvaded, had sandy loam soils. This soil type is probably a derivative of the mixed geology comprising Beaufort Group (Karoo Supergroup) sandstones and Jurassic dolerite intrusions common in the area. The sites differed in the amount of silt, coarse sand, and fine sand content. Silt content ranged from 4% to 20%, coarse sand ranged from 3% to 48%, while fine sand ranged from 25% to 66%. Soil texture is important for plant growth, mainly determining soil fertility characteristics such as water holding capacity, air circulation and root penetration (Catt, 2001). Of the 18 soil variables measured, boron, calcium, phosphorous, silt, total cations, and zinc were significantly higher ($p < 0.05$) in invaded areas than uninvaded areas (Table 3.3). All other soil variables did not differ significantly between invaded and uninvaded plots though they were generally higher in invaded plots.

Table 3.3. Results of soil variable analysis between invaded against uninvaded plots, across the 20 sites. Parameter units are indicated in brackets after the parameter.

Soil parameter	**Mean**		**Chi-Sq**	**d.f.**	**p-value**
	Invaded	**Uninvaded**			
Boron (mg/kg)	**0.27**	**0.22**	**18.76**	**1**	**0.001**
Calcium (cmol(+)/kg)	**8.31**	**7.05**	**20.62**	**1**	**0.001**
Carbon	1.63	1.86	0.53	1	0.466
Clay (%)	8.60	9.20	1.17	1	0.279
Coarse sand (%)	22.15	21.25	0.487	1	0.485
Copper (mg/kg)	4.30	4.16	0.05	1	0.819
Fine sand (%)	44.10	45.70	1.32	1	0.251
Iron	109.01	100.09	0.26	1	0.608
Magnesium (cmol(+)/kg)	2.96	2.82	1.39	1	0.239
Manganese (mg/kg)	202.22	197.39	0.05	1	0.822
Medium sand (%)	6.25	6.65	0.26	1	0.608
Available phosphorus	**69.25**	**43.85**	**10.436**	**1**	**0.001**
pH	5.51	5.46	1.117	1	0.291
Potassium (mg/kg)	391.30	332.60	2.939	1	0.086
Silt (%)	**18.90**	**17.20**	**5.898**	**1**	**0.015**
Sodium (mg/kg)	26.00	25.90	0.002	1	0.965
Total cations (cmol(+)/kg)	**12.95**	**11.55**	**9.846**	**1**	**0.002**
Zinc (mg/kg)	**6.14**	**3.65**	**7.727**	**1**	**0.005**

DISCUSSION

Nassella trichotoma invasions in montane grasslands in South Africa were found to negatively impact plant diversity and alter topsoil chemistry. This confirms what has been reported by other researchers in Australia (Badgery et al., 2008; Dassonville et al., 2008; Gibbons et al., 2017; Joubert, 1984; Vanderhoeven et al., 2005). However, this research suggests that the specific changes may depend on the initial species composition of the area being invaded. For example, *T. triandra* was found to have reduced abundance in invaded plots, this may indicate increased risk to areas that are dominated by *T. triandra* at these altitudes. The major impact of the invasion was the reduction in native species while exotic species increased. *Nassella trichotoma* could be facilitating the survival of other exotic plant species in areas it invades, which may lead to more serious impacts on native ecosystems as postulated by the invasional meltdown hypothesis (Green et al., 2011). This characteristic has also been reported in other studies before (Faithfull, 2012; Wundrow et al., 2012).

Plant diversity indices (Simpson, Inverse Simpson and Shannon) showed that the uninvaded areas were more diverse than invaded areas. However, the fewer species in *N. trichotoma* invaded plots were more evenly distributed, thus giving a higher Pielou's evenness value. This can be attributed to *N. trichotoma* creating near monoculture patches where the remaining species have a similar but very low cover abundance which is often characterised by a few weak individuals scattered throughout the patch. The uninvaded areas were dominated by a few perennials such as *T. disticha* and *T. triandra* thus leading to unevenness in these plots.

Many of the species that were recorded in the uninvaded areas were also present in the adjacent invaded areas, albeit with a much-reduced cover abundances, often below 1%, with *N. trichotoma* taking up more than 80%. Even tufted perennial grasses such as *T. disticha* and *T. triandra* which showed significant differences between invaded and uninvaded plots were recorded within *N. trichotoma* patches. In some cases, they were even seen intertwined in a single tuft. This suggests that *N. trichotoma* might be reducing them through factors such as shading and persistence.

The increase in topsoil nutrients as a result of invasion has also been reported in other research on the impacts of invasive alien species (Dassonville et al., 2008; Ehrenfeld, 2003; Gibbons et al., 2017; Vanderhoeven et al., 2005). Studies in NW Europe found that plant species, such as *Fallopia japonica,* and *Heracleum mantegazzianum*, which have a deeper rooting system than the resident vegetation, can uplift nutrients from deep soil layers to the topsoil. (Dassonville et al., 2008). *Nassella trichotoma* might be doing the same considering that it has a much higher biomass than other plants in the studied area and has a deep rooting system (Wells, 1977; Wells and De Beer, 1987). Soil-dwelling fauna are also capable of moving soil from deeper down to the surface and their presence and/or activity in the study sites should have been assessed. However, this was missed during the current study, it is therefore, recommended that it be included in future studies.

All the measured nutrients and soil parameters are essential for the health of plants and successful reproduction. These parameters interact in complex ways to affect the wellbeing of a plant (Rout and Das, 2003). Heightened levels of nutrients like boron and zinc can lead to reduced plant vigour, decrease in biomass production, delayed reproduction and poor seed production (Gupta et al., 2014; Nejad and Etesami, 2020; Rout and Das, 2003; Rudani et al., 2018). While high levels of calcium do not directly cause toxicity in plants, they can result in magnesium and potassium deficiencies leading to poor plant performance and exclusion of some species from affected areas (White, 2003). As noted by Gibbons et al. (2017), differences in soil nutrients are usually reflected in the below-ground flora and fauna. *Nassella trichotoma* develops an extensive rooting system early in its development which gives it an ability to tolerate drought much better than most grasses (Wells et al., 1983). The high cover abundance achieved by *N. trichotoma* acts as a control against excessive gas exchange between the soil and the atmosphere, thus controlling soil physiochemical and biological processes. In this way, temperature, and moisture below the plants in the invaded patches are regulated. Under these circumstances, it is reasonable to assume that the soil below invaded patches will have a different underground fauna and flora. If *N. trichotoma* is associated with specific below ground fauna and flora, this would explain its ability to recolonise areas it has been removed from. This needs to be tested as it would have a bearing on control measure options.

Boron is an important micronutrient required by plants for growth and cell wall and plasma membrane maintenance (Gupta et al., 2014; Nejad and Etesami, 2020). Plants have been reported to require Boron within a very narrow range below which it become deficient and above which it becomes toxic (Gupta et al., 2014). Calcium is an macronutrient with numerous roles in plants, which include an important role in maintaining cell wall and membranes structure as well as an intracellular second messenger (Thor, 2019). It is not known to cause toxicity in plants but can cause reduced uptake of cations which can lead to deficiencies of those nutrients (Thor, 2019; White, 2003). Phosphorus is a vital macronutrient required in energy transfer processes in plants including photosynthesis and is a component of genetic material so is important in cell division and development of new tissue (Malhotra et al., 2018). Phosphorus rarely reaches toxic concentrations in the soil but is frequently deficient because it often forms insoluble compounds (Malhotra et al., 2018). Zinc is one of the micronutrients that plants are very sensitive to and is involved in several metabolic processes such as photosynthesis, and as a component of many enzymes (Rudani et al., 2018). It causes toxicity at high concentration and deficiency may also occur.

The different vegetative cover between invaded and uninvaded areas means that the ground temperature under them will be different; this must affect water retention, decomposition rates, and nutrient dynamics and biological processes in the soil (Allison and Vitousek, 2004; Onwuka, 2018). Under *N. trichotoma*, temperatures might not fluctuate as much as in the uninvaded areas due to possible thermal insulation provided by the dense cover of the invader. With the increased moisture, decomposition will be faster and water-mediated mineralisation will be faster, facilitating increased availability of mineral nutrients in the rhizosphere (Onwuka, 2018).

CONCLUSION

Nassella trichotoma invasion is associated with lower native species richness and higher exotic species of both invasives and non-invasives. Higher soil nutrient concentrations were also noted in the rhizosphere, particularly boron, calcium, available phosphorus, silt, total cations, and zinc. These heightened soil parameters and nutrient levels might be affecting the germination and subsequent development and reproduction of native plants leading to their displacement, while facilitating further *N. trichotoma* invasion. This hypothesis could not be tested in the time available for this study but should be studied in future it has a bearing on revegetation success. It is also suspected that microbial flora will differ significantly due to these species and soil changes; this aspect was not part of the current study but would be ideal to conduct in future.

This research has shown that the soil changes that take place under *N. trichotoma* invasion do not completely exclude native plants – at least not at the sites sampled. Native plants may, however, not recolonise as fast as they would have otherwise done due to the altered soil conditions. Revegetation soon after removal of *N. trichotoma* is a possibility, but more research is required to find species that establish quickly – before *N. trichotoma* re-invades. Revegetation with locally adapted native pasture grasses should be explored as they can provide competitive cover and reduce *N. trichotoma* recolonisation. Perhaps a more sustainable, long-term solution is to vigorously pursue possible biological control methods and integrate it with soil conditioning (where possible) and strict livestock management practices. All these methods should be done as part of an area- or region-wide management effort to minimise costs (as these will be shared by many people) and reinvasion from unmanaged areas.

CHAPTER 4

THE IMPACT OF *NASSELLA NEESIANA* ON PLANT DIVERSITY, RICHNESS, AND TOPSOIL IN THE EASTERN FREE STATE

ABSTRACT

While *Nassella neesiana* is currently not a listed invader in South Africa, it is an invader of major concern in Australia and New Zealand, countries with similar socio-ecological systems to South Africa. Very little is known about *N. neesiana* distribution in South(ern) Africa, and it may be posing as a "cryptic invader" as evidenced by recent locality records. At one of the new localities – "Platberg Stream" (1621–1725 m.a.s.l., Harrismith, eastern Free State) at which the species was recently recorded by R. Soreng and S. Sylvester in 2020 – a detailed population mapping exercise was done in March 2021. The main objective of this study was to establish the distribution of *N. neesiana* in the Harrismith district and establish what impact it is having on plant species richness, diversity, and topsoil composition. Another objective was to understand the ecological aspects that might be responsible for its cryptic behaviour. Fourteen pairs of 2 x 2 m plots (total 28 plots) were sampled from all identified subpopulations, each pair comprising an invaded and a non-invaded site within 3 m of each other. In each plot the basal circumference of *Nassella neesiana* was recorded and every species and their cover abundance were recorded. Generalised mixed effect models and linear mixed effect models were used to infer the effect of invasion on diversity and soil variables. A total of seven *N. neesiana* subpopulations were recorded, four being along "Platberg Stream", and three in other open spaces. *Nassella neesiana* cover ranged from 15 % to 90 % with an average cover of 51.07 % in studied areas. Species, diversity indices did not differ significantly between invaded and uninvaded areas, but invaded areas had a lower native plant diversity ($p < 0.02$). Soil physical and chemical variables did not differ significantly between invaded and uninvaded plots, but invaded plots had slightly higher concentrations of most minerals compared to uninvaded plots. *Nassella neesiana* appears to gradually infiltrate vegetation in disturbed and riparian contexts, however its overall impact on native diversity remains unknown. The possible more extensive presence of *N. neesiana* in eastern

and southern South Africa requires investigation and further quantitative population and environmental impact studies should be conducted.

INTRODUCTION

Very little is known about the distribution of *Nassella neesiana* in South Africa since it has attracted very little interest beyond taxonomic treatments. Very few collection records are found in databases and herbaria, with most of the records being from the 1970 and more recent ones from 2010 to present. Incidental records in 2020 of *N. neesiana* have been confirmed in the Eastern Cape, Western Cape, and Free State provinces. These more recent collections extend its distributional range to new areas and suggests that it may be a cryptic invader whereby naturalised populations have gone unnoticed (Morais and Reichard, 2018), and are ending a long lag phase. It also suggests that *N. neesiana* might be more common and widespread in South Africa than records currently show. Its invasive congeners, *N. tenuissima* and *N. trichotoma,* did not have the same pattern in South Africa, whereby once introduced they spread quickly and formed monocultures in montane grassland. These two species were included on the NEM:BA (National Environmental Management: Biodiversity Act) list as category 1b invasive species. However, *N. neesiana* was not classified under NEM:BA, due to a lack of evidence of invasive populations.

Seeds of *Nassella neesiana* are poorly adapted for wind dispersal but they can spread across wide geographic ranges when transported with hay, animal skin and wool as well as clothing and farming implements due to the long, sharp awns (Bourdôt and Hurrell, 1989; Connor et al., 1993). *Nassella neesiana* can take over pastures, forming monocultures especially in overgrazed pastures (Bourdôt and Hurrell, 1989; Faithfull, 2012). Its ability to produce two types of seeds – the normal seeds (chasmogenes) and the stem seeds (cleistogenes) – gives it the ability to survive mowing and grazing much better than most other plants, ensuring its long-term survival under less favourable conditions (Connor et al., 1993; Faithfull, 2012; Kriticos et al., 2010). This gives the species a competitive advantage over other species in an invaded area.

Nassella neesiana has been shown to reduce native plant diversity when it invades disturbed natural grasslands but had no effect on other alien plant species (Faithfull, 2012). Intact natural grasslands, however, can resist invasion by *N. neesiana* even with high propagule pressure (Faithfull, 2012). While research has demonstrated the negative effect of its invasion on natural grasslands (Faithfull, 2012), no research has been done on its impact on urban and peri-urban environments that constantly experience anthropogenic disturbance. It is, therefore, important to understand the behaviour of *N. neesiana* in this type of environment where it can establish and spread from, particularly considering its reported impact on soil nutrients, water and native flora in Australia (Faithfull, 2012; Wijesuriya and Hocking, 1999).

Urban environments are often hotspots for exotic plants and often act as sources of propagules that can invade into neighbouring natural areas or even be carried to far areas (Potgieter et al., 2019). This knowledge will assist in developing management strategies that prevents it from invading into neighbouring natural ecosystems. It is hypothesised that *N. neesiana* is more widespread in the Harrismith district, South Africa, and is changing species richness, diversity, and topsoil composition, in the same way as this is occurring in other *Nassella* species in South Africa. *Nassella* species are morphologically cryptic and often go undetected as has occurred elsewhere.

MATERIALS AND METHODS

Study area

This study was carried out in Harrismith, a small town in the Thabo Mofutsanyane District, Free State province of South Africa. The presence of *N. neesiana* on this site was discovered only in 2020 by S. Soreng and S. Sylvester, and the extent of the invasion was not known until this study was conducted. This is the first detailed ecological study of *N. neesiana* that I am aware of in South Africa, and this is the only area in South Africa where *N. neesiana* is known to be invading an urban environment where livestock farming is not the major activity. The survey sites were situated along a perennial stream which rises in the Botanical Gardens / Platberg Eco Reserve at the western base of Platberg ("Flat-topped Mountain" in Afrikaans) and runs on the north-eastern edge of Harrismith (Fig. 4.1). It is a tributary of the Wilge River, which it enters on the north-eastern edge of the town; the Wilge in turn is an important tributary of the Vaal River. Harrismith was a major British military base during the Second Anglo-Boer War (1899–1902; Visser, 2012), and it is most likely that *N. neesiana* was introduced into the area during this time in hay (Henderson, 2018; Wells, 1978). If this is the case, it means this species has been present in the Harrismith vicinity since the British took control of Harrismith from the Boers in 1901 – a period of more than 120 years.

The elevation in the study area ranges from 1621 m.a.s.l. to 1725 m.a.s.l,, with Platberg overlooking the town on the north-east peaking at 2394 m.a.s.l. The annual average precipitation is about 900 mm (Merkel, 2020). Most of the rain falls during the summer period from November to March, although occasional rain associated with cold fronts can be experienced in the dry months. The daily maximum temperatures for the hot summer months (December and January) is around 18.60°C, with the lowest daily mean temperatures for the cold winter month (June and July) of −2°C to 0°C (Brand et al., 2011; Merkel, 2020). Snow occurs occasionally on Platberg , and hard frost is typical at night at lower elevations from May to September in winter (Brand et al., 2011).

The original vegetation of the general area falls within the Gm4-Eastern Free State Sandy Grasslands of the Grassland Biome (Mucina and Rutherford, 2006), still intact on most of the dryland slopes of Platberg. Brand et al. (2011) classified the vegetation of this area into 26 vegetation types and identified 551 vascular plant species with 26 of them being endemic to the Drakensberg Alpine Centre. The grasslands are characterised by *Themeda triandra, Heteropogon* *contortus* and *Eragrostis curvula* and the forbs *Commelina africana and Oxalis obliquifolia.* The terrain is generally rocky with shallow soil on the Clarens, Elliot and Molteno Formations which are part of the Karoo Supergroup. The soils are mostly sandy with gravel (Bordy, 2005; Brand et al., 2010). The vegetation of Platberg Stream (from its source in Donkey Pass on Platberg), is largely an invaded ecosystem consisting of woody non-native plants – notably *Acacia dealbata, A. mearnsii, Cotoneaster pannosus, Eucalpytus* sp*., Ligustrum* spp*., Pyracantha* spp*., Robinia pseudo-acacia, Salix babylonica*, and *S. fragilis*. Based on the few remaining indigenous woody species present, the riparian vegetation was likely a grassy-woody matrix that included *Leucosidea sericea–Buddleja salviifolia* thickets, stands of the indigenous *Salix mucronata*, and other Highveld or proto-forest species such as various *Celtis africana, Diospyros lycioides,* and *Searsia pyroides.* Indigenous woody species of value (e.g., timber, firewood) may have been removed from the system in the mid-1800s already, given the original scarcity of natural wood on the Highveld. Two privately maintained recreation areas occur in the study site, viz. “Perdegat”, and the Harrismith Golf Course, with derelict recreation areas next to the two dams in the Platberg Eco Reserve.

A detailed population mapping exercise was done in March 2021 when most plants were in flower and easy to identify. This was achieved by visiting a selection of open areas on the north-eastern side of Harrismith’s urban area as well as systematically searching for subpopulations along the stream. These areas were selected as they were most likely to have *N. neesiana* established. Any subpopulation noted was georeferenced using a handheld ETrex 10 GPS (Fig. 4.1). The extent of the subpopulation was estimated by walking around it with the GPS and calculating the area in QGIS software. The invaded areas were described in terms of the general vegetation disturbance, and soil moisture condition (estimated from observation: dry, seasonal wetland, permanent wetland) (Table 4.1). Further surveys were done from mid-November to early December to see whether any subpopulations could have been missed in March. The subpopulations discovered during this survey were described but species richness and topsoil were not assessed as was done with the seven sites determined in March.

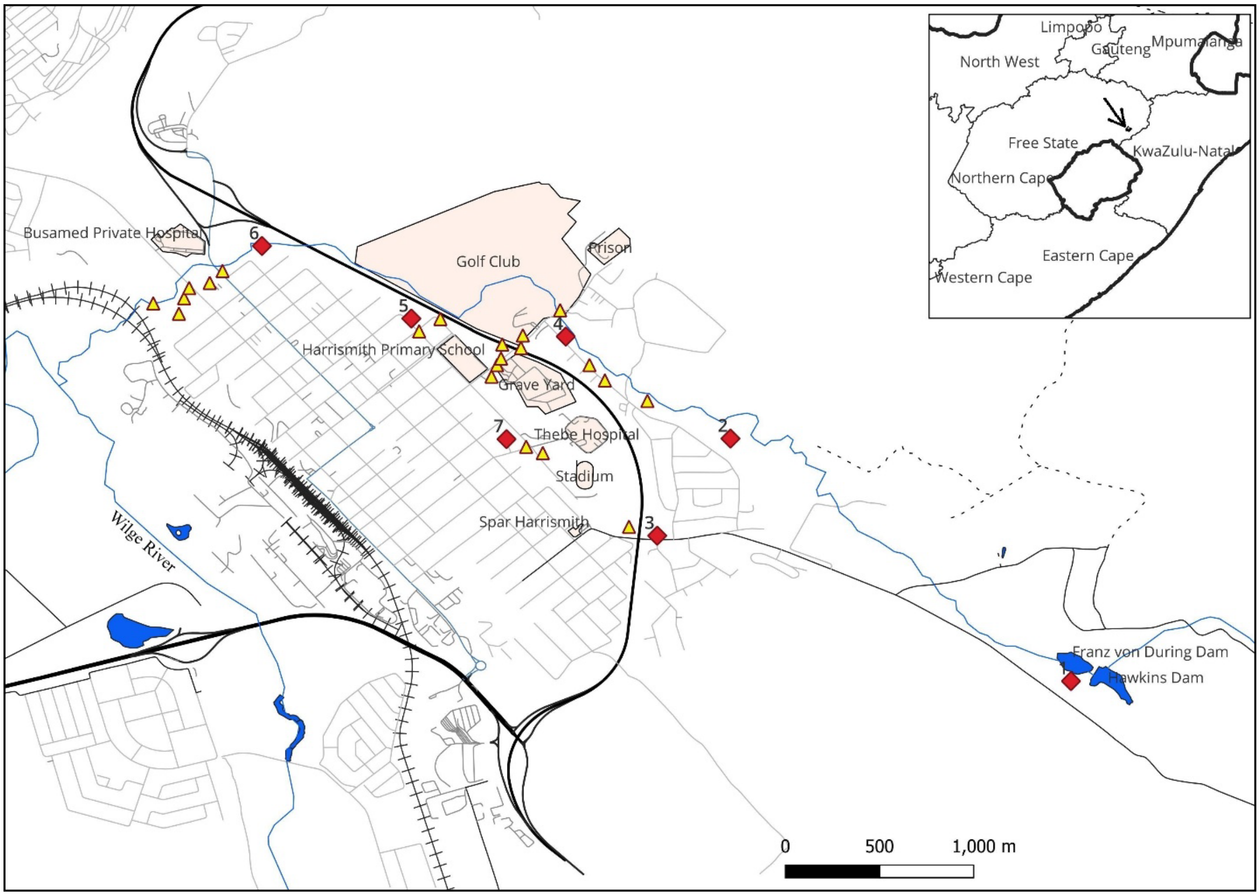


Fig. 4.1. Locations of subpopulation sites of *Nassella neesiana* along Platberg Stream Harrismith (Free State, South Africa). The red diamonds refer to studied subpopulations (the numbers against them refer to site numbers); yellow triangles refer to sites that were not studied but *N. neesiana* individuals were encountered. The location of the Harrismith is indicated by an arrow on the inset map.

Table 4.1. A list of the seven *Nassella neesiana* subpopulations studied and their characteristics (Platberg Stream, Harrismith). The common grasses are from the uninvaded areas.

Site	Altitude (m.a.s.l.)	Latitude	Longitude	General vegetation disturbance	Soil moisture
1	1724.97	-28.285146	29.164117	An old picnic site, about 50 m from edge of a weir in an old *Eucalyptus* plantation. The vegetation might have been cleared in the past and *Cynodon* planted. Grasses: *Cynodon aethiopicus, Digitaria sanguinalis, Eragrostis curvula* and *Sporobolus pyramidalis*	Seasonal wetland. An ephemeral stream located just outside the patch.
2	1645.14	-28.271861	29.145664	A dumpsite for building rubble and tree trimmings. About 100 m from houses. Mounds of soil on one side, a sign of soil having been moved using a grader. Grasses: *Aristida adscensionis*, *Cynodon dactylon, E. curvula*, *Hyparrhenia hirta* and *Paspalum scrobiculatum.*	Seasonal wetland. (Fig. 4.2).
3	1662.93	-28.275498	29.141257	Litter dumped in this area. Five metres from a property fence and stream. Partly shaded by *Salix* along the stream. Grasses: *Aristida junciformis, Cynodon aethiopicus, H. hirta* and *Sporobolus pyramidalis*.	Along stream. Gets very wet during the rainy season.
4	1632.34	-28.265923	29.136116	Area between the N3 freeway and a stream. Road-verge vegetation sometimes mowed. (Fig. 4.3). Grasses: *C. aethiopicus, E. curvula* and *Setaria pumila.*	Along a stream, but non seen growing within a metre of the stream.
5	1620.91	-28.265542	29.128414	A wetland, between houses and the highway. It was once cattle grazing area, then cleared for a golf course, but abandoned in the late 1980s. Grasses: *C. aethiopicus, E. curvula, S. pyramidalis* and *Themeda triandra*	Seasonal wetland.
6	1619.83	-28.275799	29.141317	Area between a stream and houses. It forms the western end of the old golf course mentioned in site 5 above. A lot of domestic litter – bottles, plastic and broken household goods. Grasses: *Brachiaria eruciformis, C. dactylon* and *P. scrobiculatum*	Seasonally wetland.
7	1624.27	-28.265542	29.128414	Between a small ephemeral stream and a property boundary. Several foot paths crisscross the area with tree trimmings and rubbish dumped in the area. Grasses: *C. aethiopicus, E. curvula, S. pyramidalis* and *T. triandra*	Seasonal wetland.

Fig. 4.2. Site 2, showing the density of *Nassella neesiana* with a well-maintained lawn and houses in the background. Photo: A. Mapaura.

Fig. 4.3. Site 4, showing *Nassella neesiana* in the foreground and mostly *Themeda triandra* in the background. The invaded plots were placed in the *Nassella neesiana* invaded area with the other plots in the *Themeda triandra* area. The non-native *Plantago lanceolata* is also clearly evident in the photograph. Photo: A. Mapaura.

Vegetation plots

Sites were sampled with two paired plots per invasion patch using the same datasheet as for *N. trichotoma* (Appendix B). This minimised the possibility of a few patches skewing the results. This gave an average of four plot pairs per site. Paired plots were chosen so that one plot of the pair had at least 10 % *N. neesiana* cover (invaded plot), with the other plot in a nearby area where *N. neesiana* had no cover at all (uninvaded plot). Both plots were placed so that they had similar environmental conditions and site history (aspect, elevation, rockiness, etc.) (Hejda et al., 2009; Thomaz et al., 2012; Vanderhoeven et al., 2005). Each pair was located no further than three metres apart and often within three metres of each other. This cover limit and the need to have plot pairs within three metres of each other excluded some subpopulations. *Nassella neesiana* invasion in this area is not continuous but is patchy, with dense patches and other areas with very sparse or no cover, making it possible to find areas without *N. neesiana* within the general invaded patch. A plot size of 2 m x 2 m was used for this research as this has been used in similar research (Koteen et al., 2011; Vila et al., 2006). A total of fourteen (14) paired plots (total 28 plots) were sampled in the austral summer in March 2021. One of the major criticisms of paired plot comparisons is that the differences between them may merely reflect pre-invasion conditions. Keeping both pairs within the same site and as close together as possible reduces this challenge (Maurel et al., 2010). In each plot, all vascular plant species encountered were recorded, and the cover abundance of each species visually estimated and written as a percentage cover of the whole plot. Plant species encountered were identified in the field, when this was not possible specimens were collected and identified using the Qwaqwa herbarium. The status of each species was recorded as belonging to one or more of the following categories – forbs, grasses, native species, endemic species, naturalised species, and naturalised invasive species. The status of each species was determined following the Botanical Dataset of Southern Africa (BODATSA) (Ranwashe, 2019). Other parameters measured per plot were bare ground and litter cover (percentage of plot), slope (subjectively — gentle, moderate, steep), aspect, rock cover (percentage of plot), disturbance level (subjectively — low, medium, high). These parameters, except bare ground and litter, were recorded so that the assumption that they are not different between paired plots can be verified.

In each invaded plot, the basal circumference of each *Nassella neesiana* tuft was measured to the nearest centimeter to get a sense of their resident time. An individual that has only been growing for a year or two is expected to have a small basal diameter while a large diameter suggests a longer resident time. To assess whether a fire event had occurred, all grass tufts were visually assessed to see if there were any signs of having been burnt. The follow up survey in November described any new subpopulations, however, did not assess their impacts as outlined here.

Soil sampling

Soil sampling followed Vanderhoeven et al. (2005). For every plot surveyed a soil core (20 cm deep and 5 cm diameter) was collected from near the corners of each plot and at the centre. Wherever possible, sampling was done very close or under a *N. neesiana*. The soil samples were then combined so that there was one cumulative soil sample for the invaded and uninvaded plots at each site (n = 40). The litter present was removed and discarded before soil sampling. Each composite soil sample was thoroughly mixed and a sub-sample weighing about 0.5 kg taken for soil testing. This gave a total of 14 samples (seven from the invaded plots and seven from uninvaded sites). The soil samples were collected in plastic zipper bags and kept in a fridge (4°C) until they were sent off for testing at the Soil, Water and Plant Diagnostics Laboratory, Elsenburg, Western Cape. Soil parameters tested at the lab were boron (B), calcium (Ca), carbon (C), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nitrogen (N), pH, phosphorus (P), potassium (K), sodium (Na), total cations, zinc (Zn), and particle size (5 fraction). These soil variables were selected as they are considered to have the most influence on plant growth (Vanderhoeven et al., 2005). Soil testing was carried out following the handbook by the Non-Affiliated Soil Analysis Work Committee & Soil Science Society of South Africa, (1990).

DATA ANALYSIS

All statistical analyses were performed in R-Statistical software (R Core Team, 2021). For species diversity calculations, *N. neesiana* was not included in order to assess its impact on the remaining species (Kiełtyk and Delimat, 2019; Vila et al., 2006; Wearne and Morgan, 2004).

Floristic composition

The differences in species and environmental variables between invaded and uninvaded areas were analysed using the 'mvabund' R-package (Wang et al., 2012). The model was fitted with a negative binomial error distribution with multivariate species abundances as the response variable with site and plot status (invaded/uninvaded) as categorical fixed effects. To infer the statistical significance of fixed effects, Likelihood Ratio tests (hereafter 'LRT') and pit-strap bootstrapping were used to compute P-values, using 999 bootstrap replicates. Univariate analyses, were performed to find out which species were responsible for any observed differences between invaded and uninvaded plots, using pit-strap bootstrapping to compute adjusted p-values corrected for multiple testing and correlations between species (Wang et al., 2012).

To determine which environmental variables were strongly associated with species assemblages, the manyglm function in the 'mvabund' R-package was used to model the multispecies abundances as response variable and the environmental variables as fixed effects fitted with a negative binomial distribution. Of all the environmental variables that were measured per plot, only bare ground and litter cover could be used in analysis, since the other variables like rockiness, elevation, and distance from nearest road/path were specifically selected to be similar between paired plots but were only included to test the validity of this assumption. Significant differences in underlying ecological characteristics between paired plots would make it difficult to attribute any differences between them to just the invader (Thomaz et al., 2012; Walker and Smith, 1997).

Species richness (S), Shannon index (H), Simpson's index (D), Inverse Simpson (D1), and Pielou's evenness (J) were calculated for every plot using the formulars below:

Species richness (S) = the total number of species encountered

Shannon index (H) = $-\sum p_i(\ln p_i)$

p_i = the proportion of individuals that belong to species i, which is obtained from the number of individuals in species i divided by the total number of individuals in the sample and $\mathrm{Ln}p_i$ = natural log of pi.

$$\text{Simpson's index (D)} = \frac{\Sigma(\mathrm{n}(\mathrm{n}-1)}{\mathrm{N}\,(\mathrm{N}-1)}$$

Where: n = number of individuals of each species and N = total number of individuals of all species

$$D1 = \frac{1}{D}$$

$$\text{Pielou's evenness (J)} = \frac{H}{\ln(\mathrm{S})}$$

Where: H is Shannon index and S is the total number of species in a sample

Linear mixed effect models (LMEM), using the R-package 'lme4', (Bates et al., 2015), were used to infer the effect of invasion on these diversity indices, since the distributions were normal. Generalised linear mixed effect models (GLMM) with Poisson distribution were used to assess the effect of invasion on the distribution of endemic species, forbs, and native species, all naturalised and naturalised invasive species. The differences in grass species between invaded and uninvaded plots, generalised additive mixed models (GAMM) were used with a Poisson distribution. In all cases plot status (invaded/ uninvaded), was fitted as a fixed effect and site fitted as random effect while plot pairs were fitted as nested random effect within sites. P-values were computed using LRT with 999 bootstrap replicates. Since different independent tests were performed for the different variables, the p-values were adjusted for multiple comparisons using Bonferroni correction.

Soil variables

To determine which soil physical and chemical parameters differed significantly between invaded and uninvaded plots, across the seven investigated sites, generalised additive mixed models were used for each variable separately. These models were used because they can account for the effect of soils which may arise due to the different sites having different underlying geology. Plot status (invaded/uninvaded) was fitted as a categorical fixed effect, and site was fitted as a random effect variable. Parameter significance was assessed using Likelihood Ratio Tests (LRT), with type II sum-of-squares Wald's Chi-Square test.

RESULTS

Species composition

Of the several subpopulations recorded in the area, seven *N. neesiana* subpopulations were studied in detail, four being along the stream to the northeast of Harrismith, and three in other open spaces. In mid-November 2021, a few tufts of flowering *N. neesiana* were discovered outside a house in Harrismith leading to a more thorough search. This led to more subpopulations being discovered. These 'new' subpopulations were mainly made up of a few individuals scattered among other grasses. A subpopulation of just over a hectare of *N. neesiana* was discovered, flowering profusely at that time. Some of the subpopulations which were found flowering profusely in March 2021 only had a few flowering stalks in November 2021. Subpopulations that were studied covered an area of about 357748 m^2, with subpopulation cover ranging between 32 m^2 to about 17443 m^2 and all in disturbed seasonally wet areas. *Nassella neesiana* was recorded in open grasslands with a general height of less than 50 cm. All invaded areas have had their soil disturbed at some point, either through road construction, digging, or vegetation clearing, or soil had been dumped there from construction sites elsewhere. This supports Faithfull (2012), who reported that *N. neesiana* invasion appears to be preceded by the removal of vegetation. *Nassella neesiana* does not stand out among the grasses even when it is flowering, especially when its cover is less than 10%. The inflorescence often droops below the other grasses, making the species difficult to notice. *Nassella neesiana* appears to favour areas that receive full sunlight, although a few individuals were found growing in light shade. A visit to some of the studied areas in early April showed that while other grasses had finished flowering and senescing, there were *N. neesiana* individuals still green and flowering.

Thirty-five species representing 12 families were recorded (Appendix E). Nineteen species were native, six were naturalised invaders, and 10 were naturalised alien and were listed as non-invasive species. Twenty species were recorded in the invaded area while 30 were recorded in the uninvaded plots (Table 4.2). Species composition did not vary significantly between invaded and uninvaded plots ($X^2 = 7.523$, d.f. = 1, $p = 0.244$), although uninvaded plots had a higher number of species. Litter was higher in invaded areas ($X^2 = 6.323$, d.f. = 1, $p < 0.05$). The litter was mainly from partially burnt *N. neesiana* stalks.

Nassella neesiana tuft basal circumference ranged from 2 cm to 96 cm (average 17.01 cm). *Nassella neesiana* cover ranged from 15 % to 90 % with an average cover of 51.07 % in studied areas. The densest invasions were around the golf course and prison areas (Site 4). Nearly all tufts which were evidently more than a year old, had signs of having been burnt in the past with the current culms being growth from the current season. The area had last burnt more than a year earlier, so any burnt tufts were assumed to be from either that fire or earlier fires. Some of the smaller flowering tufts, with no sign of their tufts having been burnt were assumed to have grown from seed after the fires. There was no sign of old culms on these plants and no sign of them having been burnt despite the fires of the last dry season. The larger and older tufts were breaking up with tillers coming from the edges (Fig. 4.4). These tufts showed signs of having been burnt multiple seasons before. In some cases, it was difficult to tell if tufts were coming from a single base or not after the old base has completely broken up and in such cases the tufts were considered separate.

Fig. 4.4. An old *Nassella neesiana* base (outlined in yellow) with tillers coming from the edges. Photo: A. Mapaura.

Plant richness, diversity and abundance

All diversity variables did not differ significantly between the invaded and uninvaded plots. However, invaded plots had more average alien naturalised and invasive species compared to their uninvaded plot pairs. The average number of native species, forbs, and species richness were higher in uninvaded plots than invaded plots (Table 4.2).

Table 4.2. Comparative data between *Nassella neesiana* invaded and uninvaded plots. Overall numbers are given in brackets.

Parameter	**Mean values per plot**		**Chi_sq**	**d.f.**	**p-value**
	Invaded	**Uninvaded**			
Native species	2.29 (12)	3.00 (17)	4.742	1	0.059
Forb species	2.50 (13)	3.50 (19)	2.315	1	0.128
Naturalised species	2.21 (13)	1.70 (13)	0.640	1	0.423
Naturalised invasive	1.14 (6)	1.29 (4)	0.117	1	0.731
Species richness (S)	5.64 (20)	6.57 (30)	3.158	1	0.076
Shannon index (H)	0.94	0.99	0.112	1	0.737
Simpson (D)	0.48	0.43	0.050	1	0.822
Inverse Simpson (D1)	2.18	2.33	0.063	1	0.801
Pielou's evenness (J)	0.54	0.51	0.162	1	0.687
			F		
Grass species	3.14 (12)	3.07 (11)	0.019	1	0.892

Soil physical and chemical properties

Soil physical and chemical variables did not differ significantly between invaded and uninvaded plots. All sampled areas, both invaded and uninvaded, had sandy loam soils. Even so, invaded plots had slightly higher concentrations of most minerals compared to uninvaded plots (Table 4.3). However, carbon, copper, medium sand, phosphorus, silt, and sulphur were higher in the uninvaded plots.

Table 4.3. Results of soil variable analysis between *Nassella neesiana* invaded against uninvaded plots, across the seven sites. Parameter units are indicated in brackets after the parameter.

Soil parameter	Mean		F	d.f.	p-value
	Invaded	Uninvaded			
Boron (mg/ kg)	0.213	0.187	1.554	1	0.236
Calcium (cmol(+)/ kg)	8.634	8.213	0.100	1	0.757
Carbon	3.500	3.599	0.051	1	0.826
Clay (%)	13.429	13.143	0.010	1	0.923
Coarse sand (%)	10.714	9.429	0.151	1	0.705
Copper (mg/kg)	3.753	4.027	0.362	1	0.559
Fine sand (%)	49.000	46.857	0.150	1	0.705
Magnesium (cmol(+)/ kg)	3.766	3.743	0.001	1	0.973
Manganese (mg/ kg)	242.671	238.371	0.057	1	0.816
Medium sand (%)	6.000	7.143	0.173	1	0.685
Phosphorus (mg/kg)	144.286	147.000	0.003	1	0.958
pH	6.029	5.971	0.044	1	0.838
Potassium (mg/ kg)	499.000	435.571	3.211	1	0.0999
Silt (%)	17.143	23.429	3.163	1	0.101
Sulphur	6.257	7.014	0.590	1	0.457
Sodium (mg/ kg)	39.286	101.286	1.280	1	0.281
Sotal cations (cmol(+)/ kg)	13.857	13.993	0.006	1	0.939
Zinc (mg/ kg)	14.317	13.911	0.006	1	0.938

DISCUSSION

This is the first known population and ecological study of a *N. neesiana* invasion in South Africa. Unlike most invasive species in South African urban areas (Potgieter et al., 2020), *N. neesiana* was not a deliberate introduction but is believed to be an accidental introduction in hay consignments (Henderson, 2018; Wells, 1978). Records in the Qwaqwa herbarium and checklists of the area shows that the area has been surveyed by experienced botanists before and it is expected that it would have caught the eye of at least one of them. The size diameters recorded indicated that they couldn't have grown that much in one season. The results demonstrate that *N. neesiana* is a cryptic invader. Despite its supposed introduction in the early 1900s, it had not been recorded in the study area until early 2020. No specimen could be found among the Harrismith plant collection housed in the Qwaqwa herbarium and the plant checklists of Harrismith stored in the herbarium. This is despite its wide distribution along the stream to the north-eastern part of Harrismith town (Fig 4.2). Some individuals had a basal circumference of over 80 cm being recorded in this research suggesting that they have been growing in this area for many years. The species has most likely gone unnoticed due to its growth form which superficially resembles some indigenous perennial tufted grasses (Henderson, 2018), such as *Tristachya leucothrix*, and some common non-invasive alien ruderal grasses such as *Bromus*.

Nassella neesiana was found to establish more readily along watercourses and seasonal wetlands that have experienced significant anthropogenic disturbance. This has also been noted by other researchers previously (Bedggood and Moerkerk 2002, Bourdôt and Ryde 1986, Cook 1999, Frederick 2002, Hayward and Druce 1919, Hocking 2011, Slay 2002). Vegetation clearing and availability of moisture appear to be important for the successful establishment of *N. neesiana* in this area. As noted by Faithful (2012), invasion is preceded by loss of vegetation. This was confirmed through searches in drier 'undisturbed' areas which had more native flora than exotics (with no *N. neesiana*) and close to the invaded patches.

It was observed that older *N. neesiana* tufts that were burnt and had died back were able to continue to expand due to new plants regrowing from the tuft’s edges until the clones were completely detached from each other. This strategy may give it the ability to expand even when seed germination is not possible due to factors such as shading from resident vegetation or lack of moisture. This supports Healy (1945), who observed that *N. neesiana* recovers quickly, especially from the tuft, while associated native species are often killed and need to recruit from seed. Grasses in a Grassland biome are adapted to fire since they evolved with it and the natural fires are often synchronised with their phenology. However, the invasion by *Nassella neesiana*, a C_3 cooler season grass, shifts the time these fires occur in the C_4 dominated Grasslands biome. Tufted C_4 grasses, such as *Themeda triandra*, which do not have stolons will be negatively affected by the late fires. Both the ability to withstand frequent fires and resprouting from an ever-expanding base gives *Nassella neesiana* a competitive edge over other grasses in the area. Since it was found that *N. neesiana* in the study area, remained green much longer than co-occurring grasses, it might be causing a shift in the burning season to much later in the season. This supports the findings of Faithfull (2012) and Joubert (1984) who noted that *N. neesiana* has a high biomass and alters fire regimes. The altered fire regime leads to altered regeneration of other plants which consequently leads to altered ecosystems (Dickens and Allen, 2014). Kabaš and Ljubičić, (2019), noted that in peri-urban areas *N. neesiana* increases the risk of fire.

The urban areas sampled were heavily disturbed and full of weedy aliens such as *Bidens pilosa, Bromus catharticus, Physalis viscosa, Tagetes minuta* and *Verbena brasiliensis*, which are ruderal species and well adapted to disturbance. The native species such as *Aristida junciformis, Cynodon dactylon, Eragrostis curvula, Paspalum scrobiculatum* and *Sporobolus pyramidalis* are common on disturbed areas and are considered invasives in other parts of the world (CABI, 2021). The weedy characteristics of both native and exotic flora found in the studied areas, enables them to coexist in a regularly re-disturbed context, with propagules very regularly added from illicit garden refuse dumping along the stream. *Nassella neesiana* was found to have little to no effect on plant species composition and other diversity measures in this area. This differs from the findings of research done in Australia on natural vegetation (Faithfull, 2012) where *N. neesiana* was found to reduce diversity of native species but facilitated alien species. It would therefore appear that the impact of *N. neesiana* on species richness and diversity in a disturbed environment, with mainly ruderal species may be less than in undisturbed habitats.

Although topsoil nutrient concentrations did not differ significantly between invaded and uninvaded plots, invaded plots had marginally higher average concentrations. This is consistent with the conclusions that IAPS are often associated with heightened levels of topsoil nutrients (Dassonville et al., 2008; Ehrenfeld, 2003; Gibbons et al., 2017; Vanderhoeven et al., 2005). The actual mechanism whereby *N. neesiana* achieves this is not known at this stage. However, Vanderhoeven et al. (2005) reported that plants with long roots mine nutrients as part of their development and releases them into the soil when they die thus maintaining the elevated nutrient levels in the topsoil. The higher litter levels recorded in the invaded areas compared to the adjacent uninvaded areas suggests that nutrients in the top soils are being replenished from the dead material. The raised topsoil nutrient concentrations did not appear to influence the germination and establishment of co-occurring plant species. The disjunct nature of *N. neesiana* distribution in the grasslands can be accounted for by the fact that the seeds are predisposed to fall close to their parent plant and germinate there (Bourdôt and Hurrell, 1989). The few individuals that were found growing alone in the grasslands were carried there by dispersal agents such as animals, and water.

Nassella neesiana integrates well into the resident vegetation making it difficult to spot from more than 10 m away even when flowering, unless the cover is such that the drooping inflorescences stand out. They are nearly impossible to identify when they are not flowering. Healthy looking individual *N. neesiana* plants were observed growing among other plant species. The presence of other plant species did not appear to influence their ability to grow. This is characteristic of cryptic behaviour.

The invasions in Harrismith are worrisome considering that Platberg, being an inselberg, is of conservation importance because inselbergs harbour many endemics and act as reservoirs and refugia for unique genetic material (Brand et al., 2011). Elsewhere in South Africa, *N. neesiana* is a well-integrated component of grassy mountain landscapes, such as in the Witteberg area (Eastern Cape Drakensberg), and the Boschberg (Somerset East, Eastern Cape) – so its eventual colonisation beyond Platberg Stream is not implausible (although it has not been noticed outside the current riparian zone as yet). In New Zealand, *N. neesiana* has been reported to have a lag phase spanning several decades and was still in its lag phase after more than 60 years (Bourdôt and Hurrell, 1989). The significant increase in *N. neesiana* sighting in South Africa in recent years suggests that it may be entering the geometric phase of expansion, so improved monitoring of the species is needed. A possible starting point to survey for more invaded populations is scouting for all known British posts during the Anglo-Boer war that would likely be points of introduction. Given that riparian areas are naturally ecologically dynamic zones of regular disturbance, favouring species such as *N. neesiana*, it may be that *N. neesiana* has successfully colonised much of the Wilge River system, from this Anglo-Boer War source population in Harrismith. While this study did not explore the Platberg Stream-Wilge River confluence and below-stream, this is an important future study need.

CONCLUSIONS

Nassella neesiana appears to gradually infiltrate vegetation in disturbed and riparian contexts. It has been increasing steadily over the years and its densities seem to be at a stage where it is easier to notice, suggesting that it might be exiting a long lag phase. Although its overall impact on native plant diversity and soils in South Africa remains poorly understood, it seems to have no significant impact in urban environments. The lack of impact could be due to the fact that it is invading already-disturbed environments where little original vegetation remains. The similarity of *N. neesiana* to other tufted perennial grasses and their drooping inflorescences hides them from detection. The possible more extensive presence of *N. neesiana* in eastern and southern South Africa requires investigation and further quantitative population and environmental impact studies. Its negative impact on the environment and livestock farming in Australia and New Zealand should be a strong incentive to act now. Similar research to this Harrismith one could be done at other *N. neesiana* sites in South Africa, with the aim of advising land managers on best management options, biocontrol research opportunities, and adding this species the AIS Regulations as a potential Category 1b.

CHAPTER 5

THE USE OF GENETIC BARCODING TO DISTINGUISH INVASIVE NASSELLA IN SOUTH AFRICA

ABSTRACT

The three exotic *Nassella* species occurring in the South African montane grasslands are morphologically cryptic, hence difficult to distinguish from several co-occurring tufted perennial grass species, and between one another. This makes traditional identification techniques less effective, especially when dealing with vegetative material. The efficacy of DNA barcoding in successfully discriminating morphologically cryptic species, as has been demonstrated with other plants, offers a possible solution here. The aim of this research study was to evaluate the use of DNA barcoding to identify *Nassella* species and, in turn, whether this method is a viable option for practitioners in South Africa. Two gene loci, *petL–psaJ* (*petL*) and ETS were used to discriminate the three *Nassella* species from each other, and from three native grass species (*Aristida diffusa* Trin*., Festuca caprina* Nees and *Koeleria capensis* (Steud.) Nees), and the exotic species *Jarava plumosa* (Spreng.) S.W.L. Jacobs & J. Everett. The two gene loci were found to be effective at discriminating *N. neesiana* and *N. trichotoma* from each other and from several other species in Australia. The results indicate that the *petL* locus has no power to discriminate the three species but is good at discriminating the different genera. However, *ETS* on its own was able to identify all the tested species. The cost of outsourcing the whole process to a genetic laboratory is reasonable and makes DNA barcoding a viable identification method. More invasive plants should be targeted for testing using the same gene locus in the country.

INTRODUCTION

DNA barcoding is a method that works at the molecular level to accurately identify species (DeSalle and Goldstein, 2019; Ji et al., 2020; Saddhe and Kumar, 2017; Vijayan and Tsou, 2010). DNA barcoding can augment traditional plant species identification methods which are based on morphology as it can be applied even in the absence of commonly used characters like flowers and fruits and can even be used in cases where only pieces of a plant such as root or seeds are present (Kress et al., 2005; Kress and Erickson, 2007; Ragupathy et al., 2009). The use of DNA barcoding to identify organisms relies on getting a DNA sequence of a particular locus of an unknown organism and matching it with a sequence of a known organism in a sequence repository such as the National Center for Biotechnology Information (NCBI) database (Kress and Erickson, 2007). This is not different from the traditional methods of comparing an unknown plant with known plant collection in an herbarium. As with traditional methods, identification is only possible if there is a match in the sequence repository. What is important is that the chosen gene locus/loci show high variability between species but are well conserved within species so that any intraspecies variation is insignificant (Ji et al., 2020; Vijayan and Tsou, 2010).

Unlike in animals where the mitochondrial gene, cytochrome c oxidase subunit 1 (COI) gene is fairly accurate in segregating species across group, a similar locus has not been found for plants (Hebert et al., 2003; Saddhe and Kumar, 2017). The COI gene in plants under goes a low rate of mutation which makes it a poor candidate for discriminating plant species (Hollingsworth et al., 2011). A number of DNA loci have been suggested as potential for plant species identification and have been tested for effectiveness but have generally been found to be effective in specific regions of the world and for specific groups of plants (Avin et al., 2014; Hollingsworth et al., 2011; Kress et al., 2005; Kress and Erickson, 2007; Wang et al., 2017). No single locus has been found to be effective for all plant groups (Kress et al., 2005; Saddhe and Kumar, 2017) and so a multi-locus approach has been suggested (Hollingsworth et al., 2011). However, it has been demonstrated that DNA barcoding can be effective when dealing with a limited number of species (Chen et al., 2015; Wang et al., 2017).

Nassella species have become invasive in many parts of the world after being introduced from their natural habitat in South America (Henderson, 2018; Humphries and Florentine, 2021). *Nassella tenuissima* and *N. trichotoma* have become invasive in South Africa (Henderson, 2018; Mapaura et al., 2020; Wells et al., 1983), while *N. neesiana* is also present as an alien species but is not a declared invader. All three species are not palatable and can outcompete native grasses which often leads to reducing the carrying capacity of invaded pastures.

These *Nassella* species are morphologically cryptic and therefore not easy to distinguish from several co-occurring tufted perennial grass species or between one another (Faithfull, 2012; Henderson, 2018; Wang et al., 2017). A more definitive identification is only possible when the plants are in flower, by which time seeds would have been produced and the plants are already actively spreading (Faithfull, 2012). This has made early detection impossible, thus delaying management action. The use of DNA barcoding may offer potential for determining plant identifications in cryptic species such as these. The effectiveness of DNA barcoding in discriminating cryptic species, such as grasses has already been demonstrated (Ji et al., 2020; Ragupathy et al., 2009; Steven and Subramanyam, 2009) including some *Nassella* species (Wang et al., 2017, 2014).

The negative impact of *Nassella* species on biodiversity and the economy calls for methods that facilitate early detection and rapid management response to prevent their establishment (Maxwell et al., 2009). The near impossibility of correctly identifying them when they are not in flower makes early detection difficult. The challenge though, is that DNA barcodes have not been produced for all three *Nassella* species. DNA barcodes exist for *N. neesiana* and *N. trichotoma* but not for *N. tenuissima*. In addition, the DNA loci that can be used to discriminate the three *Nassella* species have not been established in literature though the ETS and *petL* loci have been reported to successfully discriminate *N. neesiana* and *N. trichotoma* in Australia (Wang et al., 2017, 2014).

This research aimed to test the possibility of discriminating the three *Nassella* species amongst themselves and from morphologically similar species using two gene loci which have been demonstrated to be effective in distinguishing some of the *Nassella* species (Prince, 2015; Wang et al., 2017). The research also sought to explore the possibility of developing a scheme based on DNA barcoding that can be used by field practitioners to identify these invasive species in South Africa.

METHODS

Sample collection

Three co-occurring grass species, *Aristida diffusa* Trin., *Festuca caprina* Nees and *Koeleria capensis* (Steud.) Nees, all of which have been previously confused with *Nassella* species, were selected to compare with the *Nassella* species. A fourth species, *Jarava plumosa* (Spreng.) S.W.L. Jacobs & J. Everett, (an alien) was also included because it is in the same tribe as *Nassella* species and might be confused with them. *Jarava plumosa* is native to South America (Argentina, Chile, Uruguay, and southern Brazil) and has only been recorded a few times in South Africa but has potential to become invasive (Sylvester et al., 2021). A total 13 fresh leaf samples (about 1 gram each), (Table 5.1), were collected from these four species and the three *Nassella* species and dried in silica gel before analysis. The main aim of the research was to find out if the three *Nassella* species can be discriminated, using the petL and ETS gene loci. These two gene loci have been demonstrated to be good at discriminating *N. neesiana* and *N. trichotoma* from each other and from several other species in Australia (Prince, 2015; Wang et al., 2017). The species were all identified morphologically by comparing specimens with collections housed in Makhanda (formerly Grahamstown) and Qwaqwa herbaria. A voucher specimen for each sampled grass was deposited in the Qwaqwa herbarium (Table 5.1).

Table 5.1. A list of the specimens sampled for the *petL* and ETS loci and deposited at the Qwaqwa herbarium.

Species	**Collectors**	**Collector's No.**	**Location**	**Latitude**	**Longitude**	**Altitude (m)**
Nassella neesiana	A. Mapaura	1139	Senqu Local Municipality, Eastern Cape.	-30.636621	27.72374	1900.00
Nassella neesiana	A. Mapaura	1142	Boet Steenkamp's Farm, Barkly East, Eastern Cape.	-31.011580	27.475525	1698.11
Nassella neesiana	A. Mapaura	1147	Harrismith town, Free State.	-28.272033	29.145688	1645.80
Nassella tenuissima	A. Mapaura	1143	Boet Steenkamp's Farm, Barkly East, Eastern Cape.	-31.064957	27.496675	1707.71
Nassella tenuissima	A. Mapaura	1144	Gawie van Zyl 's farm, Barkly East, Eastern Cape.	-31.064957	27.496675	1707.71
Nassella trichotoma	A. Mapaura	1145	Roadside, near Dordrecht, Eastern Cape.	-31.08584	27.702326	1832.04
Nassella trichotoma	A. Mapaura	ASA01	Asante Sana Private Game Reserve, Eastern Cape.	-31.7061	25.036583	1520.07
Nassella trichotoma	A. Mapaura	ASA04	Asante Sana Private Game Reserve, Eastern Cape.	-31.70526	25.03435	1522.83
Jarava plumosa	R.J. Soreng, S.P. Sylvester, M.D.P.V. Sylvester, and A. Mapaura	30	By Tele River on road to Lundeans Nek, near Bebeza village, Eastern Cape.	-30.518347	27.649831	1460.02
Aristida diffusa	A. Mapaura, R.J. Soreng, S.P. Sylvester, and M.D.P.V. Sylvester	1141	On road to Lundeans Nek, Eastern Cape.	-30.462621	27.599741	1344.03
Festuca caprina	A. Mapaura	GG1	Golden Gate Highlands National Park, Free State.	-28.498640	28.616091	2106.31
Festuca caprina	A. Mapaura	GG2	Golden Gate Highlands National Park, Free State.	-28.49.8640	28.605674	2021.16
Koeleria capensis	A. Mapaura	1148	Phillip Erasmus's farm, Boschberg mountains, above Somerset East	-32.657374	25.610278	1394.24

DNA extraction and sequencing

Dry leaf tissue was ground using a mortar and pestle with liquid nitrogen. Approximately 0.08 g of finely ground tissue was used in a Qiagen PowerPlant® DNA extraction kit (Valencia, CA, USA) following the manufacturer's instructions. The extracted DNA was stored in TE buffer at 4 °C. The forward and reverse primers used for the specific loci were prepared at Inqaba Biotechnical Industries (Pty) Ltd, South Africa (Table 5.2).

Each polymerase chain reaction (PCR) mix contained 5 µL of KAPA Plant PCR Buffer (2X) (Kapa Biosystems/Roche), 0.3 µL of each of the primers at a 0 concentration of 10 µM, 0.04 µL of KAPA3G Plant DNA Polymerase (2.5 U/µL) (Kapa Biosystems/Roche), 0.4 µL of template DNA (50 ng/µL) and MilliQ water to a final volume of 10 µL. The PCR program had a five-minute denaturing step at 95 °C, followed by 30 cycles of 30 seconds at 95 °C, 30 second annealing at 54 °C (*petL*-IGSF - *psaJ*-IGSR) or 58.9 °C (RetS4F - 18SR) respectively, and 30 seconds extension at 72 °C. A final 10-minute extension at 72 °C was done after completion of the cycling. Aliquots of the PCR products were tested for successful amplification by visualisation on a 1.5% agarose gel stained with ethidium bromide. The PCR product was cleaned up using the Promega Wizard Clean-Up System kit following the manufacturer's instructions and submitted for sequencing at the Central Analytical Facility at Stellenbosch University using an ABI 3100® genetic analyser.

Table 5.2. PCR primers used for amplification of the ETS and *petL* loci.

Locus	Primer	Sequence	Source
petL–psaJ IGS	*petL*-IGSF *psaJ*-IGSR	TCTATTAGCGGCTTTAACTATA GCATCCGGGAATAAACGA	Prince, 2015
ETS	retS4-F 18S-R	TTGGCTACGCGAGCGCATGAG AGACAAGCATATGACTACTGGCAGG	Wang et al., 2017

Cost of DNA sequencing

To assess the feasibility of using the DNA sequencing by the general public and practitioners, one of the largest DNA sequencing laboratories was contacted for quotes and feasibility for them to carry out the whole process and give an interpreted sequence.

Data analysis

The forward and reverse sequence chromatograms at each locus were assembled in Geneious Prime 2021.2.2 (https://www.geneious.com) and the consensus sequences were manually verified. The consensus sequences were exported for alignment in MegaX ver. 10.2.6 using the MUSCLE algorithm with default settings. The ETS alignments were aligned against *N. neesiana* ETS gene locus, NCBI sequence library accession number KX281097 in Geneious Prime. The differences between each *Nassella* species and the reference sequence was noted using the base numbers of accession KX281097. The *petL* alignments were aligned against the *N. trichotoma* chloroplast complete genome, NCBI library accession number KX792500, between the *petL* and the *psaJ* genes (position 64299 to 65630).

RESULTS

Sequence characteristics

Both loci could be amplified for all tested species. After cleaning the bases, *N. neesiana* was 445 bases long, *N. tenuissima* was 447 bases long while *N. trichotoma* was 454 bases long. Sequences were also downloaded from GenBank for both *N. neesiana* and *N. trichotoma* and compared with sequences obtained here and no differences were noted. This shows that there have been no genetic sequence changes in the South African populations.

Species Discrimination

The ETS locus showed very good distinguishing power among all tested species (Supplementary Material 5.1). The three *Nassella* species differed at 22 bases to the reference sequence (Supplementary Material 5.2). *Nassella neesiana* and *N. tenuissima* differed at 17 positions, *N. tenuissima* and *N. trichotoma* differed at 13 positions, while *N. neesiana* and *N. trichotoma* differed at 15 positions. The petL to psaJ intergenic spacer was unable to distinguish *Nassella* species from each other but could distinguish the different genera tested (Supplementary Material 5.3).

Cost of DNA sequencing and interpretation

The lab that was contacted for a quote confirmed that they can carry out the whole process from genomic DNA extraction and PCR amplification to DNA sequence analysis, for a fee of R 549.92. DNA sequence analysis would cost a further R 710.63. The client must provide plant samples to work with and get the analysed sequences back from the laboratory.

DISCUSSION

This research has demonstrated that it is possible to distinguish the three *Nassella* species using just a single locus. Grasses are very uniform in appearance and their identification uses characters such as the spikelet which are small and difficult to see with the naked eye (Fish et al., 2015) thus making them difficult to identify even for experienced scientists. In addition, for most of the *Nassella* species, positive identification is only possible when they are in flower. As a result, grass taxonomists in South Africa are very few and are often overwhelmed and it can take some time before a sample is identified. In this case, DNA barcoding can be used as a quick alternative tool to identify morphologically cryptic species (Ragupathy et al., 2009). This is particularly useful when the plants are in the vegetative state and difficult to identify.

Early identification of invasive species is critical as it allows for management measures to be instituted before they become a problem. There are cases where traditional taxonomic methods fail to separate two or more morphologically cryptic species (Ragupathy et al., 2009) and this can result in invasive populations spreading without detection. The identity of some grass populations in Golden Gate Highlands National Park, which were thought to be *Nassella trichotoma,* could not be resolved for years because there was no flowering material to confidently identify them (Henderson, 2018). If DNA barcoding tools had been used at the time, a resolution could have been reached much earlier.

The *petL* locus was found to distinguish *N. neesiana* and *N. trichotoma* in Australia (Wang et al., 2014), but was not successful in this current study. This may be due to amplifying a different locus, as I could not obtain the primer sequences that they used even after making several requests. According to Wang et al 2017, they used the *psbE—petL* intergenic spacer whereas I used the petL–psaJ intergenic spacer. The primers for *petL* were then obtained from Prince (2015). The *petL* sequences downloaded from NCBI matched my sequences for the same gene, indicating that the correct locus was amplified.

The ETS gene was successful in discriminating all the species tested here. There were numerous differences between *Nassella* and the other genera which included several deletions or insertions. The current results show that this locus can also discriminate *N. tenuissima* in addition to the previously reported *N. neesiana* and *N. trichotoma* (Wang et al., 2017). Although multi-locus DNA barcodes can have more discriminatory power than a single locus (Hollingsworth et al., 2011; Kress et al., 2009), a single locus that has sufficient discriminatory power is easier to use and costs far less than multi-locus barcodes, which readily lends itself to a wider audience.

With the advances that have been made in the field of DNA sequencing and alignment, the total cost is affordable. The whole process can be out-sourced, thus removing the need for access to a laboratory and specialist knowledge. It means property owners and conservation practitioners do not need to wait for the grasses to flower before any identification can be done. In conservation areas, such as nature reserves, this tool is very important as it enables the identification of any suspected invasive species for which DNA barcodes are available before it becomes a problem. However, since successful identification using DNA sequencing requires comparison of an unknown sequence to a known sequence, it is important to sequence as many plants as possible. Only when the DNA sequence library is comprehensive can this technique become truly available for mainstream use.

CONCLUSION

This research has demonstrated that it is possible to use a single locus, ETS, to discriminate all three *Nassella* species in South Africa from one another and from some morphologically similar species. The *petL* locus was not able to distinguish the three species although it was able to distinguish them from other genera. The cost of this approach is reasonable making it a viable identification method, when the plants are found at a time that they are not in flower. Field practitioners (including farmers) can collect samples and send them directly to a laboratory and get the interpreted results back. In light of this, it is concluded that the DNA sequencing technique offers a powerful solution for the accurate identification of these morphologically cryptic invasive plants.

CHAPTER 6

CLIMATE CHANGE AND PREDICTED CHANGES IN THE DISTRIBUTION OF THE INVASIVE GRASS GENUS *NASSELLA* IN SOUTH AFRICA AND LESOTHO

ABSTRACT

Three South American *Nassella* species (*N. neesiana, N. tenuissima* and *N. trichotoma*) have naturalised in South Africa, but have not been reported in Lesotho, with *N. tenuissima* and *N. trichotoma* having been recognised as serious invaders since the 1970s. An understanding of the current and future potential distribution of these invasive species is important as a way of assessing the potential threats they pose to biodiversity in South Africa and Lesotho. Further, in assessing future distribution scenarios, the implications of climate change need to be factored in, as it is expected to influence the spread and establishment of the three species. This research sought to answer the following questions: 1. What are the bioclimatic variables controlling the geographical distribution of these *Nassella* species? 2. What is the current potential distribution of the *Nassella* species? 3. What is the future potential distribution of the three species under four carbon-emission scenarios using available estimates for the 2081 to 2100 period? Mid-range estimates for this time period suggest that temperatures will continue to rise even if carbon concentrations are to stabilize. Bioclimatic variables were downloaded from WorldClim database, (http://www.worldclim.org), at a spatial resolution of 2.5 arc minutes which is based on climate data for 1970-2000. After testing for multicollinearity among the 19 bioclimatic variables, seven environmental variables were retained for each species for the models. Species distribution modelling was done using the 'sdm' package in R. The known global distributions of the three species and an ensemble of six algorithms were used to develop the models. To project the future distribution ranges, the four future shared socio-economic pathways to the representative concentration pathways (SSP_RCP) scenarios under version six of the Model for Interdisciplinary Research on Climate (MIROC6) for the period 2081 to 2100 were run for each species separately. The research found that all three species can potentially occupy more area than they currently do under current climate envelopes. Temperature-based bioclimatic variables, especially isothermality, are important in determining the distribution of all three species. Compared to currently suitable areas, future projected suitable areas will be much less for the three species. Highly suitable areas for all species will shrink by

between 50 to 80% under low and high carbon-emission scenarios respectively. While the extent of suitable habitat for these *Nassella* species is projected to decline elsewhere in South Africa, the Maloti-Drakensberg including Lesotho will face an increase in suitable areas across all future climate-change scenarios. Given that much of Lesotho is highly degraded from poor rangeland governance, and other disturbances, which would favour *Nassella* establishment, early detection of *Nassella* incursion into Lesotho should be an extremely high priority.

INTRODUCTION

The fast pace of climate change being experienced globally poses a major threat to biodiversity and food security as the distribution and performance of species changes with the changing climate (Muluneh, 2021; Pörtner et al., 2022; Tebaldi et al., 2021). Species distributions are a result of many factors which include climate, environmental, physiological traits, and inter-specific as well as intra-specific interactions (Bezeng et al., 2017; Dubuis et al., 2013; Zhang et al., 2021). Some species have been demonstrated to shift their distribution ranges poleward especially in the northern hemisphere in response to climate change (Bellard et al., 2013), while others move upwards in elevation (Bellard et al., 2013; Esser et al., 2019; Lenoir et al., 2008; Xu et al., 2021), tracking their climate niche. This shift, however, favours species that can move at the pace of the shifting range and leads to potential extinction to those that are immobile or have a slow rate of spread (Faithfull, 2012). Knowledge of the current distribution of species under current conditions and their likely response to the projected climate change is essential in informing policy and practical responses (Tebaldi et al., 2021). Some species are projected to have an increase in their range sizes with rising temperatures (Walther et al., 2009), while others will experience range contraction (Bezeng et al., 2017; Richardson et al., 2010).

Nassella neesiana (Trin. & Rupr.) Barkworth, *N. tenuissima* (Trin.) Barkworth, and *N. trichotoma* (Nees) Hack. & Arechav. are grasses native to South America and (for *N. tenuissima*) parts of North America. All three species are invasive in other parts of the world where they invade temperate grasslands, especially in livestock farming areas where overgrazing appears to facilitate their establishment (Campbell, 1998; Faithfull, 2012; Wells and De Beer, 1987). Management of the species is particularly troublesome, as they are difficult to remove once they are established due to long-lived seeds and a deep root system (Campbell, 1998; Faithfull, 2012).

In South Africa, all three species have established, but only *N. tenuissima* and *N. trichotoma* are categorised as invasive under current legislation. Nevertheless, although current legislation does not include *N. neesiana* this species is present in the country and is already invading a number of areas and is considered a serious invader in other parts of the world (Faithfull, 2012; Giordano and Anderson, 2021; Humphries and Florentine, 2021). In South Africa, *N. neesiana* has been recorded in the Free State, Eastern and Western Cape provinces; *N. tenuissima* appears to be confined to the Eastern Cape around Barkley East and Clifford areas (Eastern Cape part of the Maloti-Drakensberg); and *N. trichotoma* has been recorded in the Eastern and Western

Cape provinces (mostly in the Cape Midlands Escarpment area; Mapaura et al., 2020). Although all three species are not known to have invaded any part of Lesotho, their presence there cannot be ruled out completely since the records for South Africa are very close to the border. All three species are considered accidental introductions from the late 1890s to the early 1900s and are invading higher elevation grasslands (Henderson, 2018; Wells, 1978). Their spread has not been monitored since the end of coordinated control efforts in early 2000 (Henderson, 2018; Mapaura et al., 2020). The current distribution of all three grasses is likely an underestimation of their true extent due to their difficulty in identification. The species are all considered morphologically cryptic whereby they are often indistinguishable from native grasses and can only be accurately identified when in flower. It seems reasonable to suspect that all three species have continued to spread unnoticed, as new localities have been recorded during this study.

Knowledge of the geographical distribution of an invasion, not just the current extent but also the potential future distribution, is important when designing species conservation strategies (Esser et al., 2019; Finch et al., 2021). It can take time for an alien species to occupy all the suitable areas in an introduced range (Peterson and Soberón, 2012), and therefore an important part of a species management is to pre-empt potentially suitable areas to prevent further spread. Having this knowledge at hand helps in avoiding surprise invasions and assists in directing surveillance (Sofaer et al., 2019). Species distribution modelling (SDM) has become a tool of choice for estimating current species distributions and projecting their distributions into the future. There are various SDM methods and techniques with varying data requirements now available (Ahmed et al., 2021; Grenouillet et al., 2011; Naimi & Araújo, 2016; Zhang et al., 2021).

Species distribution modelling links species geographical distribution with a set of environmental variables using environmental conditions at known occurrence localities (Phillips et al., 2006). This set of environmental variables is used as a predictor in a distribution model. One of the assumptions of SDMs is that species conserve their niche at both temporal and spatial scales (Liu et al., 2020a). While this may not hold in all cases, a recent review has demonstrated that invasive species tend to occupy similar climatic niches in their introduced areas as they did in their native areas (niche conservatism hypothesis) (Finch et al., 2021; Liu et al., 2020a). This means an SDM developed for one time period can be used to predict distribution in a different time period (Liu et al., 2020a, 2020b), although caution is needed when transferring models to a different area (Liu et al., 2020b). Bioclimatic data sets are an important part of SDMs either alone or augmented by other environmental variables that are deemed important for the distribution of a species (Abdelaal et al., 2020; Kariyawasam et al., 2021; Tang et al., 2021). To develop a plausible model, knowledge of the factors that control the geographic distribution of the species being modelled is required.

Nassella neesiana and *N. trichotoma* predictions for South Africa and Lesotho were indicated on maps produced as part of other projects in Australia and New Zealand (Bourdôt et al., 2012; Kriticos et al., 2004; Watt et al., 2011), and since Southern Africa was not the target, the predictions are given in broad terms and hence not suitable for use at a local level. This study aimed to use SDMs to elucidate the implications of climate change on the distribution of the three *Nassella* species in South Africa and Lesotho and produce maps that can be used to direct surveillance and management. This objective was achieved by answering the following questions, 1. What are the bioclimatic conditions driving the geographical distribution of *Nassella* species? 2. What is the current potential distribution of the three species under current climatic conditions in South Africa and Lesotho? 3. What is the future potential distribution of the three species under the four emission scenarios in South Africa and Lesotho using the latest estimates for the 2081 to 2100 period?

METHODS

Dataset sources and cleaning

Before modelling, a field survey was undertaken to confirm the existence of some *Nassella* location records in the Eastern Cape. This was especially necessary for *N. tenuissima* whose records were very few and from the 1970s and from a single locality. During the survey, additional distribution records for all three species were georeferenced using an ETrex 10 GPS as they were encountered and were included in the model.

Nassella distribution data was sourced from the Atlas of Living Australia (https://www.ala.org.au) (Australia, 2021a, 2021b, 2021c), Southern African Plant Invaders Atlas (SAPIA) (Henderson and Wilson, 2017), Global Biodiversity Information Facility (GBIF) (Occdownload Gbif.Org, 2019), Botanical Dataset of Southern Africa (BODATSA) (Ranwashe, 2019) and iNaturalist records marked as Research Grade (https://www.inaturalist.org/places/south-africa).

Occurrence data were cleaned using the ‘CoordinateCleaner’ package (Zizka et al., 2019; R Core Team, 2021). Potentially problematic records identified were analysed using QGIS software (QGIS Development Team, 2021) and Google Earth Pro (accessed in August 2021), rectified, or excluded from further analysis. For each species, all records with a locality uncertainty of more than a kilometre were removed, as were records whose coordinates fell in the Arctic and Antarctica and duplicated records. To reduce spatial survey biases in occurrence records, spatial thinning of occurrence records was done using the ‘spThin’ R package (Aiello-Lammens et al., 2015; Vilela, 2019) to ensure that the selected records have a distance greater than 3 km from each other (Abdelaal et al., 2020; Vilela, 2019). After the data cleaning process *N. neesiana* had 1045 (11 in South Africa) records, *N. tenuissima* had 363 (6 in South Africa) records and *N. trichotoma* had 1536 (15 in South Africa) records for use in the distribution modelling.

Selection of environmental predictors

Edaphic variables influence distribution of plants and are usually included in species distribution models (Dubuis et al., 2013). However, soil parameters have not been found to limit the distribution of the *Nassella* species (Mapaura et al., 2020; Wells and De Beer, 1987) and previous modelling revealed that bioclimatic variables produce very reliable predictions (Bourdôt et al., 2012; Kriticos et al., 2004; Watt et al., 2011). Consequently, only bioclimatic variables were used for modelling. To model current climatically suitable areas, 19 bioclimatic variables were downloaded from WorldClim version 2.1 database, (*http://www.worldclim.org*) (Fick and Hijmans, 2017), at a spatial resolution of 2.5 arc minute which is based on climate data for 1970-2000.

Multicollinearity among environmental predictors can lead to overfitting the model and hence reducing its predictive power (De Marco and Nóbrega, 2018; Dormann et al., 2013; Graham, 2003). Multicollinearity among the 19 bioclimatic variables was tested using the stepwise variance inflation factor (vifstep) tool in the 'usdm' package in R (Naimi et al., 2014) and all variables with a Variation Inflation Factor (VIF) threshold greater than five were removed (Guisan et al., 2017). Variation Inflation Factor measures the extent to which each predictor can be explained by other predictors. A predictor that can be explained strongly by other predictors becomes redundant and so can be removed. This resulted in seven environmental variables being retained for each species for the development of the model (Table 6.1). Each species was modelled separately since they had different environmental variables.

Table 6.1. The environmental predictors used in developing the model for the potential distribution of *Nassella neesiana*, *N. tenuissima* and *N. trichotoma* in South Africa and Lesotho. The units for each variable are indicated in brackets at the end of the description.

Variable	Variable description	Variation Inflation Factor		
		Nassella tenuissima	*Nassella neesiana*	*Nassella trichotoma*
Bio1	Annual Mean Temperature (ºC)	-	1.80	1.36
Bio3	Isothermality (BIO2/BIO7) (×100) (%)	1.86	2.28	1.78
Bio4	Temperature Seasonality (ºC)	-	1.79	1.58
Bio5	Max Temperature of Warmest Month (ºC)	1.64	-	-
Bio6	Min Temperature of Coldest Month (ºC)	3.21	-	-
Bio8	Mean Temperature of Wettest Quarter (ºC)	-	-	1.56
Bio9	Mean Temperature of Driest Quarter (ºC)	3.69	2.19	
Bio13	Precipitation of Wettest Month (mm)	2.97	2.98	-
Bio15	Precipitation Seasonality (Coefficient of Variation) (%)	2.04	1.98	1.61
Bio18	Precipitation of Warmest Quarter (mm)	3.33	-	3.00
Bio19	Precipitation of Coldest Quarter (mm)	-	2.61	2.71

To project the potential distribution of the three *Nassella* species under future climatic scenarios, future climate projections were downloaded from the WorldClim database, (https://www.worldclim.org/data/cmip6) for the period 2081 to 2100, under version six of the Model for Interdisciplinary Research on Climate (MIROC6). These are the new future pathways under the Coupled Model Intercomparison Project phase 6 (CMIP6) based on the sixth Intergovernmental Panel on Climate Change assessment report (IPCC-AR6). All four scenarios under MIROC6 were used for modelling future distribution. These scenarios relate to the shared socio-economic pathways (SSPs) to the representative concentration pathways (RCPs) (Tebaldi et al., 2021). The SSP_RCPs used are SSP126, SSP245, SSP370, and SSP585. SSP126 represents low greenhouse gas emissions while SSP585 represents high emissions, with SSP245 and SSP370 representing medium emissions. Mid-range estimates for the period 201 to 2100, suggest that temperatures will continue to rise even if carbon concentrations are to stabilize. It is important to have an idea of what the invasion scenario will be like and take pre-emptive measures.

Data processing and model evaluation

Model generation and species distribution analyses were done using the 'sdm' R package (Naimi and Araújo, 2016). Each species was analysed independently because their climatic requirements varied. The same number of pseudo-absence records as presence records were generated for each species by randomly locating them within a radius of 200 km of a presence record (Barbet-Massin et al., 2012; Lobo et al., 2010). The pseudo-absence data was necessary because the occurrence data had presence only records, yet absence data was required to successfully develop the models. An 'sdmData' object was created incorporating this pseudo-absence data and removing points located in presence sites. The sdmData object was then used to develop the model with 30 % of the data used for testing and 70% for evaluating in 10 runs of bootstrapping replication (Dakhil et al., 2021; Sillero and Barbosa, 2021).

To reduce the uncertainties associated with using a single method (Grenouillet et al., 2011; Kukunda et al., 2018; Thuiller et al., 2009), an ensemble of six algorithms that require pseudo-absence data (not background data) were used to develop the model. The methods used were boosted regression trees (brt), classification and regression trees (cart), generalised additive model (gam), multiple adaptive regression splines (mars), random forests (rf), and support vector machine (svm). The mean performance of each model using the area under the curve (AUC) of the receiver operating characteristic (ROC), a threshold-independent model evaluation indicator, and true skill statistic (TSS), a threshold-dependent measure of model accuracy, were considered a good fit if the values were above 0.90 for AUC and above 0.8 for TSS (Allouche et al., 2006). Both methods use modelled and observed presences/ absences to test sensitivity and specificity. Receiver operating characteristics curves are produced by calculating and plotting true positive (sensitivity) against false positive (1 − specificity) after considering all possible threshold possibilities (Allouche et al., 2006). AUC, which is the total area under ROC, from 0 to 1, with 0.5 and below considered no better than random guesses and those above are better predictors. AUC values of

0.5–0.7 represent poor model performance, values of 0.7–0.9 are considered moderate, and values above 0.9 represent excellent model performance. TSS, on the other hand, is defined as sensitivity + specificity – 1, and is not affected by the size of data used. Its values ranging from −1 to +1 (Allouche et al., 2006). Values of zero and below are considered no better than random guessing and 1 is a perfect prediction.

To predict the potential distribution for each species under the current climatic conditions, a prediction was run using weighted TSS and optimised using threshold criterion '2 (max(se+sp))' which maximises specificity and sensitivity. The final model predictions were also assessed by predicting the current suitable areas in South America where the species are native and assumed to have had enough time to fill most of the available niche. The model was considered a good fit if it approximated the known areas. Developing the models based on the global distribution of the species, which includes its native range, ensures that its fundamental niche is captured (Bourdôt et al., 2012; Srivastava et al., 2020). This is important in this case since the realised niche is expected to be smaller than the fundamental niche because the species may not have had enough time to occupy all the suitable areas (Bourdôt et al., 2012; Soberón and Arroyo-Peña, 2017). The final models were used to predict and project suitable areas in South Africa.

Habitat suitability maps for both present and future climate scenarios were produced and clipped to the boundaries of South Africa and Lesotho. Each map was reclassified to binary maps to indicate suitable and unsuitable areas using the minimum predicted value for the training sites (Phillips et al., 2006). This threshold-based approach works better with presence only data to identify areas of potential suitability. The suitable areas indicate regions where the species can potentially establish and become invasive. The potentially suitable areas for each map were calculated in R using the 'area' function in the 'raster package' (Hijmans, 2021). The differences between the predicted area and the projected area were calculated to produce the potential range shift in suitable areas for each species (Borges et al., 2021). A sample of the R-code used is given in Appendix F. Further map processing and annotations were performed in QGIS.

RESULTS

Model performance and variable contribution

The field surveys confirmed that *N. tenuissima* was still a serious invader in farms around Barkly East and Clifford. Some paddocks in the area were completely taken over by *N. tenuissima* and had been abandoned (Fig. 6.1). In all the areas visited, *Nassella* invasions were associated with an event that had disturbed the resident vegetation such as overgrazing, previously cultivated land that was abandoned, or road construction. This was also confirmed with collectors' notes on herbarium records.

Fig. 6.1. A paddock completely taken over by *Nassella tenuissima* just outside Barkly East, Eastern Cape province. Photo: A. Mapaura.

All the methods performed well with both AUC and TSS values above the respective cut-off points (Table 6.2). The potential distribution of all the species under current climatic conditions encompassed the realised distribution in the native areas and hence the final models were considered suitable. The highly suitable areas in South Africa also included the currently known localities suggesting that the model worked well.

Table 6.2. Model mean performance metrics per *Nassella* species, using test dataset (generated using partitioning).

Nassella species	methods	AUC	COR	TSS	Deviance
N. neesiana	rf	1	0.97	0.97	0.11
	svm	1	0.96	0.96	0.17
	gam	0.99	0.95	0.95	0.30
	mars	0.99	0.94	0.94	0.19
	cart	0.98	0.93	0.93	0.27
	brt	0.99	0.94	0.93	0.53
N. tenuissima	rf	0.99	0.93	0.93	0.22
	svm	0.98	0.86	0.88	0.37
	gam	0.98	0.87	0.88	0.41
	mars	0.98	0.86	0.88	0.38
	cart	0.94	0.83	0.83	0.49
	brt	0.98	0.85	0.87	0.59
N. trichotoma	rf	1	0.98	0.99	0.07
	svm	1	0.98	0.98	0.11
	gam	0.99	0.98	0.98	0.41
	mars	1	0.97	0.97	0.11
	cart	0.99	0.96	0.96	0.18
	brt	1	0.96	0.96	0.49

Each model had seven variables which included both temperature and rainfall variables. Table 6.3 below shows the relative contribution of each variable using Pearson correlation and AUC. Isothermality (Bio3) was important in determining the distribution of all three *Nassella* species. In addition, the distributional range of *N. neesiana* was further restricted by temperature seasonality, and annual mean temperature; while *N. tenuissima* was affected by minimum temperature of the coldest month, mean temperature of the driest quarter, and maximum temperature of warmest month; and *N. trichotoma* was mostly affected by annual mean temperature, temperature seasonality, and precipitation seasonality.

Table 6.3. The variable importance for all the models of the three *Nassella* species, averaged based on Pearson Correlation and AUC metrics.

Variable	Pearson Correlation (%)			AUC (%)		
	N. neesiana	N. tenuissima	N. trichotoma	N. neesiana	N. tenuissima	N. trichotoma
Bio1	28.3	-	21.9	19.1	-	12.2
Bio3	10.4	26.9	26.1	7.2	18.5	19.5
Bio4	37.5	-	16.6	33.0	-	9.4
Bio5	-	12.1	-	-	7.6	-
Bio6	-	32.0	-	-	27.5	-
Bio8	-	-	3.1	-	-	0.5
Bio9	6.9	13.8	-	3.7	10.3	-
Bio13	7.7	6.0	-	2.9	3.4	-
Bio15	1.6	2.3	11.0	0.4	1.1	4.6
Bio18	-	4.3	5.5	-	2.9	2.1
Bio19	3.1	-	3.9	1	-	1

All variables had a narrow range within which all three species had an optimal probability of occurrence (Fig. 6.2, Fig. 6.3, and Fig. 6.4). The probability of occurrence increased sharply as it approached the peak before falling drastically after the peak. Annual mean temperature for *N. neesiana* ranged from approximately 0ºC to 25ºC, with the highest probability between 14ºC and 16ºC (Fig. 6.2). For *N. trichotoma,* the annual mean temperature ranged from about -10ºC to 20ºC, with the highest probability at 15ºC (Fig. 6.4). All three species are sensitive to Isothermality with *N. neesiana* and *N. trichotoma* reaching their highest probabilities of occurrence at about 50%, (Fig. 6.2 and Fig. 6.4 respectively), while *N. tenuissima* is more tolerant with the best probability ranging between 53% and 65% (Fig. 6.3).

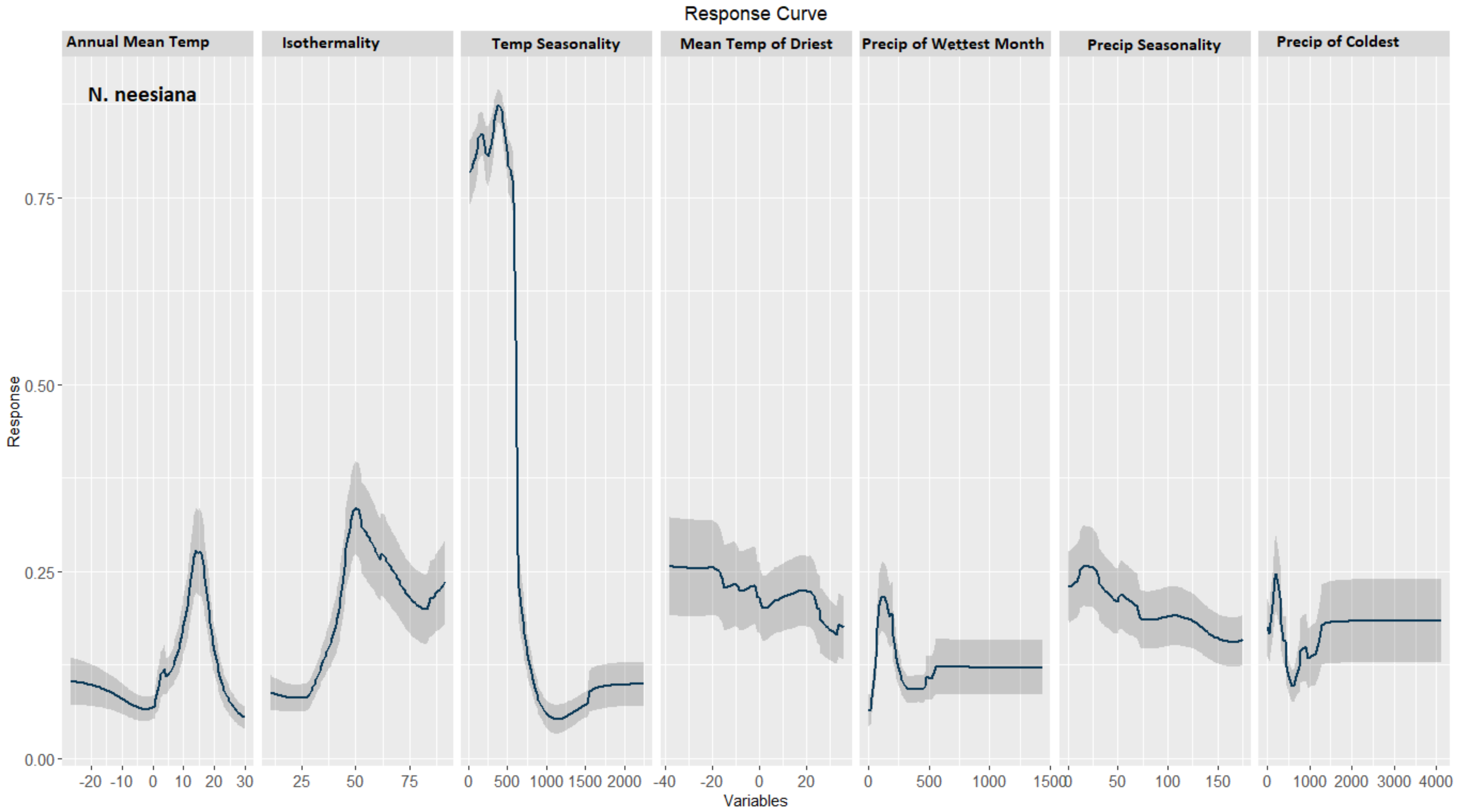


Fig. 6.2. Response curves of the predictor variables averaged across all the SDM algorithms for *Nassella neesiana* species. Plotted with a 95% confidence interval.

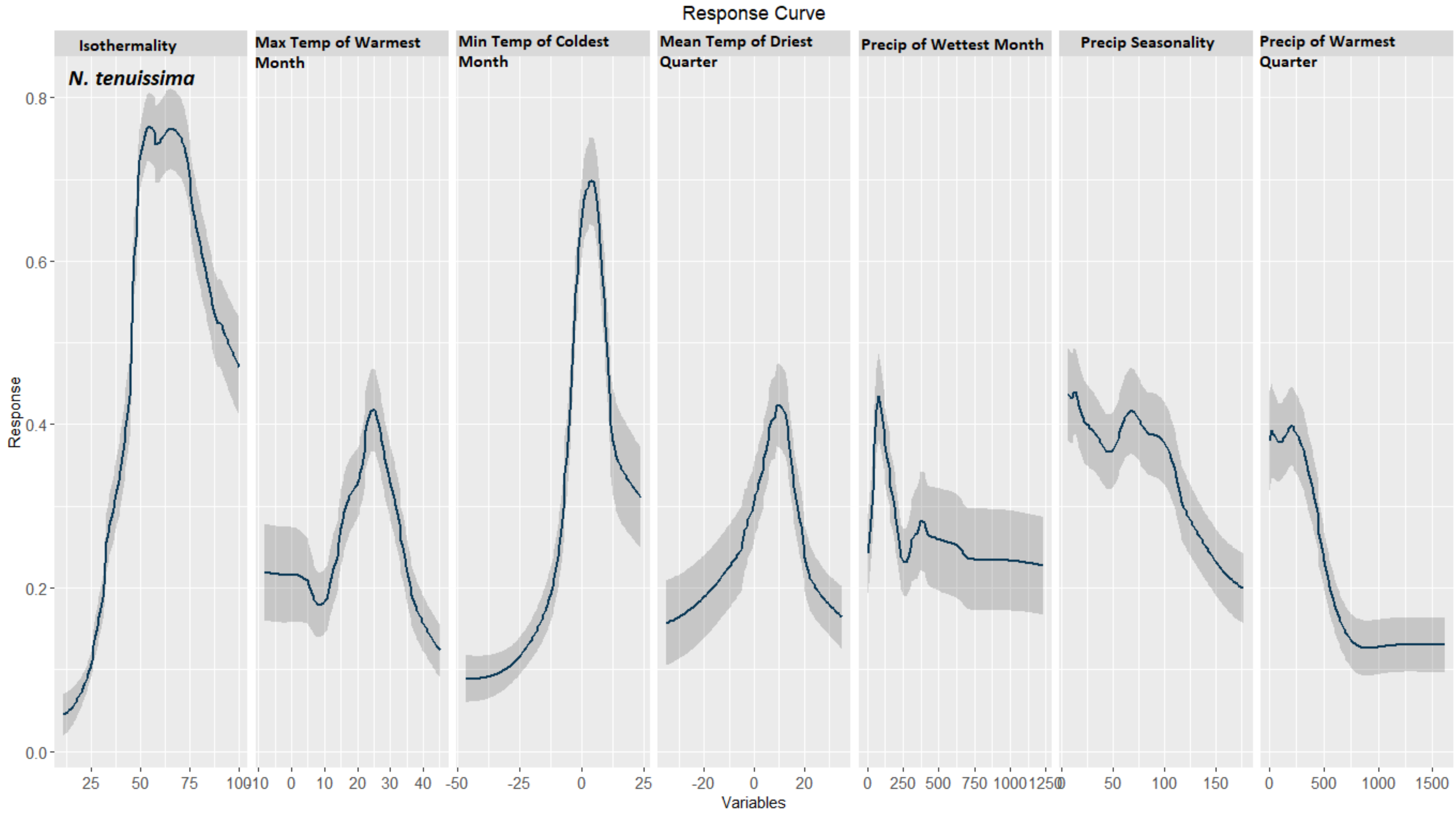


Fig. 6.3. Response curves of the predictor variables averaged across all the SDM algorithms for *Nassella tenuissima*. Plotted with a 95% confidence interval.

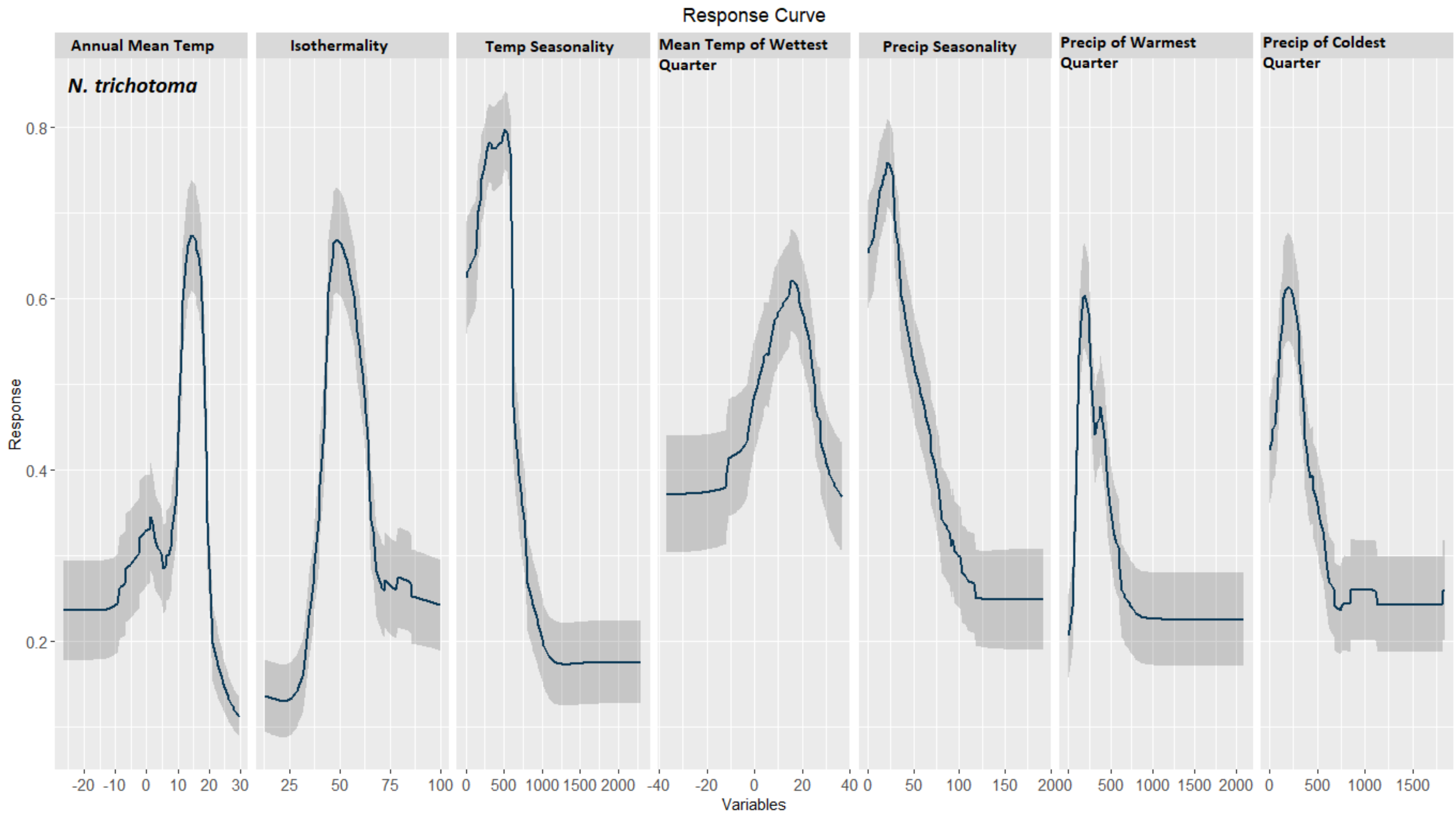


Fig. 6.4. Response curves of the predictor variables averaged across all the SDM algorithms for *Nassella trichotoma*. Plotted with a 95% confidence interval.

Potential distribution in South Africa

The models show that suitable areas in South Africa are currently much larger than the known localities. The most suitable areas are mainly in the Eastern Cape, Free State, parts of the Northern Cape and Gauteng provinces, as well as Lesotho. Suitable areas for all *Nassella* species were predicted to shrink in size under all future climate-change scenarios (Table 6.4). The reduction in size accelerates with an increase in greenhouse gas emissions. While a decrease in suitable areas in South Africa is expected by the end of the century, Lesotho is expected to face an increase in suitable areas (Fig. 6.5, Fig. 6.6, and Fig. 6.7) – notably in the highest land areas, which currently experience alpine conditions (Carbutt, 2019), but which would likely become sub-alpine / upper montane under climate change and become suitable for *Nassella*. The combination of climate change and *Nassella* invasion would be catastrophic for the exceptionally rich endemism in these areas of the Maloti-Drakensberg.

Table 6.4. Summary of the range shift (in area) of the three *Nassella* species under the four future climate- change scenarios projected by MIROC6. Area is given in km^2. Percentage change is given in brackets.

Nassella species	Current	SSP126	SSP245	SSP370	SSP585
N. neesiana	409199.7	257045.4 (-37.2)	193377.0 (-52.7)	123962.5 (-69.7)	79620.2 (-80.5)
N. tenuissima	300264.9	124899.5 (-58.4)	64544.23 (-78.5)	48225.3 (-83.9)	26057.1 (-91.3)
N. trichotoma	686265.9	443063.0 (-35.4)	316354.9 (-53.9)	183092.1 (-73.3)	101503.9 (-85.2)

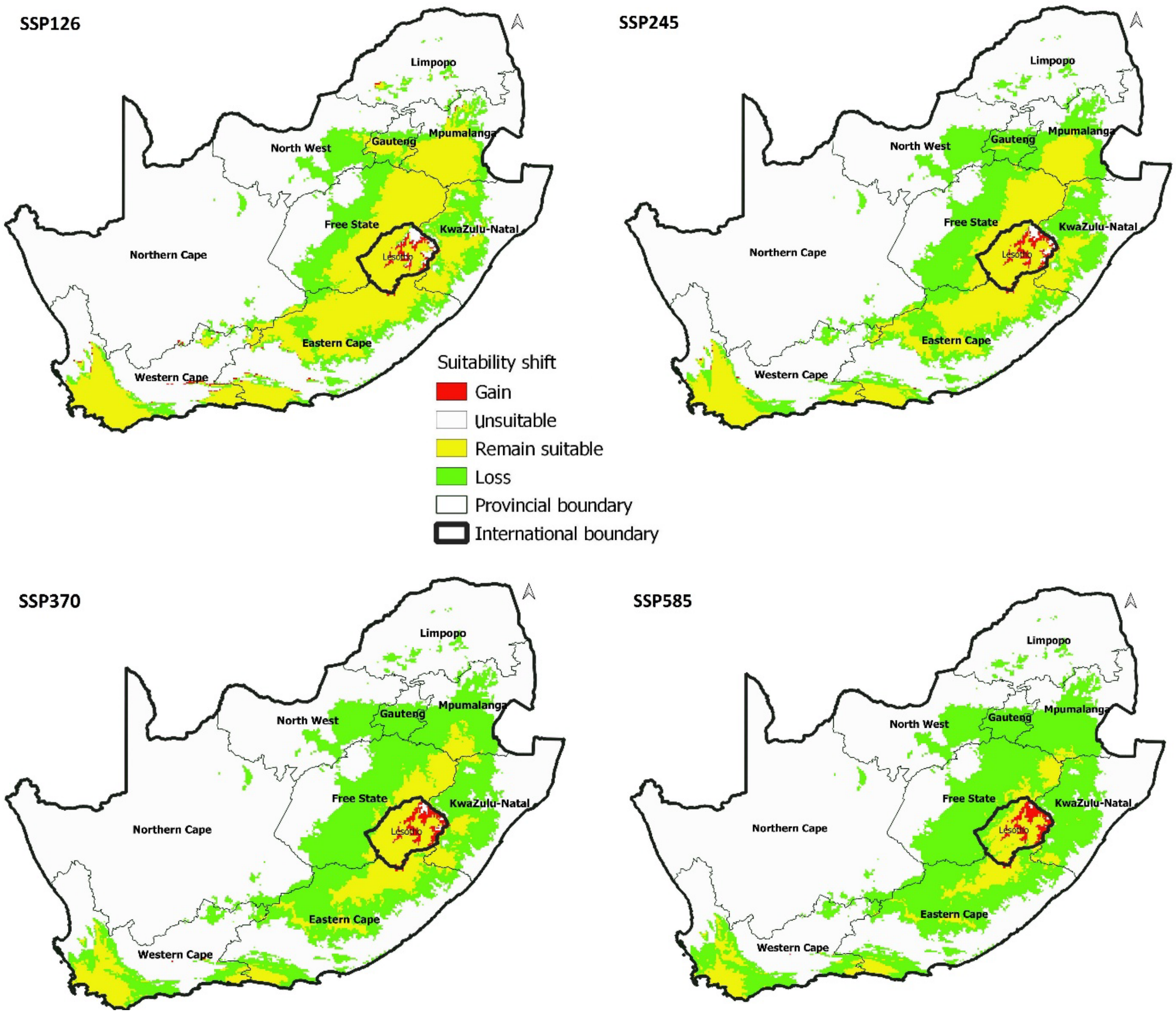


Fig. 6.5. Habitat suitability changes for *Nassella neesiana* under climate change estimates for the end of the century. Green indicates currently suitable areas that will become unsuitable in future; yellow shows areas that will remain suitable in future; and red indicates areas that are currently not suitable but will become suitable by the end of the century.

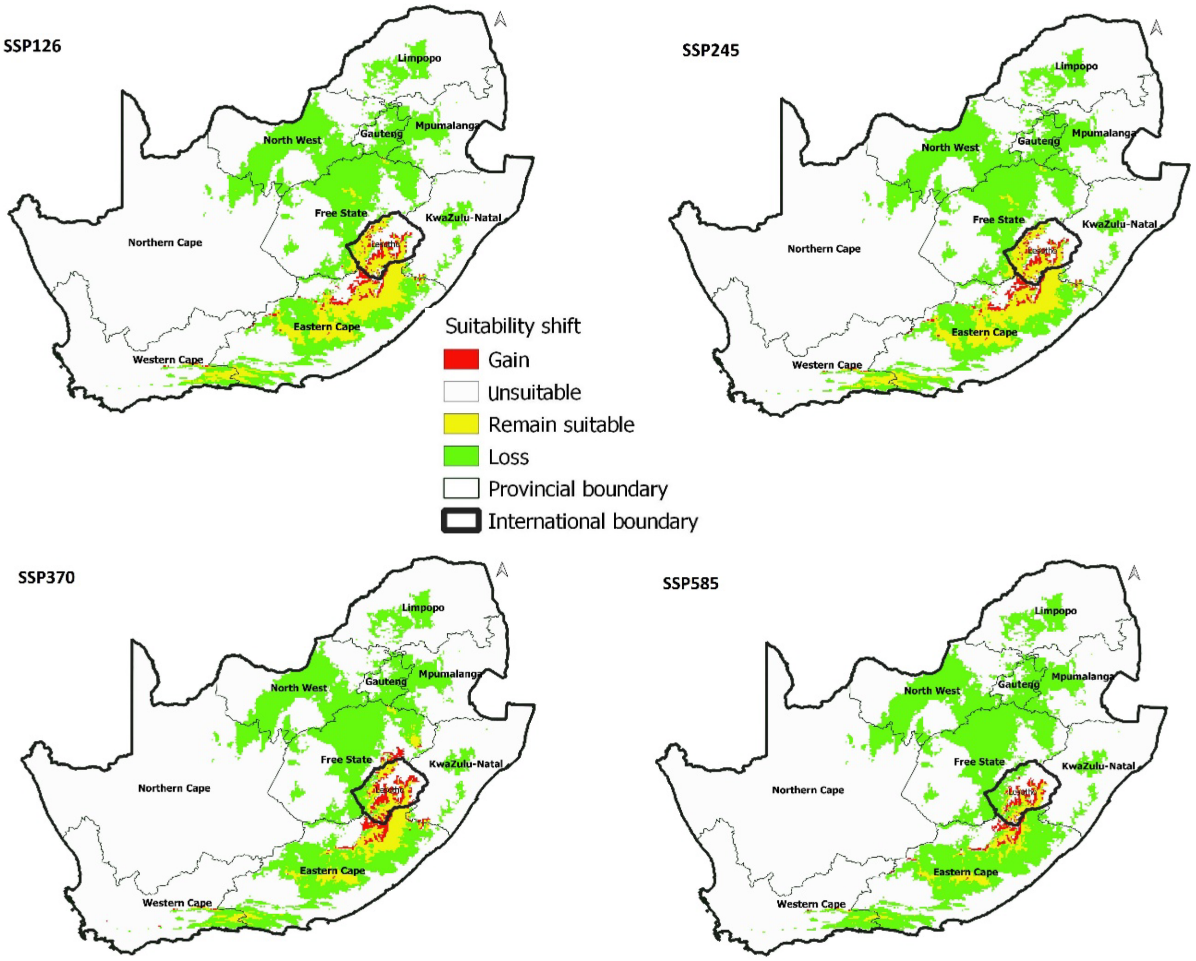


Fig. 6.6. Habitat suitability changes for *Nassella tenuissima* under climate change estimates for the end of the century. Green indicates currently suitable areas that will become unsuitable in future; yellow shows areas that will remain suitable in future; and red indicates areas that are currently not suitable but will become suitable by the end of the century.

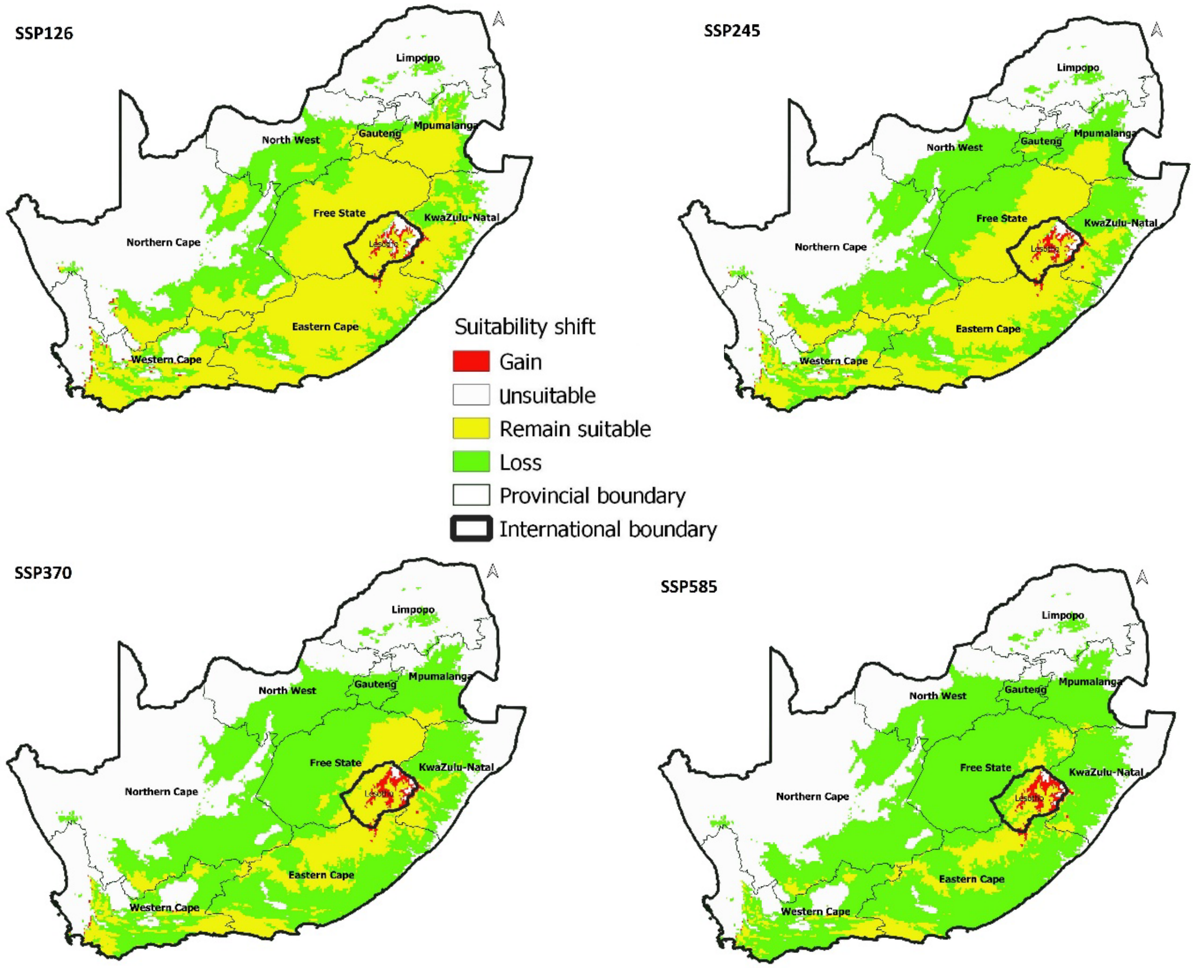


Fig. 6.7. Habitat suitability changes for *Nassella trichotoma* under climate change estimates for the end of the century. Green indicates currently suitable areas that will become unsuitable in future; yellow shows areas that will remain suitable in future; and red indicates areas that are currently not suitable but will become suitable by the end of the century.

DISCUSSION

This study used an ensemble of six modelling methods (brt, cart, gam, mars, rf, and svm), to assess the current and predicted future potential distribution of the three invasive *Nassella* species in South Africa. The high values of AUC and TSS indicate that the models used here had good predictive power (Allouche et al., 2006). All the currently known localities of the invasive species fall within the predicted highly suitable areas. The predictions also show that under current climatic conditions, all three species have not yet established across all suitable habitats in South

Africa and thus could potentially continue to spread. Areas most at risk are the Free State, Eastern and Western Cape provinces, as well as Lesotho. There is a possibility that the species are present in some of these areas but have not yet been detected, suggesting a potentially greater range than currently known. Considering the new localities recorded in recent years, this seems plausible. Search efforts for the species can be concentrated in these predicted areas to potentially get a more accurate current distribution.

The area highly suitable for *N. neesiana* under current climatic conditions is much larger than the currently known range. The known range of *N. neesiana* includes areas in the Eastern and Western Cape, as well as the recently recorded occurrences in the Free State. Under current climatic conditions, it could potentially invade a much bigger territory encompassing parts of Gauteng, Limpopo, Mpumalanga, and KwaZulu-Natal provinces. Bourdôt et al. (2012) predicted the potential global distribution of *N. neesiana* using a different modelling technique, a different set of environmental variables and using emission scenarios predicted by the IPCC fifth assessment report. While this makes the results of that modelling incompatible with the current predictions, the general trends agree, namely that there is potential expansion under current climatic conditions then contract as temperatures rise in future.

Nassella tenuissima currently appears to be confined to a limited area in the Barkly East district in the Eastern Cape. However, the SDM predicts that it has the potential to occupy a much larger area, with parts of all South African provinces and Lesotho potentially being at risk of invasion. In the areas where it has invaded, it has been recorded in natural vegetation, overgrazed paddocks, and abandoned crop fields. The reason for its restriction to a relatively small area is not clear currently, but climate does not appear to be a limiting factor. It is possible that it is still in its lag phase. The rise in temperatures under future climate change scenarios, will reduce the suitable areas for *N. tenuissima* more than the other two species. This could be due to its high sensitivity to the minimum temperature of the coldest month (Table 6.3 and Fig. 6.3), which is expected to rise as temperature increase.

While *N. trichotoma* has currently been recorded in parts of the Eastern and Western Cape provinces, highly suitable areas also exist in the Free State province and Lesotho. Kriticos et al. (2004) and Watt et al. (2011) produced maps showing the potential distribution of *N. trichotoma* in South Africa when they predicted its potential global distribution and potential distribution in New Zealand respectively. Both predictions used different modelling techniques, and a different set of environmental variables from the ones used here, as well as the emission scenarios predicted by the IPCC fifth assessment report. As the two studies were not South African-specific, and the scale of the maps produced does not provide a suitable fine-scale set of predictions, direct comparisons with these results are not possible. Notwithstanding these challenges, both models suggest that under current climatic conditions, the species can occupy more area than currently known and the area will shrink in later years. This implies that immediate ongoing management of *Nassella* as a national priority (and bilateral early warning system with Lesotho) remains a matter of urgency, and not simply a future concern.

Under the projected climate change estimates for the end of the century, areas suitable for *Nassella* species will contract under all projected scenarios (Table 6.4) – except for Lesotho, which will have an increase in suitable area. The suitable areas will retreat to high-altitude mountains, tracking cooler temperatures. This trend was also reported by Bentley and O'Connor (2018), when they assessed the projected distribution of C_3 grass species in South Africa under future climate-change scenarios. The decrease in existing suitable envelopes will accelerate with increasing emissions, rising temperatures will pull lower elevation areas out of the envelopes, while opening higher elevations to invasion potential. This agrees with the findings of Bourdôt et al. (2012), while working on the potential global distribution of *N. neesiana*. Climate change will shift the distribution of C_3 and C_4 grasses in grassy mountains due to temperature changes with the C^3 grasses being driven to higher and cooler altitude and the C_4 grasses replacing them (de Deus Vidal et al., 2021). Although C_3 grasses, such as the *Nassella* species, are expected to benefit from the rising atmospheric carbon concentration (Hamim, 2005; Wang et al., 2020), this benefit is not sufficient to counter the effect of rising temperatures (de Deus Vidal et al., 2021).

This modelling also shows that the contraction of suitable areas to higher elevations is centred on the eastern Great Escarpment, with losses in surrounding mid-elevations and gains in higher elevations, focused on the Grassland Biome and parts of the Fynbos Biome. The gains in higher elevations which include Lesotho overlaps strongly with predicted biodiversity refugia under climate change (Bentley et al., 2019) and shrinking strategic water resource areas (Nhemachena et al., 2020). Under all climate change scenarios *Nassella* remains a high risk invasive that will compete with biodiversity refugia under climate change and shrinking water production landscapes as well as shrinking economic / livelihood areas. Contractions will reduce risk in the peripheral Thicket, Savanna and Nama-Karoo Biomes and concentrate risk in higher elevation areas with predicted retention of high moisture availability and low minimum winter temperatures. It is important to note that the area of suitability under all scenarios remains large; even under the extreme-case scenarios, especially for *N. neesiana* and *N. trichotoma,* the potential suitable area is worryingly large. Even *N. tenuissima,* which does not have as large potential suitable area, and has the smallest gains under the future climate-change scenarios, should be controlled as a matter of priority, to contain the species and avoid spread into Lesotho.

The SDM modelling predicted that all three *Nassella* species could potentially spread to more areas than they are currently established in. Given this threat, it is important that management efforts are put in place to restrict *Nassella* populations such as preventing dispersal of seeds including controlling the movement of farming implements and people from invaded areas as well as preventing overgrazing. Lastly, this study has shown that there is potential for expansion into Lesotho, and there are occurrence records close to the border with this country. The fact that large areas of Lesotho are suitable for *Nassella i*nvasion, even though there are currently no recorded naturalised populations, and there is no monitoring in place to detect any populations should be a cause for concern. Efforts to improve cross-border collaboration would therefore be beneficial to both Lesotho and South Africa. It should be noted that even areas of low suitability are important to monitor since they can become sinks from which the invaders can be transferred to suitable areas. The results of this study show that all three *Nassella* species have not yet extended into all suitable areas in the country. Management efforts should ideally focus on preventing further spread of the species. Long-term control can, perhaps be achieved through biological control. Biological control research in Australia is promising (Giordano and Anderson, 2021) and South African researchers could build on that work.

CONCLUSIONS AND PERSPECTIVES

The distribution range of *Nassella* species is affected largely by temperature-related bioclimatic variables. Isothermality is an important bioclimatic variable affecting the distribution of all three species. The range of each species in South Africa and Lesotho was characterised by a unique set of bioclimatic factors. All three species can potentially occupy much more area than they currently do. However, under climate change, the extent of climatically suitable areas will shrink and will be restricted to cooler high-altitude mountains.

In interpreting these potential and projected distribution, it is important to note that they are not absolute. The establishment and persistence of *Nassella* species in a specific area depends on many other micro factors such as land use, stochastic events, vegetation type, as well as monitoring and control measures that are in place (Abdelaal et al., 2020; Kriticos et al., 2004). The predictions should be treated as guidelines to assist with monitoring and guiding policy decisions. The results of this research can be used to guide surveillance, monitoring and mitigation planning of these species. The shrinkage of suitable range for these species under climate change, offers an opportunity to control them through preventing them from establishing in novel spaces. It is also recommended that Lesotho starts monitoring the species in its own territory especially since it is faced with a possible expansion in the future.

CHAPTER 7

INTRODUCTION

This chapter reiterates the major findings reported in earlier chapters; the significance and implications of these findings are briefly discussed. The thesis concludes with recommendations for future research.

MAJOR FINDINGS

In South Africa, extensive *Nassella* research took place from the late 1970s to the late 1980s, mainly by M.J. Wells. There has been a 20 – 35-year hiatus in coordinated research and control, and all three *Nassella* species remain a major land management, conservation, and livelihood security concern in South Africa, with novel records detected in this study, some being very extensive in area and impact. Before this study, most of what was known about *Nassella* invasions came from out-dated research indicated above (Chapter 2), or from research done in Australia and New Zealand. This thesis is the first robust work in South Africa on this topic since the work of Wells (late 1970s to late 1980s).

Invasive populations of *Nassella* in South Africa pose a major risk to the country's biodiversity. The establishment of these tussock grasses can result in the displacement of native species, this is particularly concerning given that they often invade biomes with high endemism. Invasion by *N. trichotoma* reduces native vascular plant flora compared with adjacent areas of native uninvaded grassland. In contrast, *N. neesiana* did not significantly reduce native plant richness, despite the population possibly having been present for a significant period of time. However, both *N. trichotoma* and *N. neesiana* invasions are associated with heightened levels of soil variables in the rhizosphere particularly boron, calcium, available phosphorus, silt, total cations, and zinc.

Nassella neesiana was found to invade disturbed wet areas such as riparian and seasonally wet areas. Disturbance and moisture appear to be the important drivers of invasion. In these environments, *N. neesiana* can spread without detection due to: a) its morphological similarity with several co-occurring native perennial tufted grasses; b) it having a long lag phase (decades), during which time individual plants can grow among native vegetation before rapidly spreading; and c) it's low profile (<50 cm), wispy inflorescence that does not protrude above the surrounding vegetation. Given the recent novel locality records discovered in the Eastern Cape, Western Cape, and the Free State provinces, *N. neesiana* is probably more widespread than is currently known. Invasions of all three species appear to be triggered by events that disturb the native vegetation. Although this conclusion comes largely from field observations and comments from farmers, it supports findings elsewhere in the world where it is a problem species. After establishing in an area, the species become nearly impossible to eradicate.

Identification of the three *Nassella* species is problematic in South Africa, particularly when they are in their vegetative state. They are, therefore, not good candidates for citizen science observations, which reduces the chances of early detection of additional populations. These identification challenges can be overcome by using DNA barcoding. Of the two gene loci tested in this study, the ETS gene locus can effectively discriminate all three *Nassella* species from each other as well as from morphologically similar native species in South Africa. Using DNA barcoding as an identification tool for these *Nassella* species is viable given local cost-effective laboratories; the practitioner need only collect leaf samples of a suspected grass and send them to the relevant laboratory to follow the protocol outlined in this study.

From the species distribution modelling, all three species can potentially occupy a greater area than they currently do. There is therefore a need for more intensive surveys in areas where they are not yet recorded but which are predicted to be suitable for their establishment. The modelling also found that the distribution of these species is largely temperature dependant. The implication is that as temperatures rise under climate change, high-elevation mesic mountain grasslands along the eastern Great Escarpment will remain suitable for invasion, with consequences for their role in climate refugia for endemic species and rangeland-based livelihoods. In particular, the Maloti Drakensberg mountains will remain suitable for *Nassella* invasion under all projected future climate-change scenarios and should be a priority for *Nassella* early detection and action.

SIGNIFICANCE AND IMPLICATIONS OF THE FINDINGS

Nassella invasion continues to pose an immense threat to the conservation of biological diversity and rangeland-based economic viability along the eastern Great Escarpment of South Africa (including Lesotho and Eswatini) and will continue to do so under global climate change – unless resources are remobilised to rekindle an integrated research and management programme.

As invasions have not reached all suitable areas, there is still an opportunity to contain invasions of *N. tenuissima* and *N. trichotoma* to currently invaded areas. In contrast, the full extent of *N. neesiana* invasion is probably much greater than current records show, and the species should be actively looked for in the south-western Cape and throughout the mesic eastern parts of South Africa, Lesotho, and Eswatini. The findings also suggest that an integrated, co-ordinated approach is more desirable than the current *ad hoc* efforts – mostly by private landowners and practitioners. An area-wide approach may reduce the possibility of chemical resistance, lower costs, and ensure that no property will act as a sink for neighbouring properties. Early detection is key in controlling the spread of invasion. In this regard, this research also showed that Lesotho is at risk of invasion; and cross-border surveillance between Lesotho and South Africa is deemed necessary. This research has also revealed the challenges posed by the current control methods. Both chemical and mechanical control methods appear to inadvertently worsen invasions. South Africa should seek co-operation with researchers and managers in Australia and New Zealand where research on biological control of *Nassella* species is underway. The study of impacts of the species highlights how impacts can vary with ecosystems in natural and urban environments and native species. An effective management program must consider the habitat being invaded.

At a broader scale, this research confirms that invasive alien grasses in South Africa require more attention than they are currently given. Several researchers have pointed this out before (Milton, 2004; Sutton et al., 2021; Visser et al., 2017) – particularly in mountainous areas (Sylvester et al., 2021). This work is an example of one group of grasses that are morphologically cryptic. It likely represents the situation with many other alien grasses in the country whose current known localities do not reflect the true extent of their distributions. The limited grass taxonomists in South Africa only exacerbates identification challenges, leading to invasive grasses spreading undetected (Sylvester et al., 2021). DNA barcoding could offer a new tool to uncover the true distributions of many other cryptic alien grasses in the country. Efforts are being made in other parts of the world to develop these tools for use in identifying morphologically cryptic grass species (Peterson et al., 2014; Ragupathy et al., 2009; Steven and Subramanyam, 2009).

Globally, grasses are beginning to receive attention as possible targets for biological control (Anderson et al., 2017; Blossey et al., 2018; Goolsby et al., 2020), although some reservations are still being raised particularly as grasses are important as food grains and animal feed (Kiviat et al., 2019). This thesis contributes towards considerations for a future biocontrol programme for

Nassella in South Africa (Sutton et al., 2021). The information gathered during this study has led to *Nassella* species being accurately prioritised for biological control and they came out in the top 5 species to target (Sutton et al., 2021).

RECOMMENDATIONS FOR FUTURE RESEARCH

Several areas for further research have been highlighted in this thesis. These areas will improve our understanding of the drivers of *Nassella* invasions and their impacts and should lead to better *Nassella* management. They include:

1. Further investigations of potential mechanisms of invasion, particularly mycorrhizal associations. This is particularly important for explaining the spread of *N. trichotoma*. Allelopathic effects have been suggested as a possible reason for the growth habit (Faithfull, 2012; Joubert, 1984). However, allelopathic interaction investigations were not part of the current research and still needs to be investigated. This needs to be tested. Pot experiments to investigate competitive interactions of *Nassella* species and native species were set up at the greenhouses of the Centre for Biological Control at Rhodes University, Makhanda, but could not be carried through due to Covid-19 lockdown measures. This experiment is still needed to understand the mechanisms by which native species are impacted by *Nassella*.
2. It was found that *Nassella* invasion led to elevated nutrient status of soils in the rhizosphere. However, the mechanism leading to this increase and the impact of these changes needs to be investigated experimentally, and if there are implications for positive feedback for reinvasion and ecological restoration. Research on root depths and changing soil composition, especially in relation to co-occurring native species, is important to consider in future studies.
3. The ability of the ETS gene locus to discriminate *Nassella* species from four similar species was demonstrated here. This makes early identification of *Nassella* species possible even when they are not flowering. This is important in early detection and control.

4. The distributions predicted by the model used in this research of all three *Nassella* species needs to be verified on the ground. The distribution maps produced during these studies should prove helpful in this regard. Considering that the species were introduced by the British during the second Anglo-Boer War, it would make sense to start the surveys around all known British forts used during that war.
5. Research is urgently required into the use of biological control against these invasive grass species. Since work in Australia and New Zealand is already advanced, collaboration with researchers in these countries is needed to develop integrated management methods. The literature shows that the methods currently in use are failing to control the persistence and spread of *Nassella* species.
6. This research has shown that *Nassella* invasions remain a serious problem in Southern African grasslands and will remain so in the future unless effective management solutions are found. Since the fragmented control efforts by individual property owners are failing to control invasions, coordinated areawide control measures are required.

REFERENCES

Abdelaal, M., Fois, M., Dakhil, M.A., Bacchetta, G., El-Sherbeny, G.A., 2020. Predicting the potential current and future distribution of the endangered endemic vascular plant *Primula boveana* Decne. ex Duby in Egypt. Plants 9(8), 957. https://doi.org/10.3390/plants9080957

Afreen, T., Srivastava, P., Singh, H., Singh, J.S., 2018. Effect of invasion by *Hyptis suaveolens* on plant diversity and selected soil properties of a constructed tropical grassland. J. Plant Ecol. 11, 751–760. https://doi.org/10.1093/jpe/rtx045

Aguilera, A.G., Alpert, P., Dukes, J.S., Harrington, R., 2010. Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. Biol. Invasions 12, 1243–1252. https://doi.org/10.1007/s10530-009-9543-z

Ahmed, N., Atzberger, C., Zewdie, W., 2021. Species Distribution Modelling performance and its implication for Sentinel-2-based prediction of invasive *Prosopis juliflora* in lower Awash River basin, Ethiopia. Ecol. Process. 10, 1-6. https://doi.org/10.1186/s13717-021-00285-6

Aiello-Lammens, M.E., Boria, R.A., Radosavljevic, A., Vilela, B., 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography (Cop.). 38, 541–545.

Allison, S.D., Vitousek, P.M., 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawaii. Oecologia 141, 612–619. https://doi.org/10.1007/s00442-004-1679-z

Allouche, O., Tsoar, A., Kadmon, R., 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). J. Appl. Ecol. 43, 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Anderson, F.E., Barton, J., McLaren, D., 2010. Studies to assess the suitability of *Uromyces pencanus* as a biological control agent for *Nassella neesiana* (Poaceae) in Australia and New Zealand. Australas. Plant Pathol. 39, 69. https://doi.org/10.1071/AP09057

Anderson, F.E., Díaz, M.L., McLaren, D.A., 2006. Current status of research on potential biological control agents for *Nassella neesiana* and *Nassella trichotoma* (Poaceae) in Australia, in: Preston, C., Watts, J.H., Crossman, N.D. (Eds.), Proceedings of the Fifteenth Australian Weeds Conference. Weed Management Society of South Australia, pp. 591–594.

Anderson, F.E., Gallego, L., Sánchez, R.M., Flemmer, A.C., Hansen, P. V., McLaren, D., Barton, J., 2017. Plant/pathogen interactions observed during host range testing of the rust fungus *Uromyces pencanus*, a classical biological control agent for Chilean needle grass (*Nassella neesiana*) in Australia and New Zealand. Biocontrol Sci. Technol. 27, 1096–1117. https://doi.org/10.1080/09583157.2017.1384795

Anderson, F., Pettit, W., Briese D.T., McLaren, D.A., 2002. Biological control of serrated tussock and Chilean needlegrass. Plant Protection Quarterly 17: 104–111.

Australia, A.O.L., 2021a. Occurrence records downloaded on 29.08.2021. https://doi.org/https://doi.org/10.26197/ala.cd34a525-e2c6-4448-b072-1e4a57b477c0

Australia, A.O.L., 2021b. Occurrence records downloaded on 29.08.2021. https://doi.org/https://doi.org/10.26197/ala.5a49e274-0932-4235-a3bb-bfcfb3c39c6a

Australia, A.O.L., 2021c. Occurrence records downloaded on 29.08.2021. https://doi.org/https://doi.org/10.26197/ala.cf9745a1-7ab6-4ed3-8444-901675117eba

Avin, F.A., Bhassu, S., Tan, Y.S., Shahbazi, P., Vikineswary, S., 2014. Molecular divergence and species delimitation of the cultivated oyster mushrooms: Integration of IGS1 and ITS. Sci. World J. 2014, 1–10. https://doi.org/10.1155/2014/793414

Badgery, W.B., 2004. Managing competition between *Nassella trichotoma* (serrated tussock) and native grasses. PhD Thesis, Agronomy Department, University of Sydney, NSW, Australia.

Badgery, W.B., Kemp, D.R., Michalk, D.L., King, W.M., 2008. Studies of competition between *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock) and native pastures. 1. Adult plants. Aust. J. Agric. Res. 59, 226. https://doi.org/10.1071/AR07112

Badgery, W.B., Kemp, D.R., Michalk, D.L., King, W.M.C.G., 2005. Competition for Nitrogen between Australian native grasses and the introduced weed *Nassella trichotoma*. Ann. Bot. 96, 799–809. https://doi.org/10.1093/aob/mci230

Barbet-Massin, M., Jiguet, F., Albert, C.H., Thuiller, W., 2012. Selecting pseudo-absences for species distribution models: how, where and how many? Methods Ecol. Evol. 3, 327–338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Barker, N., Clark, V.R., Dold, T., 2020. FBIP: Great Escarpment Biodiversity Research Programme data mobilisation. https://doi.org/10.15468/dl.xecp8x

Barkworth, M.E., Arriaga, M.O., Smith, J.F., Jacobs, S.W.L., Valdés-Reyna, J., Bushman, B.S., 2008. Molecules and morphology in South American Stipeae (Poaceae). Syst. Bot. 33, 719–731. https://doi.org/10.1600/036364408786500235

Barkworth, M.E., Torres, M.A., 2001. Distribution and diagnostic characters of *Nassella* (Poaceae: Stipeae). Taxon 50, 439–468. https://doi.org/10.2307/1223891

Barney, J. N., Tekiela, D. R., Barrios-Garcia, M. N., Dimarco, R. D., Hufbauer, R. A., Leipzig-Scott, P., Nuñez, M. A., Pauchard, A., Pyšek, P., Vítková, M., & Maxwell, B. D. (2015). Global Invader Impact Network (GIIN): toward standardized evaluation of the ecological impacts of invasive plants. Ecology and Evolution, 5(14), 2878–2889.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using {lme4}. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Bedggood, W. and Moerkerk, M. (compilers) (2002) Integrated weed management results from a workshop on Chilean needle grass management held at Victoria University of Technology, St Albans, Victoria, 2 October 2002. Cooperative Research Centre for Weed Management Systems, Adelaide, 12 pp.

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M., Courchamp, F., 2013. Will climate change promote future invasions? Glob. Chang. Biol. 19, 3740–3748. https://doi.org/10.1111/gcb.12344

Bentley, L.K., O'Connor, T.G., 2018. Temperature control of the distributional range of five C 3 grass species in the uKhahlamba-Drakensberg Park, KwaZulu-Natal, South Africa. African J. Range Forage Sci. 35, 45–54. https://doi.org/10.2989/10220119.2018.1459841

Bentley, L.K., Robertson, M.P., Barker, N.P., 2019. Range contraction to a higher elevation: the likely future of the montane vegetation in South Africa and Lesotho. Biodivers. Conserv. 28, 131–153. https://doi.org/10.1007/s10531-018-1643-6

Bezeng, B.S., Morales-Castilla, I., van der Bank, M., Yessoufou, K., Daru, B.H., Davies, T.J., 2017. Climate change may reduce the spread of non-native species. Ecosphere 8, e01694. https://doi.org/10.1002/ecs2.1694

Bläß, C., Ronnenberg, K., Tackenberg, O., Hensen, I., Wesche, K., 2010. The relative importance of different seed dispersal modes in dry Mongolian rangelands. J. Arid Environ. 74, 991–997. https://doi.org/10.1016/j.jaridenv.2009.12.002

Blossey, B., Häfliger, P., Tewksbury, L., Dávalos, A., Casagrande, R., 2018. Host specificity and risk assessment of *Archanara geminipuncta* and *Archanara neurica*, two potential biocontrol agents for invasive *Phragmites australis* in North America. Biol. Control 125, 98–112. https://doi.org/10.1016/j.biocontrol.2018.05.019

Boardman, J., Parsons, A.., Holland, R., Holmes, P.., Washington, R., 2003. Development of badlands and gullies in the Sneeuberg, Great Karoo, South Africa. Catena 50, 165–184. https://doi.org/10.1016/S0341-8162(02)00144-3

Bordy, E.M., 2005. The contact of the Molteno and Elliot formations through the main Karoo Basin, South Africa: a second-order sequence boundary. South African J. Geol. 108, 351–364. https://doi.org/10.2113/108.3.351

Borges, F.O., Santos, C.P., Paula, J.R., Mateos-Naranjo, E., Redondo-Gomez, S., Adams, J.B., Caçador, I., Fonseca, V.F., Reis-Santos, P., Duarte, B., Rosa, R., 2021. Invasion and Extirpation Potential of Native and Invasive Spartina Species Under Climate Change. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.696333

Bourdôt, G., Basse, B., Kriticos, D., Dodd, M., 2015. Cost-benefit analysis blueprint for regional weed management: *Nassella neesiana* (Chilean needle grass) as a case study. New Zeal. J. Agric. Res. 58, 325–338. https://doi.org/10.1080/00288233.2015.1037460

Bourdôt, G.W., Hurrell, G.A., 1992. Aspects of the ecology of *Stipa neesiana* Trin. & Rupr. seeds. New Zeal. J. Agric. Res. 35, 101–108. https://doi.org/10.1080/00288233.1992.10417707

BourdôT, G.W., Hurrell, G.A., 1989. Cover of *Stipa neesiana* Trin. & Rupr. (Chilean needle grass) on agricultural and pastoral land near Lake Grassmere, Marlborough. New Zeal. J. Bot. 27, 415–420. https://doi.org/10.1080/0028825X.1989.10414122

Bourdôt, G.W., Hurrell, G.A., Saville, D.J., 1992. Eradication of nassella tussock (*Nassella trichotoma*), an unlikely outcome of grubbing. New Zeal. J. Agric. Res. 35, 245–252. https://doi.org/10.1080/00288233.1992.10427501

Bourdôt, G.W., Lamoureaux, S.L., Watt, M.S., Manning, L.K., Kriticos, D.J., 2012. The potential global distribution of the invasive weed *Nassella neesiana* under current and future climates. Biol. Invasions 14, 1545–1556. https://doi.org/10.1007/s10530-010-9905-6

Bourdôt, G.W., Ryde, D.H., 1986. Chilean needle grass Stipa neesiana. Significance, identification, control. Ministry of Agriculture and Fisheries Aglink FPP 675, 1st Edn, Information Services MAF, Wellington, 2 pp.

Bourdôt, G.W., Saville, D.J., 2019. *Nassella trichotoma* – plant growth rates and effects of timing of grubbing on populations in North Canterbury grassland. New Zeal. J. Agric. Res. 62, 224–245. https://doi.org/10.1080/00288233.2018.1483954

Brand, R.F., Brown, L.R., Du Preez, P.J., 2011. The Grassland vegetation of Platberg, eastern Free State, South Africa. Koedoe 53(1), 1-13. https://doi.org/10.4102/koedoe.v53i1.1027

Brand, R.F., Brown, L.R., Du Preez, P.J., 2010. A floristic analysis of the vegetation of Platberg, eastern Free State, South Africa. Koedoe 52(1), 1–11. https://doi.org/10.4102/koedoe.v52i1.710

Briese, D.T., Evans, H.C., 1998. Biological control of serrated tussock (Nassella trichotoma): is it worth pursuing? Plant Protection Quarterly 13(2): 94–97.

Briese, D.T., McLaren, D.A., Pettit, W.J., Zapater, M., Anderson, F., Delhey, R., Distel, R., 2000. New biological control initiatives against weeds of South American origin in Australia: Nassella tussock grasses and blue heliotrope, in: Spencer, N.R. (Ed.), Proceedings of the Tenth International Symposium on Biological Control of Weeds. Montana State University, Bozeman., Montana, USA, pp. 215–223.

CABI, 2021. Invasive Species Compendium. Wallingford , UK: CAB International. www.cabi.org/isc.

Campbell, M.H., 1998. Biological and ecological impact of serrated tussock (*Nassella trichotoma* (Nees) Arech.) on pastures in Australia. Plant Prot. Q. Rep. 13(2), 80–86.

Campbell, M.H., 1982. The biology of Australian weeds. 9: *Nassella trichotoma* (Nees) Arech. J. Aust. Inst. Agric. Sci. 48, 76–84.

Campbell, M.H., Ridings, H.I., 1988. Tolerance of grazed and ungrazed *Phalaris aquatica* to glyphosate, tetrapion and 2,2-DPA. Aust. J. Exp. Agric. 28, 747–751.

Campbell, M.H., Van de Ven, R., 1996. Tolerance of native grasses to Frenock and Round up, in: Proceedings of the Eleventh Annual Conference of the Grassland Society of NSW. Grassland Society of NSW, pp. 120–121.

Carbutt, C., 2019. The Drakensberg Mountain Centre: A necessary revision of southern Africa's high-elevation centre of plant endemism. S. Afr. J. Bot. 124, 508–529. https://doi.org/10.1016/j.sajb.2019.05.032

Catt, J.., 2001. The agricultural importance of loess. Earth-Science Rev. 54, 213–229. https://doi.org/10.1016/S0012-8252(01)00049-6

Chen, D., Hunt, H.W., Morgan, J.A.., 1996. Responses of a C3 and C4 perennial grass to CO2 enrichment and climate change: comparison between model predictions and experimental data. Ecol. Modell. 87, 11–27.

Chen, J., Zhao, J., Erickson, D.L., Xia, N., Kress, W.J., 2015. Testing DNA barcodes in closely related species of Curcuma (Zingiberaceae) from Myanmar and China. Mol. Ecol. Resour. 15, 337–348. https://doi.org/10.1111/1755-0998.12319

Chersich, M.F., Wright, C.Y., 2019. Climate change adaptation in South Africa: a case study on the role of the health sector. Global. Health 15, 22. https://doi.org/10.1186/s12992-019-0466-x

Cialdella, A.M., Sede, S.M., Romaschenko, K., Peterson, P.M., Soreng, R.J., Zuloaga, F.O., Morrone, O., 2014. Phylogeny of *Nassella* (Stipeae, Pooideae, Poaceae) based on analyses of chloroplast and Nuclear Ribosomal DNA and Morphology. Syst. Bot. 39, 814–828. https://doi.org/10.1600/036364414X681419

Clark, V.R., Barker, N.P., Mucina, L., 2009. The Sneeuberg: A new centre of floristic endemism on the Great Escarpment, South Africa. S. Afr. J. Bot. 75, 196–238. https://doi.org/10.1016/j.sajb.2008.10.010

Clark, V.R., Jr., J. de D.V., 2019. The vegetation of Asante Sana Private Game Reserve and surrounds, Sneeuberg Centre of Floristic Endemism (Eastern Cape Province, South Africa), in: Esperiana Memoir 8: The Lepidoptera Fauna of a Crater Valley in the Great Escarpment of South Africa: The Asante Sana Project. Bad Staffelstein, pp. 25–39

Connor, H.E., Edgar, E., Bourdôt, G.W., 1993. Ecology and distribution of naturalised species of

Stipa in New Zealand. New Zeal. J. Agric. Res. 36, 301–307. https://doi.org/10.1080/00288233.1993.10417727

Conolly, J., Taylor, S., 2016. Mexican feather grass (*Nassella tenuissima*) control - the ACT experience, in: Randall, R., Lloyd, S., Borger, C. (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Perth. Weeds Society of Western Australia, pp. 228–231.

Cook, C., 1998. The transmission of serrated tussock (*Nassella trichotoma*) seeds through the sheep rumen and their viability after ingestion. Rep. No. 13(2), 93.

Cook, T.,1999. Chilean needle grass: is it much different to other perennial grass weeds? 10th New South Wales Biennial Noxious Weeds Conference - Ballina 1999, pp. 91-97

Cooperative Research Centres, 2003. Alert list for environmental weed. Weed Management Guide: Chilean Needle Grass - *Nassella neesiana*. Australian Pamplet. Weed Management and the Commonwealth Department of the Environment and Heritage. pp. 6. https://nre.tas.gov.au/Documents/Chileanneedlegrass_CRC_bpmg.pdf

Corrales, X., Katsanevakis, S., Coll, M., Heymans, J.J., Piroddi, C., Ofir, E., Gal, G., 2020. Advances and challenges in modelling the impacts of invasive alien species on aquatic ecosystems. Biol. Invasions 22, 907–934. https://doi.org/10.1007/s10530-019-02160-0

Dakhil, M.A., Halmy, M.W.A., Liao, Z., Pandey, B., Zhang, L., Pan, K., Sun, X., Wu, X., Eid, E.M., El-Barougy, R.F., 2021. Potential risks to endemic conifer montane forests under climate change: integrative approach for conservation prioritization in southwestern China. Landsc. Ecol. 36, 3137–3151. https://doi.org/10.1007/s10980-021-01309-4

Dassonville, N., Vanderhoeven, S., Vanparys, V., Hayez, M., Gruber, W., Meerts, P., 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157, 131–140. https://doi.org/10.1007/s00442-008-1054-6

de Deus Vidal, J., le Roux, P.C., Johnson, S.D., te Beest, M., Clark, V.R., 2021. Beyond the Tree-Line: The C3-C4 “Grass-Line” can track global change in the world’s grassy mountain systems. Front. Ecol. Evol. 9, 760118. https://doi.org/10.3389/fevo.2021.760118

De Marco, P., Nóbrega, C.C., 2018. Evaluating collinearity effects on species distribution models: An approach based on virtual species simulation. PLoS One 13, e0202403. https://doi.org/10.1371/journal.pone.0202403

DeSalle, R., Goldstein, P., 2019. Review and interpretation of trends in DNA Barcoding. Front. Ecol. Evol. 7, 302. https://doi.org/10.3389/fevo.2019.00302

Dickens, S.J.M., Allen, E.B., 2014. Exotic plant invasion alters chaparral ecosystem resistance and resilience pre- and post-wildfire. Biol. Invasions 16, 1119–1130. https://doi.org/10.1007/s10530-013-0566-0

Dong, L.-J., Yu, H.-W., He, W.-M., 2015. What determines positive, neutral, and negative impacts of *Solidago canadensis* invasion on native plant species richness? Sci. Rep. 5, 1-9. 16804. https://doi.org/10.1038/srep16804

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., McClean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell, D., Lautenbach, S., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop.). 36, 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Dubuis, A., Giovanettina, S., Pellissier, L., Pottier, J., Vittoz, P., Guisan, A., 2013. Improving the prediction of plant species distribution and community composition by adding edaphic to topo-climatic variables. J. Veg. Sci. 24, 593–606. https://doi.org/10.1111/jvs.12002

Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibanez, I., Miller, L.P., 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. Nat. Commun. 7, 1–9.

Ehrenfeld, J.G., 2003. Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. Ecosystems 6, 503–523. https://doi.org/10.1007/s10021-002-0151-3

Epanchin-Niell, R., McAusland, C., Liebhold, A., Mwebaze, P., Springborn, M.R., 2021. Biological invasions and international trade: managing a moving target. Rev. Environ. Econ. Policy 15, 180–190. https://doi.org/10.1086/713025

Erb, H.E., 1988. *Stipa trichotoma*: Some considerations on the species, its habitat and natural distribution in Argentina. Report for the Department of Nature and Environmental Conservation of the Cape Provincial Administration. Cape Town, South Africa.

Esser, L.F., Saraiva, D.D., Jarenkow, J.A., 2019. Future uncertainties for the distribution and conservation of *Paubrasilia echinata* under climate change. Acta Bot. Brasilica 33, 770–776.

https://doi.org/10.1590/0102-33062019abb0173

Evans, H., 1991. Biological control of tropical grassy weeds, in: Baker, F.W.G., Terry, P.J. (Eds.), Tropical Grassy Weeds. CAB International, Wallingford, U.K., pp. 52–72.

Faithfull, I.G., 2012. Biodiversity impacts of Chilean needle grass *Nassella neesiana* on Australia's indigenous grasslands. PhD Thesis, Victoria University, School of Engineering and Science, St Albans, Victoria, Australia.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315. https://doi.org/10.1002/joc.5086

Finch, D.M., Butler, J.L., Runyon, J.B., Fettig, C.J., Kilkenny, F.F., Jose, S., Frankel, S.J., Cushman, S.A., Cobb, R.C., Dukes, J.S., Hicke, J.A., Amelon, S.K., 2021. Effects of Climate Change on invasive species, in: Invasive Species in Forests and Rangelands of the United States. Springer International Publishing, Cham, pp. 57–83. https://doi.org/10.1007/978-3-030-45367-1_4

Fish, L., Mashau, A.C., Moeaha, M.J., Nembudani, M.T., 2015. Identification guide to southern Africangrasses. An identification manual with keys, descriptions and distributions. Strelitzia 36, pp. 798.

Flemmer, A.C., Anderson, F.E., Hansen, P.V., McLaren, D.A., 2010. Microscopic observations of a compatible host/pathogen interaction between a potential biocontrol agent (*Uromyces pencanus*) and its target weed (*Nassella neesiana*). Mycoscience 51(5): 396–400. doi: 10.1007/S10267-010-0051-X.

Frederick, J., 2002. The North Central Chilean Needle Grass Project 1998-2002. Department of Primary Industries, Victoria, 29 pp.

Germishuizen, G., Meyer, N.L. (eds), 2003. Plants of Southern Africa: An Annotated Checklist. Strelitzia 14, 192–196. National Botanical Institute, Pretoria.

Gibbons, S.M., Lekberg, Y., Mummey, D.L., Sangwan, N., Ramsey, P.W., Gilbert, J.A., 2017. Invasive plants rapidly reshape soil properties in a grassland ecosystem. mSystems 2(2):e00178-e216. https://doi.org/10.1128/msystems.00178-16 page numbers

Giordano, L., Anderson, F.E., 2021. Detrimental effect of the rust *Uromyces pencanus* on the invasive species *Nassella neesiana* (Chilean needle grass). Australas. Plant Pathol. 50(3), 299-307. https://doi.org/10.1007/s13313-020-00773-x

Global Invasive Species Database (GISD), 2019. Species profile *Nassella tenuissima* [WWW Document]. URL http://www.iucngisd.org/gisd/species.php?sc=463 (accessed 10.08.2018).

Goolsby, J.A., Hathcock, C.R., Vacek, A.T., Kariyat, R.R., Moran, P.J., Martinez Jimenez, M., 2020. No evidence of non-target use of native or economic grasses and broadleaf plants by *Arundo donax* biological control agents. Biocontrol Sci. Technol. 30, 795–805. https://doi.org/10.1080/09583157.2020.1767038

Graham, M.H., 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84, 2809–2815. https://doi.org/10.1890/02-3114

Grech, C., McLaren, D., Lowien, J., McWhirter, L., Butler, K., Sindel, B., 2014. Effects of flupropanate application on bare ground and broadleaf weeds when used to control Chilean needle grass in introduced pastures. New Zeal. J. Agric. Res. 57, 100–109. https://doi.org/10.1080/00288233.2014.886597

Grech, C.J., McLaren, D.A., Lowien, J., McWhirter, L., Butler, K.L., Sindel, B.M., 2012. Assessment of management options for Chilean needle grass in sheep - grazing systems. Grass Forage Sci. 69, 119–128.

Green, P.T., O'Dowd, D.J., Abbott, K.L., Jeffery, M., Retallick, K., Mac Nally, R., 2011. Invasional meltdown: Invader–invader mutualism facilitates a secondary invasion. Ecology 92, 1758–1768. https://doi.org/10.1890/11-0050.1

Grenouillet, G., Buisson, L., Casajus, N., Lek, S., 2011. Ensemble modelling of species distribution: the effects of geographical and environmental ranges. Ecography (Cop.). 34, 9–17. https://doi.org/10.1111/j.1600-0587.2010.06152.x

Guisan, A., Thuiller, W., Zimmermann, N.E., 2017. Habitat Suitability and Distribution Models. Cambridge University Press, Cambridge. https://doi.org/10.1017/9781139028271

Gupta, U., Verma, P., Solanki, H.A., 2014. Impact of high boron concentration on plants. Biolife 2, 610–614.

Hamim, 2005. Photosynthesis of C3 and C4 species in response to increased CO2 Concentration and drought stress. HAYATI J. Biosci. 12, 131–138. https://doi.org/https://doi.org/10.1016/S1978-3019(16)30340-0

Hanley, N., Roberts, M., 2019. The economic benefits of invasive species management. People Nat. 1, 124–137. https://doi.org/10.1002/pan3.31

Hayward, I.M., Druce, G.C., 1919. The Adventive Flora of Tweedside. T. Buncle and Co., Arbroath, Scotland

Healy, A., 1945. Nassella tussock. Field Studies and their Agricultural Significance. DSIR, New Zealand, bulletin 91, 1-90

Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. London. Ser. B Biol. Sci. 270, 96–99. https://doi.org/10.1098/rsbl.2003.0025

Hejda, M., Pyšek, P., 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biol. Conserv. 132, 143–152. https://doi.org/10.1016/j.biocon.2006.03.025

Hejda, M., Pyšek, P., Jarošík, V., 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. J. Ecol. 97, 393–403. https://doi.org/10.1111/j.1365-2745.2009.01480.x

Hejda, M., Štajerová, K., Pyšek, P., 2017. Dominance has a biogeographical component: do plants tend to exert stronger impacts in their invaded rather than native range? J. Biogeogr. 44, 18–27. https://doi.org/10.1111/jbi.12801

Henderson, L., 2018. Shocking *Nassella* invasion threaten South Africa’s Mountain Grassland. SAPIA News Rep. No. 48, 1-8.

Henderson, L., 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37, 215–248. https://doi.org/10.4102/abc.v37i2.322

Henderson, L., 2001. Alien weeds and invasive plants: A complete guide to declared weeds and invaders in South Africa. Agricultural Research Council, Pretoria, South Africa. Plant Protection Research Institute Handbook no. 12.

Henderson, L., Wilson, J.R.U., 2017. Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. Bothalia 47. https://doi.org/10.4102/abc.v47i2.2172

Hijmans, R.J., 2021. raster: Geographic Data Analysis and Modeling. R package version 3.4-10. https://CRAN.R-project.org/package=raster

Hocking, C., 2011. National Guidelines for Minimising Waterway Infestations of Chilean Needle Grass - Filling a Major Gap in Knowledge and Advice. Final Report to Caring for Our Country Project OG083182, Australian Department of Agriculture, Fisheries and Forestry, Canberra.

Hollingsworth, P.M., Graham, S.W., Little, D.P., 2011. Choosing and using a plant DNA Barcode. PLoS One 6, e19254. https://doi.org/10.1371/journal.pone.0019254

Howell, C., Sawyer, J.W.D., 2006. New Zealand naturalised vascular plant checklist. New Zealand Plant Conservation Network, Wellington, New Zealand. 60 p

Humphries, T., Florentine, S.K., 2021. A comparative review of six invasive *Nassella* species in Australia with implications for their management. Plants 10, 1036. https://doi.org/10.3390/plants10061036

Hussaini, I.P., Lawrie, A.C., McLaren, D.A., 2000. Pathogens on and variation in *Nassella trichotoma* (Poales: Poaceae) in Australia. Spencer NR, Editor. Proceedings of the X International Symposium on Biological Control of Weeds: 269–280. Montana, USA.

Jacobs, S.W.L., Everett, J., Torres, M.A., 1998. *Nassella tenuissima* (Gramineae) recorded from Australia, a potential new weed related to Serrated Tussock. Telopea 8, 41–46.

Ji, Y., Liu, C., Yang, J., Jin, L., Yang, Z., Yang, J.-B., 2020. Ultra-Barcoding discovers a cryptic species in *Paris yunnanensis* (Melanthiaceae), a medicinally important plant. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00411

Jones, R.E., Vere, D.T., Campbell, M.H., 2000. The external costs of pasture weed spread: an economic assessment of serrated tussock control. Agric. Econ. 22, 91–103.

Joubert, D.C., 1984. The soil seed bank under *Nasella* tussock infestations at Boschberg. South African J. Plant Soil 1, 1–3. https://doi.org/10.1080/02571862.1984.10634099

Kabaš, E., Ljubičić, I., 2019. First record of *Nassella neesiana* (Trin. & Rupr.) Barkworth (Poaceae) in Croatia. BioInvasions Rec. 8, 478–486. https://doi.org/10.3391/bir.2019.8.3.02

Kariyawasam, C.S., Kumar, L., Ratnayake, S.S., 2021. Potential distribution of aquatic invasive alien plants, *Eichhornia crassipes* and *Salvinia molesta* under climate change in Sri Lanka. Wetl. Ecol. Manag. 29, 531–545. https://doi.org/10.1007/s11273-021-09799-4

Kiełtyk, P., Delimat, A., 2019. Impact of the alien plant *Impatiens glandulifera* on species diversity of invaded vegetation in the northern foothills of the Tatra Mountains, Central Europe. Plant Ecol. 220, 1–12. https://doi.org/10.1007/s11258-018-0898-z

Kiviat, E., Meyerson, L.A., Mozdzer, T.J., Allen, W.J., Baldwin, A.H., Bhattarai, G.P., Brix, H., Caplan, J.S., Kettenring, K.M., Lambertini, C., Weis, J., Whigham, D.F., Cronin, J.T., 2019. Evidence does not support the targeting of cryptic invaders at the subspecies level using classical biological control: the example of Phragmites. Biol. Invasions 21, 2529–2541. https://doi.org/10.1007/s10530-019-02014-9

Koteen, L. E., Baldocchi, D. D., & Harte, J. (2011). Invasion of non-native grasses causes a drop in soil carbon storage in California grasslands. Environmental Research Letters, 6(4), 044001. *https://doi.org/10.1088/1748-9326/6/4/044001*

Kress, W.J., Erickson, D.L., 2007. A Two-Locus Global DNA Barcode for land plants: The coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS One 2, e508. https://doi.org/10.1371/journal.pone.0000508

Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O., Bermingham, E., 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci. 106, 18621–18626. https://doi.org/10.1073/pnas.0909820106

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A., Janzen, D.H., 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. 102, 8369–8374. https://doi.org/10.1073/pnas.0503123102

Kriticos, D., Crossman, N.D., Ota, N., Scott, J.K., others, 2010. Climate change and invasive plants in South Australia. CSIRO Climate Adaptation Flagshi, Canberra, Australia. 92pp.

Kriticos, D.J., Lamoureaux, S., Bourdôt, G.W., Pettit, W., 2004. *Nassella* tussock current and potential distributions in New Zealand. New Zeal. Plant Prot. 57, 81–88.

https://doi.org/10.30843/nzpp.2004.57.6976

Kukunda, C.B., Duque-Lazo, J., González-Ferreiro, E., Thaden, H., Kleinn, C., 2018. Ensemble classification of individual *Pinus* crowns from multispectral satellite imagery and airborne LiDAR. Int. J. Appl. Earth Obs. Geoinf. 65, 12–23. https://doi.org/10.1016/j.jag.2017.09.016

Kumar Rai, P., Singh, J.S., 2020. Invasive alien plant species: Their impact on environment, ecosystem services and human health. Ecol. Indic. 111, 106020. https://doi.org/10.1016/j.ecolind.2019.106020

Kumschick, S., Gaertner, M., Vilà, M., Essl, F., Jeschke, J.M., Pyšek, P., Ricciardi, A., Bacher, S., Blackburn, T.M., Dick, J.T.A., Evans, T., Hulme, P.E., Kühn, I., Mrugała, A., Pergl, J., Rabitsch, W., Richardson, D.M., Sendek, A., Winter, M., 2015. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. Bioscience 65, 55–63. https://doi.org/10.1093/biosci/biu193

Kumschick, S., Measey, G.J., Vimercati, G., de Villiers, F.A., Mokhatla, M.M., Davies, S.J., Thorp, C.J., Rebelo, A.D., Blackburn, T.M., Kraus, F., 2017. How repeatable is the Environmental Impact Classification of Alien Taxa (EICAT)? Comparing independent global impact assessments of amphibians. Ecol. Evol. 7, 2661–2670. https://doi.org/10.1002/ece3.2877

Lamoureaux, S., Bourdôt, G.W., Verkaaik, M., 2006. Outsmarting nassella tussock - answers to often-asked questions based on recent research findings, in: Preston, C., Watts, J.H., Crossman, N.D. (Eds.), Proceedings of the Fifteenth Australian Weeds Conference. Adelaide, Australia. Weed Management Society of South Australia.

Lamoureaux, S., Bourdôt, G., 2002. Fruit viability on grubbed *Nassella trichotoma* (Nees) tussocks. Aust. J. Exp. Agric. 42, 1057. https://doi.org/10.1071/EA02032

Lamoureaux, S.L., Bourdôt, G.W., Saville, D.J., 2011. Population growth of *Nassella trichotoma* in grasslands in New Zealand slower today than in the past. Acta Oecologica 37, 484–494. https://doi.org/10.1016/j.actao.2011.06.008

Lenoir, J., Gégout, J.C., Marquet, P.A., de Ruffray, P., Brisse, H., 2008. A Significant Upward Shift in Plant Species Optimum Elevation During the 20th Century. Sci. 320, 1768–1771. https://doi.org/10.1126/science.1156831

Liu, C., Wolter, C., Xian, W., Jeschke, J.M., 2020a. Most invasive species largely conserve their climatic niche. Proc. Natl. Acad. Sci. 117, 23643–23651. https://doi.org/10.1073/pnas.2004289117

Liu, C., Wolter, C., Xian, W., Jeschke, J.M., 2020b. Species distribution models have limited spatial transferability for invasive species. Ecol. Lett. 23, 1682–1692. https://doi.org/10.1111/ele.13577

Lobo, J.M., Jiménez-Valverde, A., Hortal, J., 2010. The uncertain nature of absences and their importance in species distribution modelling. Ecography (Cop.). 33, 103–114. https://doi.org/10.1111/j.1600-0587.2009.06039.x

López Rosas, H., Moreno-Casasola, P., Mendelssohn, I.A., 2005. Effects of an African grass invasion on vegetation, soil and interstitial water characteristics in a tropical freshwater marsh in La Mancha, Veracruz (Mexico). J. Plant Interact. 1, 187–195. https://doi.org/10.1080/17429140600857693

Lusk, C.S., Hurrell, G.A., Saville, D.J., Bourdôt, G.W., 2017. Changes in plant species composition after flupropanate application for nassella tussock control, in Canterbury hill-country pastures. New Zeal. J. Agric. Res. 60, 263–276. https://doi.org/10.1080/00288233.2017.1321556

Malhotra, H., Vandana, Sharma, S., Pandey, R., 2018. Phosphorus nutrition: plant growth in response to deficiency and excess, in: plant nutrients and abiotic stress tolerance. Springer Singapore, Singapore, pp. 171–190. https://doi.org/10.1007/978-981-10-9044-8_7

Mapaura, A., Canavan, K., Richardson, D.M., Clark, V.R., Steenhuisen, S.L., 2020. The invasive grass genus *Nassella* in South Africa: A synthesis. S. Afr. J. Bot. 135, 336–348. https://doi.org/10.1016/j.sajb.2020.08.031

Masters, G., Norgrove, L., 2010. Climate change and invasive alien species, CABI Working Paper 1, 30pp. https://www.cabi.org/wp-content/uploads/CABI-Working-Paper-1.pdf (accessed 12.09.2021).

Maurel, N., Salmon, S., Ponge, J.-F., Machon, N., Moret, J., Muratet, A., 2010. Does the invasive species *Reynoutria japonica* have an impact on soil and flora in urban wastelands? Biol. Invasions 12, 1709–1719. https://doi.org/10.1007/s10530-009-9583-4

Maxwell, B.D., Lehnhoff, E., Rew, L.J., 2009. The rationale for monitoring invasive plant populations as a crucial step for management. Invasive Plant Sci. Manag. 2, 1–9. https://doi.org/10.1614/IPSM-07-054.1

McLaren, D.A., Anderson, F.E., 2011a. Protecting Australia's Agricultural Grasslands from Herbicide-Resistant Serrated Tussock. RIRDC Publication. Report No.: Pub. No. 11/070. Available at https://www.agrifutures.com.au/product/protecting-australia-s-agricultural-grasslands-from-herbicide-resistant-serrated-tussock (Accessed 08.10.2019).

McLaren, D.A., Anderson, F.E., 2011b. Biological control of serrated tussock and chilean needle grass. Rural Industries Research and Development Corporation, Canberra, Australia. Report No.: Pub. No. 11/040.

McLaren, D., Cowan, R., 2012. Biological control of serrated tussock. North Sydney: Meat and Livestock Australia Limited. Report No.: Final Report.

McLaren, D.A., Snell, K., Butler, K.L., 2008. An assessment of native tree susceptibility to the simulated aerial application of the herbicide flupropanate, for management of exotic unpalatable grasses, in: van Klinken, R.D., Osten, V.A., Panetta, F.D., Scanlan, J.C. (Eds.), Hot Topics in the Tropics. Queensland Weeds Society, Queensland, Australia, Queensland Weeds Society, Queensland, Australia.

McLaren, D.A., Stajsic, V., Gardener, M.R., 1998. The distribution and impact of South/North American stipoid grasses (Poaceae: Stipeae) in Australia. Plant Prot. Q. 13, 62–70.

McLaren, D.A., Stajsic, V., Iaconis, L., 2004. The distribution, impacts and identification of exotic stipoid grasses in Australia. Plant Prot. Q. 19, 59–66.

Merkel, A., 2020. Climate data for cities worldwide - Climate-Data.org. [WWW Document]. Online. URL https://en.climate-data.org (accessed 10.11.2021).

Miller, L., 1998. Management of serrated tussock in farming areas. Plant Prot. Q. 13, 91–93.

Milton, S.J., 2004. Grasses as invasive alien plants in South Africa. S. Afr. J. Sci. 100, 69–75.

Morais, P., Reichard, M., 2018. Cryptic invasions: A review. Sci. Total Environ. 613, 1438–1448. https://doi.org/10.1016/j.scitotenv.2017.06.133

Moretto, A.S., Distel, R.A., 1998. Requirement of vegetation gaps for seedling establishment of two unpalatable grasses in a native grassland of central Argentina. Aust. J. Ecol. 23, 419–423.

Morgan, J.W., 2001. Seedling recruitment patterns over 4 years in an Australian perennial grassland community with different fire histories. J. Ecol. 89, 908–919. https://doi.org/10.1111/j.1365-2745.2001.00617.x

Mucina, L., Rutherford, M.C., 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19. South African National Biodiversity Institute, Pretoria.

Muluneh, M.G., 2021. Impact of climate change on biodiversity and food security: a global perspective—a review article. Agric. Food Secur. 10, 36. https://doi.org/10.1186/s40066-021-00318-5

Naimi, B., a.s. Hamm, N., Groen, T.A., Skidmore, A.K., Toxopeus, A.G., 2014. Where is positional uncertainty a problem for species distribution modelling. Ecography (Cop.). 37, 191–203. https://doi.org/10.1111/j.1600-0587.2013.00205.x

Naimi, B., Araújo, M.B., 2016. sdm: a reproducible and extensible R platform for species distribution modelling. Ecography (Cop.). 39, 368–375. https://doi.org/10.1111/ecog.01881

National Environmental Management: Biodiversity Act 10 2004: Alien and Invasive Species (AIS) Regulations, 18 September 2020. (RSA)

Nejad, S.A.G., Etesami, H., 2020. The importance of boron in plant nutrition, in: metalloids in plants. Wiley, pp. 433–449. https://doi.org/10.1002/9781119487210.ch20

Nhemachena, C., Nhamo, L., Matchaya, G., Nhemachena, C.R., Muchara, B., Karuaihe, S.T., Mpandeli, S., 2020. Climate change impacts on water and agriculture sectors in southern africa: threats and opportunities for sustainable development. Water 12(10), pp. 267. https://doi.org/https://doi.org/10.3390/w12102673

Nkuna, K. V., Visser, V., Wilson, J.R.U., Kumschick, S., 2018. Global environmental and socio-economic impacts of selected alien grasses as a basis for ranking threats to South Africa. NeoBiota 41, 19–65. https://doi.org/10.3897/neobiota.41.26599

Non-Affiliated Soil Analysis Work Committee, Soil Science Society of South Africa, 1990. Handbook of standard soil testing methods for advisory purposes. Soil Science Society of South Africa, Pretoria.

Occdownload Gbif.Org, 2019. GBIF Occurrence Download.

https://doi.org/10.15468/DL.YPBDSK

Onwuka, B., 2018. Effects of soil temperature on some soil properties and plant growth. Adv. Plants Agric. Res. 8. https://doi.org/10.15406/apar.2018.08.00288

Palmer, A.R., 1990. A qualitative model of vegetation history in the eastern cape midlands, south africa. J. Biogeogr. 17, 35. https://doi.org/10.2307/2845186

Peterson, A.T., Soberón, J., 2012. Species distribution modeling and ecological niche modeling: getting the concepts right. Nat. Conserv. 10, 102–107. https://doi.org/10.4322/natcon.2012.019

Peterson, P.M., Romaschenko, K., Soreng, R.J., 2014. A laboratory guide for generating DNA barcodes in grasses: a case study of Leptochloa s.l. (Poaceae: Chloridoideae). Webbia 69, 1–12. https://doi.org/10.1080/00837792.2014.927555

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pörtner, H.-O., Roberts, D.C., Poloczanska, E.S., Mintenbeck, K., Tignor, M., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., A. Okem, 2022. IPCC, 2022: Summary for Policymakers, in: Pörtner, H.-O., Roberts, D.C., Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., A. Okem, Rama, B. (Eds.), Climate Change 2022: Impacts, adaptation, and vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. In Press.

Potgieter, L.J., Douwes, E., Gaertner, M., Measey, J., Paap, T., Richardson, D.M., 2020. Biological invasions in South Africa's urban ecosystems: patterns, processes, impacts, and management, in: biological invasions in South Africa. Springer International Publishing, Cham, pp. 275–309. https://doi.org/10.1007/978-3-030-32394-3_11

Potgieter, L.J., Gaertner, M., O'Farrell, P.J., Richardson, D.M., 2019. Perceptions of impact: Invasive alien plants in the urban environment. J. Environ. Manage. 229, 76–87. https://doi.org/10.1016/j.jenvman.2018.05.080

Powells, J., 2018. Flupropanate resistant serrated tussock (*Nassella trichotoma*) identified at multiple locations in the Monaro, New South Wales, in: Johnson, S., L., W., Wu, H., Auld, B. (Eds.), Proceedings of the Twentyfirst Australasian Weeds Conference. The Weed Society of New South Wales Inc.

Power, G., Sánchez Vilas, J., 2020. Competition between the invasive *Impatiens glandulifera* and UK native species: the role of soil conditioning and pre-existing resident communities. Biol. Invasions 22, 1527–1537. https://doi.org/10.1007/s10530-020-02202-y

Prince, L.M., 2015. Plastid Primers for Angiosperm Phylogenetics and Phylogeography. Appl. Plant Sci. 3, 1400085. https://doi.org/10.3732/apps.1400085

Pyšek, P., Pergl, J., van Kleunen, M., Dawson, W., Essl, F., Kreft, H., Weigelt, P., Wilson, J.R., Winter, M., Richardson, D.M., 2020. South Africa as a donor of naturalised and invasive plants to other parts of the world, in: Biological Invasions in South Africa. Springer International Publishing, Cham, pp. 759–785. https://doi.org/10.1007/978-3-030-32394-3_26

Pyšek, P., Richardson, D.M., 2010. Invasive species, environmental change and management, and health. Annu. Rev. Environ. Resour. 35, 25–55. https://doi.org/10.1146/annurev-environ-033009-095548

Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G.L., Williamson, M., Kirschner, J., 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53, 131–143. https://doi.org/10.2307/4135498

QGIS Development Team, 2021. QGIS Geographic Information System. Open Source Geospatial Foundation. http://qgis.org

Quattrocchi, U., 2000. CRC world dictionary of plant names. Common names, scientific names, eponyms, synonym, and etymology. Volume 3 : M - Q, 1st ed. Boca Raton : CRC Press.

R Core Team, 2021. R Foundation for Statistical Computing. R Found. Stat. Comput. ver. 4.0.5.

Ragupathy, S., Newmaster, S.G., Murugesan, M., Balasubramaniam, V., 2009. DNA barcoding discriminates a new cryptic grass species revealed in an ethnobotany study by the hill tribes of the Western Ghats in southern India. Mol. Ecol. Resour. 9, 164–171. https://doi.org/10.1111/j.1755-0998.2009.02641.x

Rahbek, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N., Nogues-Bravo, D., Whittaker, R.J., Fjeldså, J., 2019. Humboldt's enigma: What causes global patterns of mountain biodiversity? Science 365, 1108–1113. https://doi.org/10.1126/science.aax0149

Ranwashe, F., 2019. BODATSA [WWW Document]. Bot. Collect. v1.4. South African Natl. Biodivers. Institute. Dataset/Occurrence. URL http://ipt.sanbi.org.za/iptsanbi/%0Aresource?r=brahms_online&v=1.4 (accessed 03.23.2021).

Richardson, D.M., Foxcroft, L.C., Latombe, G., Le Maitre, D.C., Rouget, M., Wilson, J.R.U., 2020. The biogeography of South African terrestrial plant invasions, in: Van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R.U., Zengeya, T. (Eds.), Biological Invasions in South Africa. Springer, Berlin.

Richardson, D.M., Iponga, D.M., Roura-Pascual, N., Krug, R.M., Milton, S.J., Hughes, G.O., Thuiller, W., 2010. Accommodating scenarios of climate change and management in modelling the distribution of the invasive tree *Schinus molle* in South Africa. Ecography (Cop.). 33, 1049–1061. https://doi.org/10.1111/j.1600-0587.2010.06350.x

Richardson, D.M., Pyšek, P., 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. Prog. Phys. Geogr. Earth Environ. 30, 409–431. https://doi.org/10.1191/0309133306pp490pr

Richardson, D.M., Wilson, J.R.U., Weyl, O.L.F., Griffiths, C.L., 2011. South Africa: Invasions. In: (Eds.), Encyclopedia of Biological Invasions., in: Simberloff, D., Rejmanek, M. (Eds.), . University of California Press, Berkeley, pp. 643–651.

Romaschenko, K., Peterson, P.M., Soreng, R.J., Garcia-Jacas, N., Futorna, O., Susanna, A., 2012. Systematics and evolution of the needle grasses (Poaceae: Pooideae: Stipeae) based on analysis of multiple chloroplast loci, ITS, and lemma micromorphology. Taxon 61, 18–44. https://doi.org/10.1002/tax.611002

Romaschenko, K., Peterson, P.M., Soreng, R.J., Garcia-Jacas, N., Futorna, O., Susanna, A., 2008. Molecular phylogenetic analysis of the American Stipeae (Poaceae) resolves *Jarava* sensu lato polyphyletic: evidence for a new genus *Pappostipa*. J. Bot. Res. Inst. Texas 2, 165–192.

Rout, G.R., Das, P., 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc.

Agronomie 23, 3–11. https://doi.org/10.1051/agro:2002073

Rudani, K., Patel, V., Prajapati, K., 2018. The importance of Zinc in plant growth - A Review. Int. Res. J. Nat. Appl. Sci. 5, 38–48.

Saddhe, A.A., Kumar, K., 2017. DNA barcoding of plants: Selection of core markers for taxonomic groups. Plant Sci. Today 5, 9–13. https://doi.org/10.14719/pst.2018.5.1.356

Sillero, N., Barbosa, A.M., 2021. Common mistakes in ecological niche models. Int. J. Geogr. Inf. Sci. 35, 213–226. https://doi.org/10.1080/13658816.2020.1798968

Slay, M.W.A., 2002. Chilean Needle Grass Control in Hawke's Bay. Chemical Topping. Report prepared for the Hawke's Bay Chilean Needle Grass Group, Homelea AgTechnology and Advisory Services and AgResearch Poukawa, Hastings and Havelock North, New Zealand, July, 30 pp.

Smith, L.P., Lamoureaux, S.L., 2006. When science meets policy - Nassella tussock in New Zealand as a case study, in: Proceedings of the Fifteenth Australian Weeds Conference. Weed Management Society of SA, pp. 450–453.

Soberón, J., & Arroyo-Peña, B. (2017). Are fundamental niches larger than the realized? Testing a 50-year-old prediction by Hutchinson. PLOS ONE, 12(4), e0175138. https://doi.org/10.1371/journal.pone.0175138

Sofaer, H.R., Jarnevich, C.S., Pearse, I.S., Smyth, R.L., Auer, S., Cook, G.L., Edwards, T.C., Guala, G.F., Howard, T.G., Morisette, J.T., Hamilton, H., 2019. Development and delivery of species distribution models to inform decision-making. Bioscience 69, 544–557. https://doi.org/10.1093/biosci/biz045

Soreng, R., Peterson, P., Davidse, G., Judziewicz, E., Zuloaga, F., Filgueiras, T.S., Morrone, O., 2009. Tropicos - name search. [WWW Document]. URL http://www.tropicos.org/Name-%0ASearch.aspx (accessed 05.08.2019).

Srivastava, V., Liang, W., Keena, M. A., Roe, A. D., Hamelin, R. C., & Griess, V. C. (2020). Assessing Niche Shifts and Conservatism by Comparing the Native and Post-Invasion Niches of Major Forest Invasive Species. Insects, 11(8), 479. https://doi.org/10.3390/insects11080479

Stefanowicz, A.M., Majewska, M.L., Stanek, M., Nobis, M., Zubek, S., 2018. Differential influence of four invasive plant species on soil physicochemical properties in a pot experiment. J. Soils Sediments 18, 1409–1423. https://doi.org/10.1007/s11368-017-1873-3

Steven G., N., Subramanyam, R., 2009. Testing plant barcoding in a sister species complex of pantropical *Acacia* (Mimosoideae, Fabaceae). Mol. Ecol. Resour. 9, 172–180. https://doi.org/10.1111/j.1755-0998.2009.02642.x

Stricker, K.B., Hagan, D., Flory, S.L., 2015. Improving methods to evaluate the impacts of plant invasions: lessons from 40 years of research. AoB Plants 7. https://doi.org/10.1093/aobpla/plv028

Sutton, G., Bownes, A., Visser, V., Mapaura, A., Canavan, K., 2021. Progress and prospects for the biological control of invasive alien grasses poaceae) in South Africa. African Entomol. 29. https://doi.org/10.4001/003.029.0837

Sutton, G.F., Canavan, K., Day, M.D., den Breeyen, A., Goolsby, J.A., Cristofaro, M., McConnachie, A., Paterson, I.D., 2019. Grasses as suitable targets for classical weed biological control. BioControl 64, 605–622. https://doi.org/10.1007/s10526-019-09968-8

Sylvester, S.P., Soreng, R.J., Sylvester, M.D.P.V., Mapaura, A., Ralph Clark, V., 2021. New records of alien and potentially invasive grass (Poaceae) species for southern Africa. Bothalia, African Biodivers. Conserv. 51(2), a1. https://doi.org/10.38201/btha.abc.v51.i2.1

Tang, S.-L., Song, Y.-B., Zeng, B., Dong, M., 2021. Potential distribution of the extremely endangered species *Ostrya rehderiana* (Betulaceae) in China under future climate change. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-021-16268-1

Taylor, N.J., 1987. Ecological aspects of Nassella tussock (*Stipa trichotoma*). Bot. Div. Lincoln, New Zealand. DSR, Lincoln, New Zealand.

Taylor, S., Conolly, J., Gruber, A., 2016. Chilean needle grass (*Nassella neesiana*) control - the ACT experience., in: Randall, R., Lloyd, S., Borger, C. (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Weeds Society of Western Australia, Perth., pp. 223–227.

Tebaldi, C., Debeire, K., Eyring, V., Fischer, E., Fyfe, J., Friedlingstein, P., Knutti, R., Lowe, J., O'Neill, B., Sanderson, B., van Vuuren, D., Riahi, K., Meinshausen, M., Nicholls, Z., Tokarska, K.B., Hurtt, G., Kriegler, E., Lamarque, J.-F., Meehl, G., Moss, R., Bauer, S.E., Boucher, O., Brovkin, V., Byun, Y.-H., Dix, M., Gualdi, S., Guo, H., John, J.G., Kharin, S., Kim, Y., Koshiro, T., Ma, L., Olivié, D., Panickal, S., Qiao, F., Rong, X., Rosenbloom, N., Schupfner, M., Séférian, R., Sellar, A., Semmler, T., Shi, X., Song, Z., Steger, C., Stouffer, R., Swart, N., Tachiiri, K., Tang, Q., Tatebe, H., Voldoire, A., Volodin, E., Wyser, K., Xin, X., Yang, S., Yu, Y., Ziehn, T., 2021. Climate model projections from the Scenario Model Intercomparison Project (ScenarioMIP) of CMIP6. Earth Syst. Dyn. 12, 253–293. https://doi.org/10.5194/esd-12-253-2021

Thomaz, S.M., Agostinho, A.A., Gomes, L.C., Silveira, M.J., Rejmanek, M., Aslan, C.E., Chow, E., 2012. Using space-for-time substitution and time sequence approaches in invasion ecology. Freshw. Biol. 57, 2401–2410. https://doi.org/10.1111/fwb.12005

Thor, K., 2019. Calcium—nutrient and messenger. Front. Plant Sci. 10:440. https://doi.org/10.3389/fpls.2019.00440

Thuiller, W., Lafourcade, B., Engler, R., Araújo, M.B., 2009. BIOMOD - a platform for ensemble forecasting of species distributions. Ecography (Cop.). 32, 369–373. https://doi.org/10.1111/j.1600-0587.2008.05742.x

Torres, A., Alarcón, P., Rodríguez-Cabal, M., Nuñez, M., 2018. Secondary invasions hinder the recovery of native communities after the removal of nonnative pines along a precipitation gradient in Patagonia. Forests 9, 394. https://doi.org/10.3390/f9070394

Vanderhoeven, S., Dassonville, N., Meerts, P., 2005. Increased Topsoil Mineral Nutrient Concentrations Under exotic invasive plants in Belgium. Plant Soil 275, 169–179. https://doi.org/10.1007/s11104-005-1257-0

Vere, D.T., Campbell, M.H., 1984. Economics of controlling serrated tussock in the southeastern Australian rangelands. J. Range Manag. 37, 87–93.

Verloove, F., 2005. A synopsis of *Jarava* Ruiz and Pav. and *Nassella* E. Desv. (Stipa L. s.l.) (Poaceae: Stipeae) in southwestern Europe. Candollea 60, 97–117.

Vijayan, K., Tsou, C., 2010. DNA barcoding in plants: taxonomy in a new perspective. Curr. Sci. 99, 1530–1541.

Vila, M., Tessier, M., Suehs, C.M., Brundu, G., Carta, L., Galanidis, A., Lambdon, P., Manca, M., Medail, F., Moragues, E., Traveset, A., Troumbis, A.Y., Hulme, P.E., 2006. Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. J. Biogeogr. 33, 853–861. https://doi.org/10.1111/j.1365-2699.2005.01430.x

Vilela, B., 2019. Niche overlap: update to Silva et al. 2014 supplementary matterial - R tutorials for macroecology [WWW Document]. URL https://rmacroecology.netlify.app/2019/01/21/niche-overlap-update-to-silva-et-al-2014-supplementary-matterial (accessed 08.22.2021).

Viljoen, B.D., 1999. Influence of adjuvants on tetrapion efficacy for Nassella tussock (*Stipa trichotoma* Nees) control and subsequent seedling establishment: a preliminary study. S. Afr. J. Plant Soil 16, 102–105. https://doi.org/10.1080/02571862.1999.10634855

Viljoen, B.D., 1987. Effect of rate and time of application of tetrapion on Nassella tussock (*Stipa trichotoma* Nees) in South Africa. S. Afr. J. Plant Soil 4, 79–81.

Visser, D., 2012. The Anglo-Boer War 1899–1902: White man's war, black man's war, traumatic war. S. Afr. J. Mil. Stud. 40, 171-173. https://doi.org/10.5787/40-2-1003

Visser V, Wilson JRU, Canavan K, Canavan S, Fish L, Le Maitre D, Nänni I, Mashau C, O'Connor T, Ivey P, et al. 2017. Grasses as invasive plants in South Africa revisited: Patterns, pathways and management. Bothalia 47(2), 29 pp. doi: 10.4102/abc.v47i2.2169.

Visser, V., Wilson, J.R.U., Fish, L., Brown, C., Cook, G.D., Richardson, D.M., 2016. Much more give than take: South Africa as a major donor but infrequent recipient of invasive non-native grasses. Glob. Ecol. Biogeogr. 25, 679–692. https://doi.org/10.1111/geb.12445

Volery, L., Blackburn, T.M., Bertolino, S., Evans, T., Genovesi, P., Kumschick, S., Roy, H.E., Smith, K.G., Bacher, S., 2020. Improving the Environmental Impact Classification for Alien Taxa (EICAT): a summary of revisions to the framework and guidelines. NeoBiota 62, 547–567. https://doi.org/10.3897/neobiota.62.52723

Walker, L.R., Smith, S.D., 1997. Impacts of invasive plants on community and ecosystem properties. pp. 69–86. https://doi.org/10.1007/978-1-4612-1926-2_7

Walther, G.-R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z., Bugmann, H., 2009. Alien species in a warmer world: risks and opportunities. Trends Ecol. Evol. 24, 686–693. https://doi.org/10.1016/j.tree.2009.06.008

Wang, A., Gopurenko, D., Wu, H., Lepschi, B., 2017. Evaluation of six candidate DNA barcode loci for identification of five important invasive grasses in eastern Australia. PLoS One 12, e0175338. https://doi.org/10.1371/journal.pone.0175338

Wang, A., Gopurenko, D., Wu, H., Stanton, R., Lepschi, B.J., 2014. DNA barcoding for identification of exotic grass species present in eastern Australia, in: Baker, M. (Ed.), Proceedings of the Nineteenth Australasian Weeds Conference. Tasmanian Weed Society, Tasmania, pp. 444–447.

Wang, A., Wu, H., Gopurenko, D., 2016. Chloroplast genome of serrated tussock (*Nassella trichotoma*): structure and evolution, in: Randall, R., Lloyd, S., Borger, C. (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Perth. Weeds Society of Western Australia, pp. 152–156.

Wang, F., Gao, J., Yong, J.W.H., Wang, Q., Ma, J., He, X., 2020. Higher atmospheric CO2 levels favor C3 plants over C4 plants in utilizing ammonium as a nitrogen source. Front. Plant Sci. 11, 1877. https://doi.org/https://doi.org/10.3389/fpls.2020.537443

Wang, Y., Naumann, U., Wright, S.T., Warton, D.I., 2012. mvabund - an R package for model-based analysis of multivariate abundance data. Methods Ecol. Evol. 3, 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x

Watt, M.S., Kriticos, D.J., Lamoureaux, S.L., Bourdôt, G.W., 2011. Climate change and the potential global distribution of serrated tussock (*Nassella trichotoma*). Weed Sci. 59, 538–545. https://doi.org/10.1614/WS-D-11-00032.1

Wearne, L.J., Morgan, J.W., 2004. Community-level changes in Australian subalpine vegetation following invasion by the non-native shrub *Cytisus scoparius*. J. Veg. Sci. 15, 595–604. https://doi.org/10.1111/j.1654-1103.2004.tb02301.x

Weller, S., Florentine, S., Sillitoe, J., Grech, C., McLaren, D., Chauhan, B.S., 2015. Detecting the Seeds of *Nassella neesiana* in Large Round Hay Bales, by Means of Non-Destructive Core Sampling. PLoS One 10, e0137343. https://doi.org/10.1371/journal.pone.0137343

Weller, S.L., Florentine, S.K., Sillitoe, J.F., Grech, C.J., McLaren, D.A., 2016. An investigation of the effects of stage of ensilage on *Nassella neesiana* seeds, for reducing seed viability and injury to livestock. Sci. Rep. 6, 22345. https://doi.org/10.1038/srep22345

Wells, M.J., 1978. Nassella tussock, in: Stirton, C.H. (Ed.), Plant Invaders: Beautiful but Dangerous. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town, South Africa, pp. 140–143.

Wells, M.J., 1977. Progress with research on Nassella tussock. Proc. Second Natl. Weeds Conf. South Africa. 47–55.

Wells, M.J., De Beer, H., 1987. Nassella Tussock. Agricultural Research Council. Weeds

A.21/1987.

Wells, M.J., Engelbregcht, V.M., Balsinhas, A.A., Stirton, C.H., 1983. Weed flora of South Africa 2: power shifts in the veld. Bothalia 14, 961–965. https://doi.org/10.4102/abc.v14i3/4.1269

Westbrooks, R.G., Cross, G., 1993. Serrated Tussock (*Nassella trichotoma*) in the United States. Weed Technol. 7, 525–528.

White, P.J., 2003. Calcium in Plants. Ann. Bot. 92, 487–511. https://doi.org/10.1093/aob/mcg164

Wijesuriya, S., Hocking, C., 1999. Why do weeds grow when you dig up native grasslands? The effects of physical disturbance on available nutrients, mineralisation and weed invasion in native grasslands, in: Craigie, V., Hocking, C. (Eds.), Down to Grass Roots. Victoria University, St Albans, Victoria, pp. 31–37.

Wundrow, E.J., Carrillo, J., Gabler, C.A., Horn, K.C., Siemann, E., 2012. Facilitation and competition among invasive plants: a field experiment with alligatorweed and water hyacinth. PLoS One 7, e48444. https://doi.org/10.1371/journal.pone.0048444

Xu, Y., Huang, Y., Zhao, H., Yang, M., Zhuang, Y., Ye, X., 2021. Modelling the effects of climate change on the distribution of endangered *Cypripedium japonicum* in China. Forests 12, 429. https://doi.org/10.3390/f12040429

Zhang, Y., Tang, J., Ren, G., Zhao, K., Wang, X., 2021. Global potential distribution prediction of *Xanthium italicum* based on Maxent model. Sci. Rep. 11, 16545. https://doi.org/10.1038/s41598-021-96041-z

Zizka, A., Silvestro, D., Andermann, T., Azevedo, J., Duarte Ritter, C., Edler, D., Farooq, H., Herdean, A., Ariza, M., Scharn, R., Svantesson, S., Wengström, N., Zizka, V., & Antonelli, A. (2019). CoordinateCleaner: Standardized cleaning of occurrence records from biological collection databases. Methods in Ecology and Evolution, 10(5), 744–751. https://doi.org/10.1111/2041-210X.13152

APPENDICES

Appendix A. A synthesis of available information about *Nassella* invasions in South Africa and identified research gaps

South African Journal of Botany 135 (2020) 336–348

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

The invasive grass genus *Nassella* in South Africa: A synthesis

Anthony Mapaura[a,*], Kim Canavan[b], David M. Richardson[c], Vincent R. Clark[d], Sandy-Lynn Steenhuisen[a]

[a] Afromontane Research Unit and Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba 9866, South Africa
[b] Centre for Biological Control, Department of Entomology and Zoology, Rhodes University, Grahamstown 6140, South Africa
[c] Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch 7602, South Africa
[d] Afromontane Research Unit and Department of Geography, University of the Free State, Qwaqwa Campus, Phuthaditjhaba 9866, South Africa

ARTICLE INFO

Article History:
Received 21 April 2020
Revised 6 July 2020
Accepted 28 August 2020
Available online xxx

Edited by S Geerts

Keywords:
Biological invasions
Invasive grasses
Nassella neesiana
Nassella tenuissima
Nassella trichotoma
Poaceae

ABSTRACT

Three species of *Nassella* have naturalized in South Africa. *Nassella trichotoma* and *N. tenuissima* are declared weeds under category 1b of the National Environmental Management: Biodiversity Act (NEM:BA) and occur mainly in the montane grasslands of the Western and Eastern Cape provinces. *Nassella neesiana* is not listed in NEM:BA but is naturalized in the Eastern Cape, Western Cape and Free State provinces. Research conducted in the 1970s and 1980s led to vigorous government-funded awareness and control campaigns which ended in 2000. No research on *Nassella* distribution or control has been undertaken since then. Despite this hiatus, *Nassella* remains a dangerous genus in southern Africa, given the serious impacts of these species in similar social-ecological systems in Australia and New Zealand. This paper presents a synthesis of available information about *Nassella* invasions in South Africa and identifies research gaps. It specifically addresses these questions: What identification issues exist? What is the current spatial distribution of *Nassella*? What is the autecology of the genus? What are the social-ecological impacts of *Nassella*? What control measures are currently applied and what are their strengths and limitations? What do we know about *Nassella* distribution and its response to climate change? This paper highlights many knowledge gaps about *Nassella*, such as the species' current distribution range, field identification and detection difficulties, and the uncoordinated control efforts that require urgent research to inform an effective management response.

1. Introduction

The genus *Nassella* (Trin.) E. Desv. belongs to the grass family, Poaceae (Gramineae), subfamily Pooideae in the tribe Stipeae (spear grasses). *Nassella* comprises at least 116 species (Barkworth et al., 2008; Soreng et al., 2009; Romaschenko et al., 2012). Species currently placed in this genus were originally included in the genus *Stipa* s.l. and for many years researchers held mixed views regarding the segregation of these genera. However, with strong support from molecular studies, their separation appears to be largely settled, save for a few species (Romaschenko et al., 2012; Cialdella et al., 2014). The name 'Nassella' comes from the Latin noun *nassa* which means "a fish basket" (Quattrocchi, 2000), probably because these grasses were used for making fishing baskets.

According to Barkworth and Torres (2001), with confirmation from researchers such as Romaschenko et al. (2012) and Soreng et al. (2009), most *Nassella* species are native to South America, while six are also found in the United States and Canada. The species are mostly native to Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Paraguay, Peru, Uruguay, and Venezuela. The highest species diversity of *Nassella* occurs in north-western Argentina which is home to 72 species (Barkworth and Torres, 2001).

Eleven *Nassella* species have been recorded as growing outside their natural ranges (Barkworth and Torres, 2001). Of these, *N. neesiana, N. tenuissima* and *N. trichotoma* have naturalised (*sensu* Pyšek et al., 2004) and become invasive in Australia, Europe, New Zealand, South Africa and the USA (Howell and Sawyer, 2006; Henderson, 2018; Ranwashe, 2019). The other species which have naturalised outside their natural ranges are not invasive (Barkworth and Torres, 2001). *Nassella neesiana, N. tenuissima* and *N. trichotoma* invade disturbed areas, particularly overgrazed pastures, and also indigenous grasslands, including those dominated by *Themeda triandra* Forrsk in temperate mountains (Faithfull et al., 2012; Taylor et al., 2016). *Nassella trichotoma* is regarded as a major invader in Australia, New Zealand and South Africa (Wells and De Beer, 1987).

There are no indigenous species of *Nassella* in South Africa. Three species are known to have naturalised in the country: *N. neesiana, N. tenuissima* and *N. trichotoma* (Fish et al., 2015; Visser et al., 2017; Ranwashe, 2019). All three species are thought to have been

---

* Corresponding author.
E-mail address: amapaura@gmail.com (A. Mapaura).

https://doi.org/10.1016/j.sajb.2020.08.031
0254-6299/© 2020 SAAB. Published by Elsevier B.V. All rights reserved.

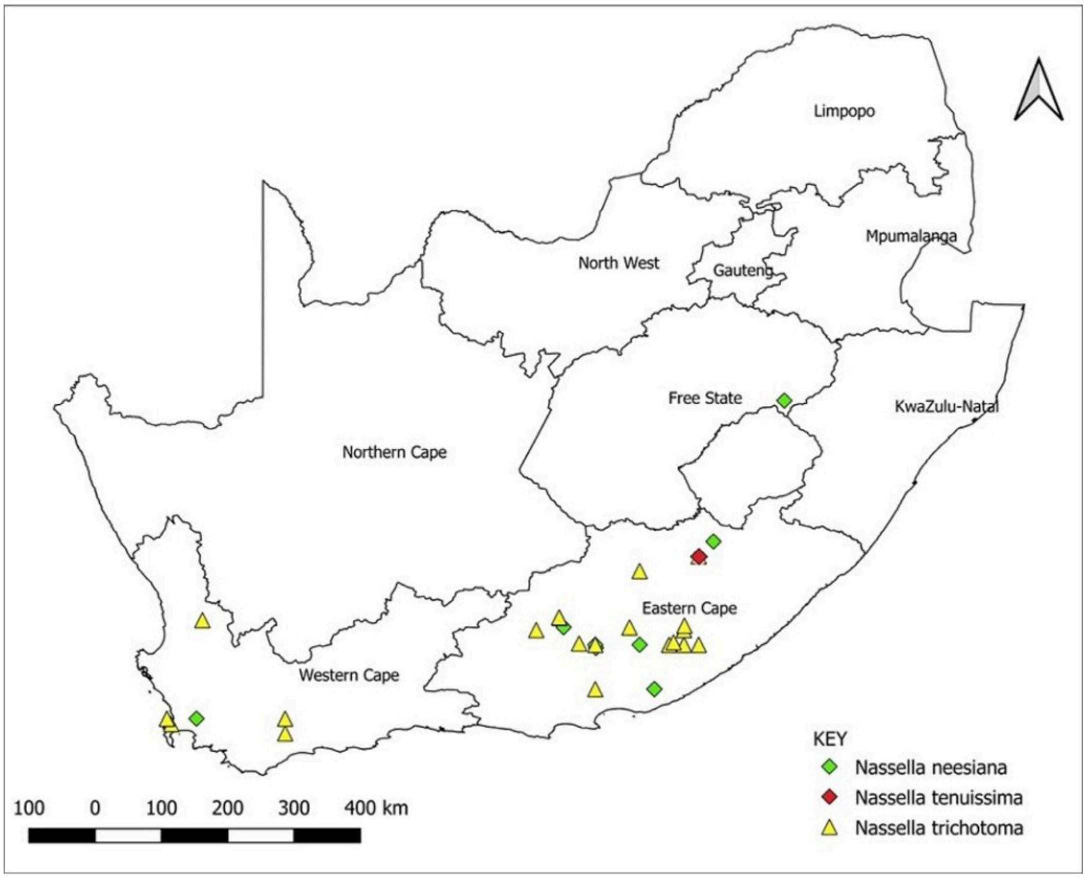


Fig. 1. The known distribution of *Nassella neesiana, N. tenuissima* and *N. trichotoma* in South Africa. Based on records from SAPIA, Fish et al. (2015), GBIF and Research Grade iNaturalist observations.

Table 1
Description of the defining features of three naturalised *Nassella* species in South Africa (Barkworth and Torres, 2001; Fish et al., 2015).

Character	*Nassella neesiana*	*Nassella tenuissima*	*Nassella trichotoma*
Synonyms	*Stipa neesiana* Trin. and Rupr	*Stipa tenuissima* Trin.	*Stipa trichotoma* Nees
Common names	Chilean needle grass	Mexican feather grass, white tussock, witpolgras	Nassella tussock; serrated tussock, Nassella-polgras
General	A perennial tussock forming grass, up to 1 m high.	A perennial tussock forming grass, up to 1 m high. Tussock distinctly white in winter. Culms slender and wiry.	A perennial tussock forming grass, up to 0.65 m high. Mature tussocks droop
Leaves	Flat, 300 mm long and 1-5 mm wide, strongly ribbed on adaxial surface with rough margins	Very tightly rolled inward, and up to 700 mm long and 0.5 mm wide, appear as thin wiry filaments which are rough to touch.	Tightly rolled, 80–500 mm long and 0.5 mm wide, hairless with very fine serrations. Rough when stroked downwards.
Ligules	Truncate with a few hairs	Acute	Obtuse
Inflorescence	Open panicle	Slender, compact panicle rarely extending above leaves.	Open, much-branched panicle, detaching at maturity, leaving the plants free of inflorescences for most of the year.
Spikelet	15–17 mm long (excluding awn)	4–5 mm long (excluding awn)	6–8.5 mm long (excluding awn)
Glume	Longer than lemma		
Lemma		Elliptic	Obovate
Awns	50–120 mm long, geniculate	45-90 mm long, unnoticeably geniculate, centrally placed on lemma	15–35 mm long, off centre of lemma
Time of flowering	November to March	January	August to March

accidentally introduced to South Africa by the British Army during the Anglo-Boer War (1899–1902), through the ports of East London and Port Elizabeth, possibly as seed in hay from Argentina for horse fodder (Wells, 1978; Henderson, 2018). Only *N. trichotoma* and *N. tenuissima* are currently listed as invasive species in category 1b of the National Environmental Management: Biodiversity Act (NEM:BA, 10/2004): Alien and Invasive Species (AIS) Regulations of 1 October 2014. These regulations stipulate that they cannot be traded or planted in any form and should be removed and destroyed wherever possible.

Research conducted on *Nassella* in South Africa in the 1970s and 1980s, especially by M.J. Wells, led to widespread awareness and many control campaigns. Effective enforcement of control of *Nassella* species ended in 2000 when the government subsidy stopped (Henderson, 2018). The termination of these subsidies and the coordinated control efforts also appears to have ended research efforts, the gathering of new information and formal reporting on *Nassella* invasions.

This paper collates all available information about *Nassella* invasions in South Africa and identifies research gaps. It specifically seeks to shed light on these questions:

What identification issues exist? What is the current spatial distribution of *Nassella*? What is the autecology of the genus? What are the social-ecological impacts of *Nassella*? What control measures are currently applied and what are their strength and limitations? What do we know about *Nassella* distribution and its response to climate change?

2. Materials and methods

The taxonomic delimitations used in this review of *Nassella* follow Barkworth and Torres (2001), supplemented with insights from Fish et al. (2015). Data were gathered from published and grey literature. Several methods were pursued in order to unearth as much literature as possible. These methods included searching repositories for the three species, following-up on literature cited in references, searching for articles by authors whose works were frequently referenced, and by consulting relevant research institutes in the country. Landowners, practitioners, and rangeland scientists also gave their own perceptions, thoughts and experiences during reconnaissance visits and informal interpersonal and email communications. These were also included, together with the observations and experiences of the authors when no published data could be found. *Nassella* research in South Africa effectively stopped in the 1980s and no recent scientific research could be found in the literature. However, Australia and New Zealand have similar socio-ecological systems to South Africa (Pyšek et al. 2020), and much research on the invasion ecology of the genus has been undertaken in these countries. This research was extensively consulted for this review, and the implications of this work were cautiously interpreted with reference to the South African context.

Distributional data were gathered from databases such as the Southern African Plant Invaders Atlas (SAPIA) (Henderson and Wilson, 2017), the Global Biodiversity Information Facility (GBIF) (Occdownload Gbif.Org, 2019), Botanical Dataset of Southern Africa (BODATSA) (Ranwashe, 2019) and iNaturalist records marked as Research Grade. These data were used to produce current distribution maps of the three *Nassella* species (Fig. 1). Unverified records, such as those given by word of mouth were not included. The distributional data were converted to degree decimals and plotted on a map using the Free and Open Source QGIS software.

3. Description

Nassella are best distinguished from other members of the tribe Stipeae by the strongly convolute lemma and short, glabrous palea (Barkworth and Torres, 2001). They are perennial tussock grasses with membranous ligules which are sometimes pubescent or ciliate. The inflorescences are panicles. The *Nassella* species themselves are similar and difficult to distinguish from each other, particularly outside the flowering season; this is especially problematic for *N. trichotoma* and *N. tenuissima* which have very similar ecologies and growth habits (Jacobs et al., 1998) (Table 1).

3.1. Similar species in the field

Nassella highlights the challenges presented by cryptic invaders that are not readily distinguishable from native grasses (Henderson and Wilson, 2017). They are very similar to native *Stipa* species (Connor et al., 1993; Global Invasive Species Database GISD, 2019) of which South Africa has four indigenous species, including the endemic *S. dregeana* Steud. var. *dregeana* (Ranwashe, 2019). As highlighted by Henderson (2018), *N. trichotoma* looks superficially like *Festuca caprina* and *Tenaxia stricta* and has been confused with these species even by experienced researchers. Henderson (2018) also reports that *N. trichotoma* often shares its habitat with *T. stricta* in rocky mountainous areas. The short, white, hairless ligule with a rounded apex found on *N. trichotoma*, differentiates it from *T. stricta* and similar tussock species which either lack the ligule or possess a ring of hairs around the top of the ligule (Henderson, 2018). As mentioned above, the three *Nassella* species are also difficult to distinguish from one another. This difficulty in species identification explains why no records are submitted through citizen science such as SAPIA (Henderson and Wilson, 2017). On 8 September 2020 there are 7 observations of *Nassella* species: 3 (2) for *N. neesiana*, 2 for *N. pulchra* and 2 (1) for *N. trichotoma* (numbers of "Research Grade" observations are in brackets). *Nassella pulchra* has not naturalized and is not known to be invasive.

4. Distribution and ecology

Nassella species are C_3 grasses, and are therefore generally restricted to temperate regions of the world where they inhabit a wide ecological range (Milton, 2004; Romaschenko et al., 2008). The species occur mainly in degraded pastures but also in natural grasslands and open woodlands (Henderson, 2001; Taylor et al., 2016). Temperature tolerance ranges from as low as −5 °C to about 25 °C and they can survive short periods of frost and ice cover (Healy, 1945; Bourdôt et al. 2012). They favour areas with annual rainfall between 300 and 800 mm (Healy, 1945; Wells and De Beer, 1987; Henderson, 2001). They are therefore better at tolerating droughts than most pasture species in areas where they occur (Wells, 1977; Miller, 1998; Grech et al., 2012). *Nassella* species have been shown to grow in a variety of soil types and fertility ranging from poor well-drained acidic soils (Campbell, 1998) to fertile soils (Wells and De Beer, 1987; Badgery et al., 2005). The distribution of the species is influenced by soil disturbance and reduction in native perennial grasses (Healy, 1945; Badgery et al., 2005).

In South Africa *Nassella* is restricted to the montane grasslands of the Eastern and Western Cape mountains (Fig. 1).

Nassella neesiana is native to Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, Peru, and Uruguay (Barkworth and Torres, 2001; Soreng et al., 2009). It has naturalised in Australia, Europe, New Zealand, and South Africa (Barkworth and Torres, 2001; Fish et al., 2015; Henderson, 2018; Howell and Sawyer, 2006; Verloove, 2005). While it is considered a serious invader in Australia and New Zealand (Howell and Sawyer, 2006; Henderson, 2018; Ranwashe, 2019), it is not currently listed in the NEM:BA regulation although it could be a threat in South Africa judging from the distribution pattern shown on Fig 1. It is known to have naturalised in the Eastern Cape, Western Cape and the Free State provinces (Fig. 1) at altitudes between 600 m and 1700 m (Germishuizen and Meyer, 2003).

Nassella tenuissima is native to Argentina, Chile, Mexico, and USA (Barkworth and Torres, 2001; Jacobs et al., 1998; Soreng et al., 2009). It has become naturalised in New Zealand, Australia, Europe, South Africa and the USA (outside the natural range) (Barkworth and Torres, 2001). *Nassella tenuissima* has been widely distributed mostly as a result of its value as a horticultural grass. In South Africa, the species has so far only been recorded from a single locality in the Eastern Cape Drakensberg Mountains (Fig. 1) (Henderson, 2001; Fish et al., 2015; Ranwashe, 2019). A search in February 2020, by a team of grass taxonomists and one of the authors could not locate the species. While in other parts of the world it has been recorded at altitudes ranging from near sea level to 2900 m (Jacobs et al., 1998; Occdownload Gbif.Org, 2019), the recorded populations in South Africa occur at altitudes between 2000 and 2600 m (Germishuizen and Meyer, 2003) and only in grassland (Henderson, 2007).

Nassella trichotoma is native to Argentina, Bolivia, Brazil, Chile, Peru and Uruguay (Wells, 1977; Westbrooks and Cross, 1993; McLaren et al., 1998; Barkworth and Torres, 2001). It is invasive in Australia, New Zealand and South Africa (McLaren et al., 2004; Howell and Sawyer, 2006; Henderson, 2018; Ranwashe, 2019), with some invasive populations in parts of Europe (Campbell, 1982; Barkworth and Torres, 2001). In South Africa, it has been recorded as being invasive in the Western, Eastern and Northern Cape provinces (Fig. 1), and there are unconfirmed records from Limpopo, Gauteng, Free State and Mpumalanga (Ranwashe, 2019). By 1985 it had invaded about 70 000 ha with just over 9000 ha of this being moderately to heavily invaded (Wells and De Beer, 1987). Wells and De Beer (1987) projected that by the mid-2020s about 2 million ha of South Africa could be invaded. The possibility of this projection coming true is unknown as data on the extent of invasion are not available. It has been recorded at altitudes between 100 and 1600 m (Germishuizen and Meyer, 2003). It is commonly encountered in savanna and grassland but also occurs in fynbos, Nama-karoo and succulent karoo vegetation types (Henderson, 2007).

5. Reproduction, dispersal and germination

5.1. Reproduction

Nassella species appear to rely exclusively on sexual reproduction via seeds (Campbell, 1982) which are produced in large quantities. For example, *N. trichotoma* has been shown to produce as many as 100 000 seeds per plant per year (Wells, 1977; Wells, 1978; Campbell, 1982; Wells and De Beer, 1987), giving an estimated production of 900–3400 million seeds per hectare in heavily invaded areas (Healy, 1945).

Nassella neesiana and *N. tenuissima* produce chasmogenes ('normal seeds') and cleistogenes (seeds produced in closed stem flowers), and can also self-pollinate (Connor et al., 1993; Jacobs et al., 1998; Faithfull et al., 2012). The cleistogenes are reportedly more common when the production of chasmogenes is suppressed, such as during prolonged mowing and overgrazing (Kriticos et al., 2010). Cleistogamy ensures that the species persist and keeps the seed bank replenished during those periods when the production of inflorescences is subdued.

5.2. Dispersal

Seeds can be dispersed over long distances, especially in the case of *N. trichotoma* whose seeds are borne on inflorescences that detach readily when seeds mature and can be blown for great distances, as much as 16–30 km per day depending on wind speed and physical impediments (Wells, 1978; Campbell, 1982; Joubert, 1984). However, *N. neesiana* and *N. tenuissima* are usually only dispersed for short distances because their seeds remain attached to the plant. This could explain why *N. tenuissima* seems not to have spread from the single locality from where it has been reported in the Eastern Cape.

Seeds are also dispersed by many other agents such as agricultural machinery, floodwaters, in mud or soil, clothing, trains, and cars (Wells, 1978; Campbell, 1982; Cooperative Research Centres, 2003). Seeds can pass through the digestive system of cattle (Healy, 1945) and sheep (Cook, 1998) unharmed and thus can be spread in animal faeces. However, the consumption of *Nassella* by livestock is limited and mostly occurs by accident during the process of grazing more palatable forage (Bläßet et al., 2010). Seeds can also be spread through silage and hay if proper care is not taken. This is particularly important in cases where fodder has to be moved large distances to feed livestock due to a lack of available forage. (Weller et al., 2016, Weller et al., 2016).

5.3. Germination and growth

Most of the seed bank for *Nassella* species is found in the top 2.5 cm of the soil; seeds close to the soil surface lose viability more rapidly than seeds buried deeper, and deep-buried seeds experience stronger dormancy (Bourdôt and Hurrell, 1992; Faithfull, 2012; Joubert, 1984). Once in the soil the seeds of *N. trichotoma* can lie dormant for up to 20 years (Wells, 1977; Wells, 1978; Wells and De Beer, 1987). However, the viability of seeds declines quickly. Taylor (1987) found that after 6 months viability declined to between 20 and 61% under field conditions in New Zealand. This is not the case with all *Nassella* species; for example *N. neesiana* seeds lose viability at a rate of 38% per annum (Connor et al., 1993) and so may not last for more than a few years in the soil (Bourdôt and Hurrell, 1992). The soil seed bank can be immense, ranging from 1700 to more than 42 000 seeds/m^2 in New Zealand (Healy, 1945) and up to 75 000 seeds/m^2 for *N. trichotoma* in South Africa (Joubert, 1984) although they are normally found in much lower quantities with a high proportion showing damage from factors such as animal trampling and insect predation and hence probably not viable (Joubert, 1984). While *N. trichotoma* seeds are dispersed everywhere, seedlings do not take hold in healthy native grasslands, possibly due to shading (Campbell, 1982; Joubert, 1984; Faithfull, 2012). Germination or survival of seedlings is probably prevented by shading, with evidence of higher germination under unshaded conditions in undamaged seed (Taylor, 1987). Most seeds that germinate do not survive the first summer due to competition for moisture with established plants (Badgery, et al. 2008). These two mechanisms work together to ensure that *N. trichotoma* does not establish easily in dense native grasslands. Some stakeholders in the Eastern Cape have pointed out that *N. trichotoma* invasions in South Africa start from a point of initial establishment and expand outwards from that point as new plants establishing themselves close to already established plants (Fig. 2). This suggests that most of the seeds fall close to parent plants even though they have the potential to be dispersed further.

Seeds can germinate at any time of the year, but mostly in autumn and winter in South Africa (Joubert, 1984). Germination appears to be regulated by rainfall and temperature, with low temperatures and droughts limiting germination (Bourdôt and Hurrell, 1992). Seeds germinate over a wide range of conditions and over a long period (Healy, 1945), probably as a result of dormancy mediated by the permeability of testa (Campbell, 1998). The need for dormancy is demonstrated by the fact that fresh seeds show lower germination rates compared to seeds set in the previous years (Lamoureaux and Bourdôt, 2002; Lamoureaux et al., 2006). Removing the testa and cutting off the awn-end of seed increases germination rate in the seeds (Campbell, 1998).

Nassella trichotoma grows more slowly than desirable pasture grasses and also flowers several years after germination (Campbell, 1998). Research in New Zealand has shown that tussock expansion is much faster than previously believed but is rarely noticed until flowering starts (Bourdôt and Saville, 2019). The seeds of *N.*

Fig. 2. A *Nassella trichotoma* patch (tufted grasses in background) expanding in an overgrazed paddock in the Boschberg near Somerset East, Eastern Cape, South Africa.

trichotoma are viable well before the panicles extend above the tussock canopy (Lamoureaux and Bourdôt, 2002; Lamoureaux et al., 2006). It produces a very strong rooting system, reaching depths of 20 cm, which makes uprooting difficult even for young plants (Wells, 1977; Wells and De Beer, 1987).

Nassella neesiana, on the other hand, grows so rapidly that it is often difficult to distinguish the seedling and juvenile stages (Faithfull, 2012). Seeds can germinate and plants mature and produce viable seeds within a single year (Bourdôt and Hurrell, 1992; Faithfull, 2012). On poor soils, however, flowering may be delayed until the third or fourth year due to poor vegetative growth (Campbell, 1998). The fast growth rate ensures that the plants outcompete all other grasses and occupy open spaces rapidly. Moretto and Distel (1998), reported that *N. tenuissima* is a poor root and shoot competitor, and so establishes and thrives in vegetation gaps where there is no competition from other grasses, and where seed dormancy is broken by fluctuating temperatures. They also noted that germination was concentrated during the rainy season since the seeds are highly susceptible to desiccation.

6. Uses

Since their accidental introduction into South Africa, *Nassella* species have not been used for anything. Several uses have, however, been noted around the globe, especially in the native range of the taxa. All species are occasionally grazed by goats and cattle when they are still young and in the active vegetative growth stage (Campbell, 1982; Westbrooks and Cross, 1993). *Nassella tenuissima* is also used as an ornamental grass in some countries, but this use is illegal in many countries due to the possibility of it escaping and becoming invasive. In Argentina, *N. tenuissima* is reportedly used for thatching (Global Invasive Species Database GISD, 2019).

7. Impact

Research to date suggests that invasive alien grasses generally have a greater socio-economic impact than environmental impact in South Africa (Visser et al., 2017; Nkuna et al., 2018). However, since the majority of recorded impacts are on crop and animal production there is a bias towards socio-economic data (Nkuna et al., 2018). The abundance of *Nassella* plants can increase rapidly, leading to them assuming dominance over whole pastures, completely displacing both planted and natural grass species (Fig. 3) (Faithfull 2012). Of the three *Nassella* species, *N. trichotoma* is the most devastating and widespread in South Africa, and known to completely smother even planted pastures (Wells, 1977). Its feat as an invader led Wells (1977) to conclude that: '…this is almost the perfect weed, a beautifully adapted, highly efficient organism: a self-perpetuating factory of valueless fibres.'

7.1. Environmental impact

There is a general lack of data on the environmental impacts of *Nassella* species, especially in South Africa (Visser et al., 2017). In general, *Nassella* invasions are associated with reduced invertebrate diversity, altered fire regimes, and the suppression of co-occurring native grasses (Faithfull, 2012). *Nassella neesiana*, and possibly all *Nassella* species, has an increasing impact on native grass diversity as its residence time increases (Faithfull, 2012). It has also been reported that even though numerous native generalist insect species consume it in Australia (Faithfull, 2012), it significantly reduced invertebrate abundance, composition, and richness compared to native-grass dominated areas (Faithfull, 2012). However, much of the loss of diversity in invaded areas probably precedes invasion caused by anthropogenic disturbances such as mowing, major soil disturbance, and previous control with herbicides (like flupropanate or glyphosate) that also kill many of the native species (Faithfull, 2012).

Invasion of *N. trichotoma* has been reported to be associated with altered fire regimes, for example in the Geelong region of Victoria, Australia, invaded stands resulted in extending the fire season by as much as two months (McLaren et al., 2004). The altered fire regime has major impacts on the regeneration of native species through changing the soil carbon and nitrogen status and soil microbial structure and function (Dickens and Allen, 2014).

Fig. 3. A paddock invaded by *Nassella trichotoma* on the Boschberg near Somerset East, Eastern Cape Province, South Africa (Photo A. Mapaura).

Soil seed banks in areas invaded by *Nassella* species include seeds of other species, both native and non-native, but many of these species are not represented in the above-ground plant assemblage (Joubert, 1984; Wells and De Beer, 1987; Faithfull, 2012). Joubert (1984) suggested that this could be due to allelopathic interactions, but no research has been done to confirm this type of interaction. However, it is not clear that all invasive populations of *Nassella* have such an effect; for example, *N. trichotoma* in natural montane grasslands in the Eastern Cape co-occurs with several species of native grasses (A. Mapaura, pers. obs.). The non-germination of indigenous grasses in densely invaded patches of *Nassella* could also be a result of the dense cover of *Nassella* which prevents light from reaching the soil to stimulate seed germination, and also competition for other resources, particularly water. The effect of canopy cover is more likely considering that after clearing the *Nassella* plants seeds of other native plant species are able to germinate and establish (Joubert, 1984). The mechanisms whereby *Nassella* impacts native species are not understood, although much research is currently underway, especially in Australia and New Zealand. Research is needed to determine the factors leading to the suppression of native grasses in *Nassella*-invaded patches.

7.2. Socio-economic impact

In pastures the more palatable grasses and plants are generally smothered by less palatable and poisonous species as the former are preferentially eaten by animals (Wells et al., 1983). This progressively reduces the quality of the pastures, impacting negatively on the pastures' carrying capacities (Viljoen, 1999; Richardson et al., 2011; Nkuna et al., 2018). As palatable pasture plants decrease, inedible and indigestible plants such as *Nassella* species increase, reducing native grass and forb richness by as much as 50% (Morgan, 2001; Faithfull, 2012). *Nassella* species have been reported to impact native species more than other non-native species present in an area where they invade (Faithfull, 2012).

Nassella species are not palatable and hence increase as the palatable grasses decrease in pastures, especially where there is bad pasture management (Wells, 1977; Wells and De Beer, 1987). *Nassella trichotoma* has a very high fibre content (as high as 86%) and very low nutritive value (protein content as low as 4%), making it non-nutritional hence seldom grazed by animals (Wells and De Beer, 1987). Furthermore, if animals are forced to graze it, the fibres, which are indigestible, can form boles in the rumen, causing rapid loss of health, which may lead to death (Wells and De Beer, 1987; Campbell, 1998). This reduction in pasture quality can be massive, leading to sub-economic carrying capacities (Campbell, 1998; McLaren et al., 2004; Anderson et al., 2010). In New Zealand, carrying capacity reductions of up to 90% were reported on some farms, leading to the abandonment of sheep farming (Healy, 1945). In worst case scenarios in New South Wales, Australia, some farms were so heavily invaded that the cost of control was higher than the value of the farm, leading farmers to abandon farming or to sell their farms and seek alternative employment (Campbell, 1998). Some farmers in the Eastern Cape, South Africa, have lost the use of part of their farms to *Nassella* invasion (A. Mapaura pers. obs.).

In monetary terms, losses can be huge. Productivity losses and management costs were estimated to range from as little as $ AUD7.00 ha^{-1} for light-invaded areas controlled by hand to $ AUD122.00 ha^{-1} for heavy invasions in SE Australia in the early 1980s (Vere and Campbell, 1984). Most of the losses were suffered by the wool and lamb industries due to reduced carrying capacity on invaded farms (Vere and Campbell, 1984). Young animals, especially lambs, can have their skin or eyes damaged by the sharp awns, while the awns are difficult to remove from wool and reduce its quality (Anderson et al., 2010; Campbell, 1998).

8. Management

8.1. General

South Africa seems to be less susceptible to invasion by non-native grasses than many other parts of the world (Visser et al., 2016). Nonetheless, invasions of several species of non-native grasses are having major, and rapidly growing, impacts on human livelihoods and biodiversity. Several researchers have made urgent calls for more effective management of invasive grasses, including *Nassella*, in South Africa (Wells et al., 1983; Milton, 2004; Henderson and Wilson, 2017; Visser et al., 2017; Henderson, 2018; Nkuna et al., 2018). The spread of invasive alien species is facilitated by the increasing global movement of people and goods, while anthropogenic environmental changes facilitate their establishment and proliferation (Early et al., 2016). In light of limited resources and a multiplicity of competing needs, it is important to evaluate both monetary and non-monetary benefits of all available control methods before embarking on any action, or deciding to take no action (Bourdôt et al., 2015; Hanley and Roberts, 2019).

The ability to control any invasive species depends on the ability of managers to identify it correctly at every stage of its growth and their ability to notice it before it has become a problem (Wells, 1977; Wang et al., 2016). However, as discussed above, *Nassella* species are cryptic; their presence is often noticed after the species are well established and are causing dramatic impacts (Wells, 1977; McLaren et al., 2004; Smith and Lamoureaux, 2006). Late flowering in *N. trichotoma*, for example, makes early detection difficult since the species is easily distinguishable when in flower, meaning that by the time they are detected they will be already established and difficult to control (Campbell, 1998; Smith and Lamoureaux, 2006).

Following the pioneering work of M.J. Wells and others in the 1970s and 1980s, *N. trichotoma* and *N. tenuissima*, were listed as invasive species in South Africa, and were placed in category 1b in the NEM:BA regulations. Vigorous awareness and control campaigns were launched by the Department of Agriculture in the 1980s and 1990s. The Government provided landowners with task teams and subsidised herbicides (Henderson, 2018). However, all progress made with tackling these invasions was lost when the subsidy was removed, and the herbicide flupropanate was withdrawn from sale in 1997; government-driven control and control enforcement thus effectively stopped. Since then there has been no centrally coordinated effort to control *Nassella* invasions in South Africa, and farmers have borne the burden of addressing these invasions alone, often leading to individual farmers working independently of their neighbours (Henderson, 2018), or not at all. The ideal control measure will be one that reduces the soil seed bank, while at the same time minimising re-invasion from neighbouring areas (Joubert, 1984; Bourdôt and Hurrell, 1992). To prevent re-invasion, an area-wide control system is crucial, to ensure that neighbouring property owners and managers cooperate to deal with the invasion (Miller, 1998).

8.2. Cultural control

Nassella grasses are unpalatable – most animals will not ordinarily graze them (Wells and De Beer, 1987; Anderson et al., 2006); this greatly reduces the potential for using livestock to control *Nassella* invasions (Grech et al., 2012). Goats have, however, been reported to graze actively growing low *Nassella* weed population densities of less than 20% ground cover but due to low nutritive value grazing is avoided unless there are no other options (Campbell, 1982; Westbrooks and Cross, 1993). *Nassella neesiana* reportedly produce good feed in the early vegetative stage and so livestock can be used to reduce their productivity (Westbrooks and Cross, 1993; Miller, 1998; Cooperative Research Centres, 2003). However, *N. neesiana* has a very short juvenile stage which means that it is available for grazing for a very brief period (Faithfull, 2012). To reduce the potential of spreading the seeds through silage, the silage can be quarantined for at least 6 weeks to ensure that the seeds are not viable and also to diminish the sharpness of the seeds which can be injurious to the intestinal lining of animals (Weller et al., 2016).

Afforestation has been used in Australia and New Zealand to control *Nassella* especially in cases where agriculture was no longer possible due to heavy invasions (Healy, 1945; Campbell, 1982; Miller, 1998; Jones et al., 2000). However, it takes up to six years to shade-out the weed and prevent flowering, and another four years before the tussocks die (Wells, 1977; Campbell, 1982; Miller, 1998). It would take many more years for the seedbank to be depleted. To speed up the establishment of tree seedlings, flupropanate can be sprayed to kill *Nassella* without affecting the tree seedlings (McLaren et al., 2008). As noted, before, *Nassella* species invade grassland ecosystems, and afforestation is not always possible, practical nor desirable in these ecosystems. Tree species that could potentially be used for such purposes are mainly non-native species that themselves cause major problems as invasive species (Richardson et al., 2020). All these reasons make afforestation an unsuitable control option for South Africa.

Native species may lack or have transient seed banks which limits their recruitment and reestablishment, hence are not efficient at naturally colonising open spaces (Morgan, 2001). Experiments in Australia concluded that native grasses have low survival rate and natural densities making them poor competitors against *Nassella* species. (Morgan, 2001). Seeding of pastures with native grass species after *Nassella* removal has, therefore, been suggested as a possible solution to cover the bare ground left after *Nassella* removal (Miller, 1998; Cooperative Research Centres, 2003). However, seedlings have little to no effect in smothering *Nassella* since most of the native grasses grow much slower than *Nassella* (Faithfull, 2012; Grech et al., 2012). Unlike most C_3 grasses, *Nassella* species. are more drought hardy and tolerate poor soils better than most good pasture grasses, hence for an effective seeding method to work a grass with similar or better traits has to be found to suppress *Nassella* both in the short and long term (Miller, 1998; Grech et al., 2012).

Burning the dead organic material on the soil surface may destroy between 18% to 20% of the *Nassella* seeds present in the top soil (Joubert, 1984; Faithfull, 2012), suggesting that this treatment may be used to reduce the seed bank, bearing in mind that fire also stimulates seed germination. However, although *Nassella* grasses burn readily, they also recover quickly especially if the tuft is not burnt completely, while associated native species are often killed (Healy, 1945). Fire also reduces the seed bank of good pasture species in the soil leaving the ground bare and creating ideal conditions for reinvasion by *Nassella* species (Campbell, 1982; Joubert, 1984; Wells and De Beer, 1987). Burning as a control measure may, therefore, inadvertently increase rather than decrease *Nassella* populations. More research is required to ascertain how different regimes of burning affect *Nassella* invasion dynamics (Badgery, 2004). *Nassella* often invades fire adapted natural grassland ecosystems dominated by *T. triandra*, in both Australia and South Africa (Faithfull, 2012; Faithfull et al., 2012) which makes fire exclusion as a control method unviable. The hot fires that would eventually result after years of fire exclusion, are often not a problem for *Nassella*, which always has some seeds left unburnt due to high seed bank, and seeds can be reintroduced by wind from distant areas and take advantage of the reduced cover (Healy, 1945; Badgery, 2004; Faithfull, 2012).

8.3. Mechanical control

Small, low-density patches and isolated plants can be controlled by hand weeding or digging when equipment is cleaned meticulously and the clippings are destroyed (Healy, 1945; Wells, 1977; Cooperative Research Centres, 2003; Taylor et al., 2016). However, the potential exists for exacerbating the invasions (Faithfull 2012; Taylor et al., 2016). These methods are of little use in natural grasslands where native grasses such as *T. triandra* have vulnerable growing points and lack the ability to grow horizontal tillers compared to *Nassella* (Faithfull et al., 2012). Grubbing as a management tools cannot eradicate *Nassella* but can reduce densities or keep population levels static (Bourdôt et al., 1992; Smith and Lamoureaux, 2006). Grubbing was also found to be more effective against medium sized tufts as opposed to large and small plants but more research is required to determine the best time to perform it based on *Nassella* population dynamics (Smith and Lamoureaux, 2006; Bourdôt and Saville, 2019).

Cultivating or ploughing has been suggested as a possible control mechanism for managing *Nassella* invasions (Wells, 1977). *Nassella* seeds are concentrated in the top 2.5 cm of the soil (Joubert, 1984; Bourdôt and Hurrell, 1992; Faithfull, 2012), so ploughing has the potential to bury them to depths they cannot emerge from (Campbell, 1982), but this has to be done with care since the seeds can attain a state of permanent dormancy (Bourdôt and Hurrell, 1992). However, ploughing may inadvertently exacerbate the problem by bringing deep-buried seeds to the surface where they can germinate (Wells, 1977; Westbrooks and Cross, 1993). For this method to be successful, ploughing must be done when the soil is dry and must be repeated every year for many consecutive years until the soil seed bank has been exhausted (Wells, 1977; Bourdôt and Hurrell, 1992). In Australia, cultivation has been used successfully in areas where the land is arable and soil fertility is reasonable. Foraging crops are planted for 2-3 years to reduce the soil seedbank, followed by sowing of competitive pasture species (Campbell, 1998). However, *Nassella* is often found in areas which are rocky and difficult to access, making ploughing futile (Grech et al., 2012). In natural rangelands, such as those being invaded by *Nassella* in South Africa, ploughing is undesirable as it would have major impacts on forb diversity (which is the richest component of grassland flora).

8.4. Chemical control

Several herbicides have been used to manage *Nassella* invasions in different parts of the world. Most of the herbicides are based on formulations that include glyphosate or flupropanate as active ingredients (Viljoen, 1987; Wells and De Beer, 1987; Grech et al., 2012). Flupropanate has been championed as the panacea for *Nassella* invasions for many years because it was shown to have over 90% efficiency in killing *Nassella* over a wide range of environmental conditions (Wells, 1977; Viljoen, 1987; Campbell, 1998; Viljoen, 1999; Conolly and Taylor, 2016). Although it can take more than a year to kill the plants completely, it is easy to use, is non-toxic to mammals and was once considered highly selective, especially when applied to young *Nassella* plants (Viljoen, 1987; Wells and De Beer, 1987; Campbell and Ridings, 1988). However, recent research shows that it is not as specific as previously thought as it impacts many native species (Campbell and Van de Ven 1996; Grech et al., 2014; Lusk et al., 2017). Glyphosate has also been used extensively and has been shown to be effective against seed-head production (Campbell, 1998), but it is a broad spectrum herbicide that is indiscriminate in its impacts on non-target species (Grech et al., 2012). While glyphosate and flupropanate are effective independently, their effectiveness will be reduced if they are mixed Taskforceherbicide, 2020).

Viljoen (1987) found that flupropanate was effective at lower rates than recommended with less effect on non-target species, which also greatly reduced costs (Viljoen, 1987; Wells and De Beer, 1987; Campbell and Ridings, 1988; Campbell, 1998). Other researchers also reported that once applied, the chemical has a long residual action that prevents germination and establishment of seedlings for 3–5 years, (Viljoen, 1987; Campbell, 1998; Viljoen, 1999). However, recent studies seem to suggest that these results may not always hold true. For example, flupropanate has been shown to negatively impact beneficial pasture grasses and forbs even at the recommended dosage (Grech et al., 2014; Lusk et al., 2017). Furthermore, research in New Zealand showed that the residual effect was shorter than widely believed (Bourdôt et al., 2017).

Application of flupropanate was found to increase both bare ground and broad leaf weeds especially in grazed areas (Wells and De Beer, 1987; Grech et al., 2014; Lusk et al., 2017). This can lead to reinvasion by *Nassella* once the residual effect has diminished (Badgery et al., 2008; Lusk et al., 2017) and so follow-up spraying is required (Grech et al., 2012). However, follow-up spraying would have to be done over many years to deplete the seedbank, making re-spraying uneconomical (Viljoen, 1987). The increase in bare ground, (Fig. 4), and in broadleaved species reduces pasture palatability and quality (Grech et al., 2014); this could be addressed by seeding treated areas with productive pasture species.

An increasing number of *N. trichotoma* populations are reportedly becoming resistant to flupropanate in Australia due to continuous use of the herbicide over long periods (McLaren and Anderson, 2011; ; Powells, 2018). Flupropanate resistance in South Africa has not yet been investigated.

8.5. Biological control

There has been a perception that the uniform and simplistic architecture of grasses and lack of or limited secondary metabolites in grasses makes polyphagy common, limiting the use of classical biological control (hereafter referred to as biocontrol) for grasses in general (Evans, 1991). A further deterrent has been the concern over potential non-target damage on important grass crops (Briese et al., 2000). However, a recent study by Sutton et al. (2019) has demonstrated that many grasses have suitably specific and damaging herbivores to warrant consideration for biocontrol.

A biocontrol programme was initiated on *Nassella* in South Africa and the first surveys for herbivores were done by Wells (1977) in the native range in Argentina looking for potential biocontrol agents to be released on *N. trichotoma*. This survey determined high levels of herbivory, and a follow up survey by Erb (1988) identified ten species of weevil (Coleoptera) and two moth species (Lepidoptera). However, none of these herbivores were found to be suitably host-specific and this programme was discontinued.

Since then, research into biocontrol on *Nassella*, specifically *N. neesiana* and *N. trichotoma*, has been undertaken in Australia and New Zealand. Surveys in the native range in Argentina since 1995 have found an additional 22 potential agents (Table 2). To date, only one of these agents, a fungus (*Uromyces pencanus*), has been approved for release after completion of host-specificity testing and is due for release in New Zealand pending export permits (Anderson et al. 2017). Many of the potential agents on *Nassella* have been found to be host-specific to each species and therefore each *Nassella* species will likely require its own biocontrol agent.

8.6. Integrated control

Integrated control of invasive plants involves a combination of at least two primary control methods *viz*. cultural, mechanical, chemical, and biological. For many weed species, the best control is achieved when a combination of control measures is used (Cooperative Research Centres, 2003).

Wells (1978), working in South Africa, detailed an integrated strategy based on prevention of seeding by removing and burning seed heads, killing mature tussocks by chipping, ploughing or spot-spraying and prevention of seed dispersal by planting windbreaks, keeping stock away at seeding time, maintaining a good plant cover, particularly on the veld, by not burning. This strategy is essentially the same as that advocated for Australia (Campbell, 1998; Miller, 1998), where it has been concluded that a plan to limit seed reinvasion must be in place to support other control measures, such as using appropriate growing regimes, fertilisers and spot-spraying. Underpinning this integrated control tactic is the realisation that a competitive environment needs to be maintained to prevent (re) invasion (Wells, 1978; Campbell, 1998).

Fig. 4. A patch of *Nassella trichotoma* treated with aerial spraying of flupropanate in the Boschberg near Somerset East, Eastern Cape Province, South Africa showing the bare ground left as *Nassella* dies (Photo: A. Mapaura).

Because of the difficulty and poor performance of most conventional control methods, research into management systems that utilises *Nassella* but minimises its spread needs to be pursued (Grech et al., 2012). To counter and reduce incidences of chemical resistance, integrated approaches which utilise different chemicals for short periods coupled with mechanical and cultural methods would be desirable (Wells, 1977; Powells, 2018). A multi-faceted, multi-disciplinary *Nassella* control program involving biological,

Table 2
Potential and tested biocontrol agents against *Nassella* weeds.

Biocontrol agent	Type of agent	Target *Nassella* species	Notes and References
Alternaria sp. (Pleosporaceae)	Fungus	*N. trichotoma*	Not tested (McLaren and Cowan, 2012)
Ascochyta leptospira var.*variispora* (Didymellaceae)	Bacteria	*N. trichotoma*	Potential (Briese and Evans, 1998; Hussaini et al., 2000)
Corticiaceae sp.	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998; Anderson et al., 2002; McLaren and Anderson, 2011; McLaren and Cowan, 2012)
Dinemasporium sp. (Chaetosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Hussaini et al., 2000)
Epicoccum sp. (Didymellaceae)	Fungus	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Fusarium oxysporum (Nectriaceae)	Fungus	*N. trichotoma*	Not effective (Hussaini et al., 2000; McLaren and Cowan, 2012)
Hendersonula sp. (Botryosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Mucor sp. (Mucoraceae)	Fungus	*N. trichotoma*	Not tested (McLaren and Cowan, 2012)
Paratrichodorus sp. (Trichodoridae)	Nematode	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Phytophthora cryptogea (Pythiaceae)	Fungus	*N. trichotoma*	(McLaren and Cowan, 2012)
Puccinia graminella (Pucciniaceae)	Fungus	*N. neesiana*	Not effective (Anderson et al., 2011, 2006)
Puccinia nassellae (Pucciniaceae)	Fungus	*N. neesiana, N. trichotoma*	Not host specific and not sufficiently pathogenic (Anderson et al., 2002; Anderson et al., 2011, 2006; McLaren and Anderson, 2011; McLaren and Cowan, 2012)
Puccinia saltensis var. *saltensis* (Pucciniaceae)	Fungus	*N. neesiana, N. tenuissima*	Potential (Briese and Evans, 1998)
Rhizoctonia sp. (Ceratobasidiaceae)	Fungus	*N. trichotoma*	Not tested (Briese and Evans, 1998; McLaren and Cowan, 2012)
Rotylenchus sp. (Hoplolaimidae)	Nematode	*N. trichotoma*	Not host specific (McLaren and Cowan, 2012)
Septoria sp. (Mycosphaerellaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Stagonospora sp. (Phaeosphaeriaceae)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Tranzscheliella spp. (Ustilaginaceae)	Fungus	*N. trichotoma N. neesiana*	Not sufficiently pathogenic (McLaren and Anderson, 2011; McLaren and Cowan, 2012)
Uredo sp. (*incertae familiae*)	Fungus	*N. trichotoma*	Potential (Briese and Evans, 1998)
Uromyces pencanus (Pucciniaceae)	Fungus	*N. neesiana*	Approved for release in New Zealand (on 22 June 2011) pending export permit. Australia requires more host range tests before introduction and release permit can be given. (Briese and Evans, 1998; Anderson et al., 2006, 2010, 2011, 2017; Flemmer et al., 2010)
Ustilago hypodytes (Ustilaginaceae)	Fungus	*N. neesiana, N. trichotoma*	Not specific (Briese and Evans, 1998)
Ustilago sp. (Ustilaginaceae)	Fungus	*N. trichotoma*	More research needed (Briese and Evans, 1998; Anderson et al., 2002)

ecological, economic, social and legal issues will probably be the most effective (Anderson et al., 2003; Early et al., 2016).

9. Climate change and projected future distribution

The frequent droughts and extreme weather events such as cyclones and extreme temperatures being witnessed in southern Africa are the manifestation of climate change (Chersich and Wright, 2019). These weather events provide invasive plants with opportunities for dispersal and growth as native species can be negatively impacted (Masters and Norgrove, 2010). With frequent droughts, grazing pressure will remove good pasture species at a rate higher than recruitment which could favour non-palatable invasive species like *Nassella* (Faithfull, 2012; Grech et al., 2012; Morgan, 2001).

South Africa is in a unique situation in that the effects of climate change are taking place concurrently with socio-economic transformation coupled with rapid change in land use brought by majority rule (Richardson et al., 2000). The effects of climate change maybe more rapid and pronounced in such a situation than would otherwise be the case. This will have serious implications on species distribution and is of particular interest in the future control of invasive species such as *Nassella*. Global distribution models for *N. neesiana* and *N. trichotoma* have been done using CLIMEX models but not for *N. tenuissima*.

9.1. *Nassella neesiana*

Bourdôt et al. (2012), produced a CLIMEX model of the projected global distribution of *N. neesiana*. This model showed that the distribution will vary between countries and continents. In the Northern Hemisphere, suitable areas will generally expand with some current suitable areas becoming less suitable while in the Southern Hemisphere there is a general decline in suitable areas. In South Africa, suitable areas are expected to shrink marginally with the majority of the area remaining suitable. More importantly, *N. neesiana* has not yet saturated its current suitable area, and apparently currently occupies a small portion of it.

9.2. *Nassella trichotoma*

A model of suitable geographic range for *N. trichotoma* under current climate conditions indicates that there is great potential for expansion (Watt et al., 2011). An estimate of the global potential distribution under projected future climate change scenarios to 2080, using CLIMEX showed a general increase in Europe and a decrease in the Southern Hemisphere. For South Africa, the projections show the suitable area marginally decreasing and Lesotho remaining optimal (Watt et al., 2011). The trend is that the lower reaches of the mountains will become unsuitable while areas higher up will remain suitable.

10. Discussion

Our review has highlighted the invasive nature, ecology and major impacts that *Nassella* invasions cause in terms of biodiversity, soil productivity, and the economy based on both local and foreign literature. Dense invasions of *Nassella* could lead to huge losses and costs to the animal husbandry industry, tourism, and severe impacts on biodiversity in the temperate montane grassland regions of South Africa. Globally, mountain areas are very important and contribute immensely to biodiversity and are already under pressure from population expansion and so cannot afford the threat from these invasive species (Rahbek et al., 2019). In South Africa, these mountains are home to many endemic, range-restricted and threatened species, and the continued expansion of these invasive grass species will likely contribute to their decline. The need to understand the status of these species in South Africa and to institute control measures is important and urgent.

A major problem is that *Nassella* species are difficult to differentiate from several native tussock species and from each other. It is very likely that some of the literature on *N. trichotoma* could actually be mistakenly referring to other *Nassella* species as very little information was found for the other two species. The available identification keys rely heavily on the presence of inflorescences. The problem is that when the inflorescence appears the seeds are mature, and it is too late to prevent propagation. Easy-to-use keys that do not rely heavily on inflorescences and which make it possible to identify the species before they flower are central prerequisites to the implementation of a successful control program (Taylor et al., 2016; Wang et al., 2016). The development of such a key, its distribution in areas susceptible to *Nassella* invasions, and training of stakeholders are crucial requirements.

The use of DNA techniques to distinguish similar species is rapidly becoming a reality. In South Africa, these techniques could be useful for separating the three *Nassella* species from any morphologically similar species. Sufficient phylogenetic distance exists among the three *Nassella* species (Cialdella et al., 2014) and it was demonstrated in a pilot study in Australia that *N. neesiana* and *N. trichomata* could be separated using the *petL* plastid gene (Wang et al., 2014). This gene needs to be sequenced in *N. tenuissima* as well. This would make it feasible to identify the species using these DNA markers.

The true extent of the current distribution and abundance of all three *Nassella* species in South Africa is not known. However, information gathered from stakeholders indicates that *Nassella* invasions have been increasing since the last published distribution records. The situation may be worse than currently thought considering that *Nassella* is highly mobile, especially the more invasive *N. trichotoma*. There is therefore an urgent need to determine the current distribution and densities of *Nassella* species in South Africa.

Chemical control has been the most widely used method and is considered the most effective way to keep *Nassella* invasions in check. The herbicides currently being used lack specificity, causing damage to desirable species in grassland and thus inadvertently giving *Nassella* species a competitive advantage. Also, the repeated use of flupropanate has led to chemical resistance in Australia. This development means research that integrates flupropanate into an integrated control strategy or reduces its use to a bare minimum might be required. The need for alternate integrated methods becomes even more compelling when it is considered that the chemicals on the market are not as target specific as initially thought even at the minimum recommended concentrations. This calls for more research into the best way to use these chemicals. For example, how often can spraying be repeated before it increases the possibility of resistance developing? What is the effect of environmental conditions such as rainfall, slope and wind on the efficacy of chemicals applied aerially? The possibility of incorporating adjuvants such as Citowett, Frigate and Reverseal 9 into herbicides, as suggested by Viljoen (1999), needs to be investigated.

Biological control research has highlighted a number of potential agents for invasive *Nassella* populations. Biocontrol offers a long-term and sustainable management option and can be used in concert with other control efforts. *Uromyces pencanus*, a rust fungus that has been approved in New Zealand for *N. neesiana,* can be tested using the standard procedures to verify its suitability for South Africa. Native range surveys also identified a number of agents that are specific to *N. tenuissima* and *N. trichotoma* that could be tested in South Africa.

With the predictions of climate change becoming a reality, information on the physiological responses of these invasive species to changing conditions is urgently needed. Models of currently suitable geographic areas show that *Nassella* species are far from saturating suitable areas. Most of these suitable areas are high mountainous areas, suggesting that the invasions could become a serious problem if no action is taken. Projecting into the future, existing models predict a decline in the suitable areas for *Nassella* in South Africa. The decline would likely be in the western fringes of the montane areas in the Eastern and Western Cape and the Free State provinces. To be able to effectively take action against *Nassella* invasion in South Africa, fine-scale predictions that take into account local variables are required. Such models could provide useful inputs to implementable coping strategies.

It is also important to understand the response of invasive species in relation to their co-occurring species especially as it pertains to their photosynthetic pathways. *Nassella* species (C_3) have been recorded invading natural montane grasslands dominated by *T. triandra* (C_4) and *Merxmuellera* (C_3) (Faithfull, 2012; Clark and Vidal, 2019). Under the projected increase in temperature and atmospheric CO_2, C_3 plants are expected to become more vigorous while C_4 plants will have marginal responses since they are already operating at near maximum rate but the net gain of C_3 will be offset by the frequent droughts (Kriticos et al., 2010). The response of each specific species is affected by factors such as intra-specific and inter-species competition, herbivory pressure and management (Chen et al., 1996; Kriticos et al., 2010). These traits need to be included in species distribution models that seek to estimate responses of invasive species to climate change and hope to result in effective control programs (Chen et al., 1996).

11. Conclusions

The three *Nassella* species discussed in this paper are difficult to distinguish from each other and from other tussock grasses, especially when not flowering. This has had a significant effect on invasion detection and there is an urgent need for a new approach to collect more accurate distribution data. Identification guides that are accessible to non-scientists are required to encourage citizens to contribute data through platforms like iNaturalist. Current control efforts are uncoordinated and depend on the interest and the ability of individual property owners to fund control measures. As a result, efforts are failing to reduce existing invasions or prevent future spread. Invasive grasses have not generally been considered good targets for biological control however the prospects for *Nassella* species are promising and should be explored for South Africa. With climate change already a reality, models to predict future distribution ranges are needed to guide the prioritization of regions for management intervention.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was financially supported by the Department of Science and Innovation-National Research Foundation (DSI-NRF), Centre of Excellences (CoE), Centre for Invasion Biology (CIB, Stellenbosch University), as well as the Afromontane Research Unit (ARU, University of the Free State). DMR specifically thanks the NRF (grant 85417) and the Oppenheimer Memorial Trust (grant 18576/03) for financial support to support this project through the CIB; KC thanks the Working for Water (WfW) programme of the Department of Environment, Forestry and Fisheries: National Resource Management (DEFF: NRM) programmes and the South African Research Chairs Initiative of the Department of Science and Technology (DST) and the National Research Foundation (NRF) for financial support to support this project through the CBC; AM thanks Rohan Kruger (Game Works Helicopters) for financial support for the project.

We thank David Currie, Dave Davenport, Danie and Phillip Erasmus, Gideon Gravenstein and Kitty and Richard Viljoen for providing valuable insights into the *Nassella* problems in the Eastern Cape Province and allowing access to their properties. Rohan Kruger (Game Works Helicopters) shared valuable insights on the spread and aerial control of *Nassella trichotoma*. Maryke Stern facilitated contact with farmers with invaded farms in the Eastern Cape. Sincere thanks are due to Lyn Fish and Aluoneswi Caroline Mashau for their assistance with aspects of identification and providing literature. Nicholas Le Maitre provided helpful insights on the potential use of DNA techniques for separating *Nassella* species.

References

Anderson, F., Pettit, W., Briese, D.T., McLaren, D.A., 2002. Biological control of serrated tussock and Chilean needlegrass. Plant Protection Quarterly 17, 104–111.

Anderson, F.E., Barton, J., McLaren, D., 2010. Studies to assess the suitability of *Uromyces pencanus* as a biological control agent for *Nassella neesiana* (Poaceae) in Australia and New Zealand. Australasian Plant Pathology 39, 69–78. https://doi.org/10.1071/AP09057.

Anderson, F.E, Díaz, M.L., Barton, J., Flemmer, A.C., Hansen, P.V., McLaren, D.A., 2011. Exploring the life cycles of three South American rusts that have potential as biocontrol agents of the stipoid grass *Nassella neesiana* in Australasia. Fungal Biology 115 (4–5), 370–380. https://doi.org/10.1016/j.funbio.2011.01.008.

Anderson, F.E., Díaz, M.L., McLaren, D.A., 2006. Current status of research on potential biological control agents for *Nassella neesiana* and *Nassella trichotoma* (Poaceae) in Australia. In: Preston, C, Watts, JH, Crossman, ND (Eds.), Proceedings of the Fifteenth Australian Weeds Conference. Adelaide, South Australia. Weed Management Society of South Australia, pp. 591–594.

Anderson, F.E., Gallego, L., Sánchez, R.M., Flemmer, A.C., Hansen, P.V., McLaren, D., Barton, J., 2017. Plant/pathogen interactions observed during host range testing of the rust fungus *Uromyces pencanus*, a classical biological control agent for Chilean needle grass (*Nassella neesiana*) in Australia and New Zealand. Biocontrol Science and Technology 27 (9), 1096–1117. https://doi.org/10.1080/09583157.2017.1384795.

Anderson, G.L., Delfosse, E.S., Spencer, N.R., Prosser, C.W., Richard, R.D., 2003. Lessons in developing successful invasive weed control programs. Rangeland Ecology and Management / Journal of Range Management Archives 56 (1), 2–12.

Badgery, W.B., 2004. Managing competition between *Nassella trichotoma* (serrated tussock) and native grasses. PhD thesis The University of Sydney.

Badgery, W., Kemp, D.K., MIchalk, D.L., King, W.M.C.G., 2005. Competition for nitrogen between Australian native grasses and the introduced weed *Nassella trichotoma*. Annals of Botany 96, 799–809. https://doi.org/10.1093/aob/mci230.

Badgery, W.B., Kemp, D.R., Michalk, D.L., King, W.M., 2008. Studies of competition between *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock) and native pastures. 2. Seedling responses. Australian Journal of Agricultural Research 59, 237–246.

Barkworth, M., Arriaga, M., Smith, J., Jacobs, S., Valdés-Reyna, J., Bushman, B., 2008. Molecules and morphology in South American Stipeae (Poaceae). Systematic Botany 33 (4), 719–731. https://doi.org/10.1600/036364408786500235.

Barkworth, M., Torres, M., 2001. Distribution and diagnostic characters of *Nassella* (Poaceae: Stipeae). Taxon 50 (2 part 4), 439–468. https://doi.org/10.2307/1223891.

Bläßet, C., Ronnenberg, K., Tackenberg, O., Hensen, I., Wesche, K., 2010. The relative importance of different seed dispersal modes in dry Mongolian rangelands. Journal of Arid Environments 74, 991–997. https://doi.org/10.1016/j.jaridenv.2009.12.002.

Bourdôt, G., Basse, B., Kriticos, D., Dodd, M., 2015. Cost-benefit analysis blueprint for regional weed management: *Nassella neesiana* (Chilean needle grass) as a case study. New Zealand Journal of Agricultural Research 58 (3), 325–338. https://doi.org/10.1080/00288233.2015.1037460.

Bourdôt, G.W., Hurrell, G.A., 1992. Aspects of the ecology of *Stipa neesiana* Trin. and Rupr. seeds. New Zealand Journal of Agricultural Research 35 (1), 101–108. https://doi.org/10.1080/00288233.1992.10417707.

Bourdôt, G.W., Hurrell, G.A., Saville, D.J., 1992. Eradication of nassella tussock (*Nassella trichotoma*), an unlikely outcome of grubbing. New Zealand Journal of Agricultural Research 35 (3), 245–252. https://doi.org/10.1080/00288233.1992.10427501.

Bourdôt, G.W., Jackman, S., Saville, D.J., 2017. Plant mortality and seedling recruitment responses to flupropanate in grassland populations of *Nassella trichotoma*. New Zealand Plant Protection 70, 160–164. https://doi.org/10.30843/nzpp.2017.70.42.

Bourdôt, G.W., Lamoureaux, S.L., Watt, M.S., Manning, L.K., Kriticos, D.J., 2012. The potential global distribution of the invasive weed *Nassella neesiana* under current and future climates. Biol Invasions 14 (8), 1545–1556. https://doi.org/10.1007/s10530-010-9905-6.

Bourdôt, G.W., Saville, D.J., 2019. *Nassella trichotoma* – plant growth rates and effects of timing of grubbing on populations in North Canterbury grassland. New Zealand Journal of Agricultural Research 62 (2), 224–245. https://doi.org/10.1080/00288233.2018.1483954.

Briese, D.T., Evans, H.C., 1998. Biological control of serrated tussock (*Nassella trichotoma*): is it worth pursuing? Plant Protection Quarterly 13 (2), 94–97.

Briese, D.T., McLaren, D.A., Pettit, W.J., Zapater, M., Anderson, F., Delhey, R., Distel, R., 2000. New biological control initiatives against weeds of South American origin in Australia: Nassella tussock grasses and blue heliotrope. In: Spencer, NR (Ed.), Proceedings of the Tenth International Symposium on Biological Control of Weeds. Montana State University, Bozeman., Montana, USA, pp. 215–223.

Campbell, M.H., 1982. The biology of Australian weeds. 9: *Nassella trichotoma* (Nees) Arech. Journal of the Australian Institute of Agricultural Science 48 (2), 76–84.

Campbell, M.H., 1998. Biological and ecological impact of serrated tussock (Nassella trichotoma (Nees) Arech.) on pastures in Australia. Australia: Plant Protection. Plant Protection Quarterly ReportNo. 13 (2).

Campbell, M.H., Ridings, H.I., 1988. Tolerance of grazed and ungrazed *Phalaris aquatica* to glyphosate, tetrapion and 2,2-DPA. Australian Journal of Experimental Agriculture 28, 747–751.

Campbell, M.H., Van de Van, R., 1996. Tolerance of native grasses to Frenock® and Round up®. In: Proceedings of the Eleventh Annual Conference of the Grassland Society of NSW. Grassland Society of NSW, pp. 120–121.

Chersich, M.F., Wright, C.Y., 2019. Climate change adaptation in South Africa: a case study on the role of the health sector. Global Health 15, 22. https://doi.org/10.1186/s12992-019-0466-x.

Chen, D., Hunt, H.W., Morgan, J.A., 1996. Responses of a C_3 and C_4 perennial grass to CO_2 enrichment and climate change: comparison between model predictions and experimental data. Ecological Modelling 87, 11–27.

Cialdella, A.M., Sede, S.M., Romaschenko, K., Peterson, P.M., Soreng, R.J., Zuloaga, F.O., Morrone, O., 2014. Phylogeny of *Nassella* (Stipeae, Pooideae, Poaceae) based on analyses of chloroplast and nuclear ribosomal DNA and morphology. Systematic Botany 39 (3), 1–15. https://doi.org/10.1600/036364414X681419.

Clark, V.R., Vidal, J.D., 2019. The vegetation of Asante Sana Private Game Reserve and surrounds, Sneeuberg Centre of Floristic Endemism (Eastern Cape Province, South Africa). Esperiana Memoir 8, 25–38.

Connor, H.E., Edgar, E., Bourdôt, G.W., 1993. Ecology and distribution of naturalised species of *Stipa* in New Zealand. New Zealand Journal of Agricultural Research 36 (3), 301–307.

Conolly, J., Taylor, S., 2016. Mexican feather grass (*Nassella tenuissima*) control – the ACT experience. In: Randall, R., Lloyd, S., Borger, C. (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Perth. Weeds Society of Western Australia, pp. 228–231.

Cooperative Research Centres, 2003. Alert list for environmental weed. Weed Management Guide: Chilean Needle Grass – *Nassella neesiana*. Australian Pamplet. Weed Management and the Commonwealth Department of the Environment and Heritage.

Dickens, S.J.M., Allen, E.B., 2014. Exotic plant invasion alters chaparral ecosystem resistance and resilience pre- and post-wildfire. Biological Invasions 16 (5), 1119–1130. https://doi.org/10.1007/s10530-013-0566-0.

Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibanez, I., Miller, L.P., et al., 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications 7 (12485), 1–9.

Cook, C., 1998. The transmission of serrated tussock (Nassella trichotoma) seeds through the sheep rumen and their viability after ingestion. Report No. 13 (2).

Erb, H.E., 1988. Stipa trichotoma: Some considerations on the species, its habitat and natural distribution in Argentina. Report for the Department of Nature and Environmental Conservation of the Cape Provincial Administration. Cape Town. South Africa.

Evans, H., 1991. In: Baker, FWG, Terry, PJ (Eds.), CAB International, Wallingford. Tropical grassy weeds.

Faithfull, I.G., 2012. Biodiversity impacts of Chilean needle grass, *Nassella neesiana* to Australia's indigenous grasslands [PhD]. Victoria University of Technology, Australia.

Faithfull, I.G., Hocking, C., McLaren, D.A., 2012. In: Eldershaw, V (Ed.), Weed Society of Victoria Inc, Melbourne, Victoria, Australia. Available at http://www.caws.org.au/awc/2012/awc201210591.pdf. Accessed 2012 Oct 8.

Fish, L., Mashau, A.C., Moeaha, M.J., Nembudani, M.T., 2015. Identification Guide to Southern African Grasses: An Identification Manual with Keys, Descriptions, and Distributions, 36. SANBI, Pretoria. Strelitzia.

Flemmer, A.C., Anderson, F.E., Hansen, P.V., McLaren, D.A., 2010. Microscopic observations of a compatible host/pathogen interaction between a potential biocontrol agent (*Uromyces pencanus*) and its target weed (*Nassella neesiana*). Mycoscience 51 (5), 396–400. https://doi.org/10.1007/S10267-010-0051-X.

Global Invasive Species Database (GISD). 2019. Apr 18. Species profile Nassella tenuissima. Available at http://www.iucngisd.org/gisd/species.php?sc=463. Accessed 8 October 2018.

Germishuizen, G., Meyer, N.L., NBI, 2003. Plants of Southern Africa: An Annotated Checklist. National Botanical Institute, Pretoria, South Africa.

Grech, C.J., McLaren, D.A., Lowien, J., McWhirter, L., Butler, K.L., Sindel, B.M., 2012. Assessment of management options for Chilean needle grass in sheep–grazing systems. Grass and Forage Science 69, 119–128.

Grech, C.J., McLaren, D.A., Lowien, J., McWhirter, L., Butler, K.L., Sindel, B.M., 2014. Effects of flupropanate application on bare ground and broadleaf weeds when used to control Chilean needle grass in introduced pastures. New Zealand Journal of Agricultural Research 57 (2), 100–109. https://doi.org/10.1080/00288233.2014.886597.

Hanley, N., Roberts, M., 2019. The economic benefits of invasive species management. People and Nature 1 (2), 124–137. https://doi.org/10.1002/pan3.31.

Healy, A.J., 1945. Nassella tussock (*Nassella trichotoma* (Nees.) Hack.). Field studies and their agricultural significance. Department of Science and Industrial Research(22), 1–90 Bulletin No. 91.

Henderson, L., 2001. Alien Weeds and Invasive Plants: A Complete Guide to Declared Weeds and Invaders in South Africa. Agricultural Research Council, Pretoria, South Africa. Plant Protection Research Institute Handbook no. 12.

Henderson, L., 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37 (2), 215–248.

Henderson, L., 2018. Shocking *Nassella* Invasion Threatens South Africa's Mountain Grasslands. ARC–Plant Protection Research Institute, Pretoria. SAPIA News Report No.: 48.

Henderson, L., Wilson, J.R.U., 2017. Changes in the composition and distribution of alien plants in South Africa : an update from the Southern African Plant Invaders Atlas. Bothalia – African Biodiversity and Conservation 47 (2), 1–26. https://doi.org/10.4102/abc.v47i2.2172.

Howell, C.J., Sawyer, J.W.D., 2006. New Zealand Naturalised Vascular Plant Checklist. Plant Conservation Network, Wellington: New Zealand.

Hussaini, I.P., Lawrie, A.C., McLaren, D.A., 2000. Pathogens on and variation in *Nassella trichotoma* (Poales: Poaceae) in Australia. In: Spencer, N.R. (Ed.), Proceedings of the Tenth International Symposium on Biological Control of Weeds. Montana, USA. Bozeman, Montana State University, Montana, USA, pp. 269–280.

Jacobs, S.W.L., Everett, J., Torres, M.A., 1998. *Nassella tenuissima* (Gramineae) recorded from Australia, a potential new weed related to serrated tussock. Telopea 8 (1), 41–46.

Jones, R.E., Vere, D.T., Campbell, M.H., 2000. The external costs of pasture weed spread: an economic assessment of serrated tussock control. Agricultural Economics 22 (1), 91–103.

Joubert, D.C., 1984. The soil seed bank under nassella tussock infestations at Boschberg. South African Journal of Plant and Soil 1 (1), 1–3.

Kriticos, D.J., Crossman, N.D., Ota, N., Scott, J.K., 2010. Climate Change and Invasive Plants in South Australia. CSIRO Climate Adaptation Flagshi, Canberra, Australia. p.

Lamoureaux, S., Bourdôt, G.W., 2002. Fruit viability on grubbed *Nassella trichotoma* (Nees) tussocks. Australian Journal of Experimental Agriculture 42, 1057–1061. https://doi.org/10.1071/ea02032.

Lamoureaux, S., Bourdôt, G.W., Verkaaik, M., 2006. Outsmarting nassella tussock – answers to often-asked questions based on recent research findings. In: Preston, C, Watts, JH, Crossman, ND (Eds.), Proceedings of the Fifteenth Australian Weeds Conference; Adelaide, Australia. Weed Management Society of South Australia.

Lusk, C.S., Hurrell, G.A., Saville, D.J., Bourdôt, G.W., 2017. Changes in plant species composition after flupropanate application for nassella tussock control, in Canterbury hill-country pastures. New Zealand Journal of Agricultural Research 60 (3), 263–276. https://doi.org/10.1080/00288233.2017.1321556.

Masters, G., Norgrove, L., 2010. Climate change and invasive alien species. CABI Working Paper, pp. 1–30.

McLaren, D., Anderson, F.E.., 2011. Protecting Australia's Agricultural Grasslands from Herbicide-Resistant Serrated Tussock. RIRDC Publication.. Report No.: Pub. No. 11/070. Available at https://www.agrifutures.com.au/product/protecting-australia-s-agricultural-grasslands-from-herbicide-resistant-serrated-tussock/. Accessed 8 Oct 2019.

McLaren, D., Cowan, R., 2012. Biological control of serrated tussock. North Sydney: Meat and Livestock Australia Limited. Report No.: Final Report.

McLaren, D.A., Anderson, F.E., 2011. Biological Control of Serrated Tussock and Chilean Needle Grass. Rural Industries Research and Development Corporation, Canberra, Australia. Report No.: Pub. No. 11/040.

McLaren, D.A., Snell, K., Butler, K.L., 2008. An assessment of native tree susceptibility to the simulated aerial application of the herbicide flupropanate, for management of exotic unpalatable grasses. In: van Klinken, RD, Osten, VA, Panetta, FD, Scanlan, JC (Eds.), Hot Topics in the Tropics. Queensland Weeds Society, Queensland, Australia.

McLaren, D.A., Stajsic, V., Gardener, M.R., 1998. The distribution and impact of South/North American stipoid grasses (Poaceae: Stipeae) in Australia. Plant Protection Quarterly 13 (2), 62–70.

McLaren, D.A., Stajsic, V., Iaconis, L., 2004. The distribution, impacts and identification of exotic stipoid grasses in Australia. Plant Protection Quarterly 19 (2), 59–66.

Miller, L., 1998. Management of serrated tussock in farming areas. Plant Protection Quarterly 13 (2), 91–93.

Milton, S.J., 2004. Grasses as invasive alien plants in South Africa. South African Journal of Science 100, 69–75.

Moretto, A.S., Distel, R.A., 1998. Requirement of vegetation gaps for seedling establishment of two unpalatable grasses in a native grassland of central Argentina. Australian Journal of Ecology 23, 419–423.

Morgan, J.W., 2001. Seedling recruitment patterns over 4 years in an Australian perennial grassland community with different fire histories. Journal of Ecology 89, 908–919. https://doi.org/10.1111/j.1365-2745.2001.00617.x.

Nkuna, K.V., Visser, V., Wilson, J.R.U., Kumschick, S., 2018. Global environmental and socio-economic impacts of selected alien grasses as a basis for ranking threats to South Africa. NB 41, 19–65. https://doi.org/10.3897/neobiota.41.26599.

Occdownload Gbif.Org, 2019. GBIF occurrence download-*Nassella* tenuissima. The Global Biodiversity Information Facility. https://doi.org/10.15468/DL.YPBDSK.

Powells, J., 2018. Flupropanate resistant serrated tussock (*Nassella trichotoma*) identified at multiple locations in the Monaro, New South Wales. In: Johnson, S, Weston L, Wu, H, Auld, B (Eds.), Proceedings of the Twenty-first Australasian Weeds Conference. The Weed Society of New South Wales Inc, p. 397.

Pyšek, P., Pergl, J., van Kleunen, M., et al., 2020. South Africa as a donor of naturalized and invasive plants to other parts of the world. In: van Wilgen, BW, Measey, J, Richardson, DM, Wilson, JR, Zengeya, TA (Eds.), Biological Invasions in South Africa. Springer, Berlin, pp. 755–782. doi.org/10.1007/978-3-030-32394-3_26.

Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G.L., Williamson, M., Kirschner, J., 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53, 131–143. https://doi.org/10.2307/4135498.

Quattrocchi, U., 2000. CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. Vol. 3: M - Q. CRC Press, Boca Raton, FL.

Rahbek, C., Borregaard, M.K., Colwell, R.K., Dalsgaard, B., Holt, B.G., Morueta-Holme, N., Nogues-Bravo, D., Whittaker, R.J., Fjeldså, J., 2019. Humboldt's enigma: what causes global patterns of mountain biodiversity? Science 365 (6458), 1108–1113. https://doi.org/10.1126/science.aax0149.

Ranwashe, P., 2019. BODATSA: Botanical Collections v1.4. South African National Biodiversity Institute. Dataset/Occurrence. Available at http://ipt.sanbi.org.za/iptsanbi/resource?r=brahms_online&v=1.4. Accessed 27 July 2019.

Richardson, D.M., Bond, W.J., Dean, W.R.J., Higgins, A.I., Midgley, G., Milton, S.J., 2000. Invasive alien species and global change: a South African perspective. In: Mooney, HA, Hobbs, RJ (Eds.), Invasive Species in a Changing World. Island Press, Washington, DC, pp. 303–350.

Richardson, D.M., Foxcroft, L.C., Latombe, G., Le Maitre, D.C., Rouget, M., Wilson, J.R.U., 2020. The biogeography of South African terrestrial plant invasions. In: Van Wilgen, BW, Measey, J, Richardson, DM, Wilson, JRU, Zengeya, T (Eds.), Biological Invasions in South Africa. Springer, Berlin. in press.

Richardson, D.M., Wilson, J.R.U., Weyl, O.L.F., Griffiths, C.L., 2011. South Africa: Invasions. In: Simberloff, D, Rejmánek, M (Eds.), Encyclopedia of Biological Invasions. University of California Press, Berkeley, pp. 643–651.

Romaschenko, K., Peterson, P., Soreng, R., Garcia-Jacas, N., Futorna, O., Susanna, A., 2012. Systematics and evolution of the needle grasses (Poaceae: Pooideae: Stipeae) based on analysis of multiple chloroplast loci, ITS, and lemma micromorphology. Taxon 61, 18–44. https://doi.org/10.1002/tax.611002.

Romaschenko, K., Peterson, P.M., Soreng, R.J., Garcia-Jacas, N., Futorna, O., Susanna, A., 2008. Molecular phylogenetic analysis of the American Stipeae (Poaceae) resolves *Jarava* sensu lato polyphyletic: evidence for a new genus. Pappostipa. Journal of the Botanical Research Institute of Texas 2 (1), 165–192.

Smith, L.P., Lamoureaux, S.L., 2006. When science meets policy – nassella tussock in New Zealand as a case study. In: Proceedings of the Fifteenth Australian Weeds Conference proceedings. Weed Management Society of SA, pp. 450–453.

Soreng, R., Peterson, P., Davidse, G., Judziewicz, E., Zuloaga, F., Filgueiras, T.S., Morrone, O., 2009. Tropicos – name search. Available at http://www.tropicos.org/NameSearch.aspx. Accessed 8 May 2019.

Sutton, G.F., Canavan, K., Day, M.D., den Breeyen, A., Goolsby, J.A., Cristofaro, M., McConnachie, A., Paterson, I.D., 2019. Grasses as suitable targets for classical weed biological control. BioControl 64, 605–622. https://doi.org/10.1007/s10526-019-09968-8.

Taylor, N.J., 1987. Ecological aspects of nassella tussock (*Stipa trichotoma*). Botany Division, Lincoln, New Zealand. DSR.

Taylor, S., Conolly, J., Gruber, A., 2016. Chilean needle grass (*Nassella neesiana*) control – the ACT experience. In: Randall, R, Lloyd, S, Borger, C (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Perth. Weeds Society of Western Australia, pp. 223–227.

Vere, D.T., Campbell, M.H., 1984. Economics of controlling serrated tussock in the southeastern Australian rangelands. Journal of Range Management 37 (1), 87–93.

Verloove, F., 2005. A synopsis of *Jarava* Ruiz and Pav. and *Nassella* E. Desv. (*Stipa* L. s.l.) (Poaceae: Stipeae) in southwestern Europe. Candollea 60 (1), 97–117.

Viljoen, B.D., 1987. Effect of rate and time of application of tetrapion on nassella tussock (*Stipa trichotoma* Nees) in South Africa. South African Journal of Plant and Soil 4 (2), 79–81.

Viljoen, B.D., 1999. Influence of adjuvants on tetrapion efficacy for nassella tussock (*Stipa trichotoma* Nees) control and subsequent seedling establishment: a preliminary study. South African Journal of Plant and Soil 16 (2), 102–105. https://doi.org/10.1080/02571862.1999.10634855.

Visser, V., Wilson, J.R.U., Canavan, K., Canavan, S., Fish, L., Le Maitre, D., Nänni, I., Mashau, C., O'Connor, T., Ivey, P., et al., 2017. Grasses as invasive plants in South Africa revisited: Patterns, pathways and management. Bothalia 47 (2), 29. https://doi.org/10.4102/abc.v47i2.2169 pages.

Visser, V., Wilson, J.R.U., Fish, L., Brown, C., Cook, G.D., Richardson, D.M., 2016. Much more give than take: South Africa as a major donor but infrequent recipient of invasive non–native grasses. Global Ecology and Biogeography 25, 679–692. https://doi.org/10.1111/geb.12445.

Wang, A., Gopurenko, D., Wu, H., Stanton, R., Lepschi, B.J., 2014. DNA barcoding for identification of exotic grass species present in eastern Australia. In: Baker, M (Ed.), Proceedings of the Nineteenth Australasian Weeds Conference (2014). Tasmanian Weed Society, pp. 444–447.

Wang, A., Wu, H., Gopurenko, D., 2016. Chloroplast genome of serrated tussock (*Nassella trichotoma*): structure and evolution. In: Randall, R, Lloyd, S, Borger, C (Eds.), Proceedings of the Twentieth Australasian Weeds Conference. Perth. Weeds Society of Western Australia, pp. 152–156.

Watt, M.S., Kriticos, D.J., Lamoureaux, S.L., Bourdôt, G.W., 2011. Climate change and the potential global distribution of serrated tussock (*Nassella trichotoma*). Weed Science 59 (4), 538–545. https://doi.org/10.1614/WS-D-11-00032.1.

Weller, S.L., Florentine, S.K., Sillitoe, J.F., Grech, C.J., McLaren, D.A., 2016. An investigation of the effects of stage of ensilage on *Nassella neesiana* seeds, for reducing seed viability and injury to livestock. Scientific Report 6 (22345), 1–7. https://doi.org/10.1038/srep22345.

Weller, S.L., Florentine, S., Sillitoe, J.F., Grech, C., McLaren, D., Chauhan, B.S., 2016. Detecting the Seeds of *Nassella neesiana* in Large Round Hay Bales, by Means of Non-Destructive Core Sampling. PLoS One 10 (9), 1–15. https://doi.org/10.1371/journal.pone.0137343.

Wells, M.J., 1977. Progress with research on *Nassella* tussock. In: Proceedings of the Second National Weeds Conference of South Africa. Stellenbosch, Cape Town. Stellenbosch, Cape Town, South Africa, pp. 47–55.

Wells, M.J., 1978. Nassella tussock. In: Stirton, CH (Ed.), Plant Invaders: Beautiful but Dangerous. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town, South Africa, pp. 140–143.

Wells, M.J., De Beer, H., 1987. Nassella Tussock. Agricultural Research Council. Weeds A.21/1987.

Wells, M.J., Engelbrecht, V.M., Balsinhas, A.A., Stirton, C.H., 1983. Weed flora of South Africa 2: power shifts in the veld. Bothalia 14 (3/4), 961–965.

Westbrooks, R.G., Cross, G., 1993. Serrated Tussock (*Nassella trichotoma*) in the United States. Weed Technology 7 (2), 525–528.

Taskforceherbicide, 2020. TASKFORCE® herbicide: frequently asked questions. Available at: http://taskforceherbicide.com/faq. Accessed 24 Jan 2020.

Appendix B. The field datasheet used during field data collection for *Nassella neesiana* and *Nassella trichotoma*

Nassella Research Plot Data Recording Sheet

Study Site:				
Date:	Recorder:	Waypoint:	Field No.	Final No.
Co-ordinates	S	E	Altitude (m):	

Species	Cover (Estimate % of plot)	Notes/herbivory

SOIL TYPE		SLOPE		ASPECT	
clay		Level (0–2º)		North	
Loam		Gentle (2–10º)		South	
Sand		Moderate (10–45º)		West	
Humus		Steep (>45º)		East	
				Northwest	
				Northeast	
				Southwest	
				Southeast	

BARE GROUND	
LITTER COVER	
SOIL (tick when collected)	

NOTES:

Land use: (e.g. paddock, cultivated)

Disturbance: (e.g. overgrazed, burnt, mowed)

Moisture Regime:

Appendix C. A list of all plant species recorded in the Eastern Cape during the study of *Nassella trichotoma* in March 2020 and March 2021. South African endemic species are indicated with a star.

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ajuga ophrydis Burch. ex Benth.	Lamiaceae							X	X		X	X									
Alchemilla bicarpellata Rothm.	Rosaceae		X																		
Anthospermum monticola Puff	Rubiaceae			X			X	X	X			X									
Arctotis arctotoides (L.f.) O.Hoffm.	Asteraceae		X	X	X	X	X	X				X									
Argyrolobium candicans Eckl. & Zeyh.	Fabaceae											X									
Aristida congesta Roem. & Schult. subsp. *barbicollis* (Trin. & Rupr.) De Winter	Poaceae			X				X													
Aristida diffusa Trin. subsp. burkei (Stapf) Melderis	Poaceae															X	X	X	X	X	X
Berkheya buphthalmoides (DC.) Schltr.	Asteraceae						X														
Berkheya multijuga (DC.) Roessler	Asteraceae																X		X		X

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bromus catharticus Vahl	Poaceae		X			X				X	X						X				
Bulbostylis hispidula (Vahl) R.W.Haines	Cyperaceae															X	X	X			
Carex glomerabilis V.I.Krecz.	Cyperaceae		X		X																
Chenopodium album L.	Amaranthaceae	X	X	X	X	X	X	X	X		X	X									
Chrysocoma oblongifolia DC.	Asteraceae					X					X	X	X		X						X
Cliffortia strobilifera L.	Rosaceae				X	X															
**Clutia impedita* Prain	Peraceae		X			X				X											
Commelina africana L. var. *africana*	Commelinaceae													X							
Conyza albida Willd. ex Spreng.	Asteraceae														X						
Conyza podocephala DC.	Asteraceae	X	X		X		X	X							X						X
Cotula hispida (DC.) Harv.	Asteraceae		X	X							X				X			X			X
Cynodon dactylon (L.) Pers.	Poaceae													X	X	X	X	X	X	X	
**Cynodon incompletus* Nees	Poaceae	X		X	X						X										
Cynoglossum hispidum Thunb.	Boraginaceae	X								X											
Datura stramonium L.	Solanaceae										X										
Ehrharta longigluma C.E.Hubb.	Poaceae	X	X		X	X		X												X	

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
**Ehrharta ramosa* (Thunb.) Thunb. subsp. *aphylla* (Schrad.) Gibbs Russ.	Poaceae	X								X		X									
Eragrostis capensis (Thunb.) Trin.	Poaceae									X				X		X	X				
Eragrostis curvula (Schrad.) Nees	Poaceae	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Erica sp.	Ericaceae			X		X			X			X									
**Felicia filifolia* (Vent.) Burtt Davy subsp. *bodkinii* (Compton) Grau	Asteraceae							X	X		X	X									
Felicia muricata (Thunb.) Nees subsp. *cinerascens* Grau	Asteraceae	X						X	X												
Geum capense Thunb.	Rosaceae		X	X	X		X	X	X		X	X		X							
Gnaphalium griquense Hilliard & B.L.Burtt	Asteraceae					X	X														
Gomphocarpus fruticosus (L.) W.T.Aiton subsp. *fruticosus*	Apocynaceae	X		X	X	X	X				X										
Haplocarpha scaposa Harv.	Asteraceae														X	X	X		X		X

Species	Family	Site number																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Helichrysum cymosum (L.) D.Don	Asteraceae																X	X			
Helichrysum cymosum (L.) D.Don subsp. *calvum* Hilliard	Asteraceae												X	X	X	X	X	X	X	X	X
Helichrysum odoratissimum (L.) Sweet var. *odoratissimum*	Asteraceae																		X		
Helichrysum psilolepis Harv.	Asteraceae		X	X	X	X				X											
Helichrysum rugulosum Less.	Asteraceae												X	X	X	X	X		X	X	X
Helichrysum spiralepis Hilliard & B.L.Burtt	Asteraceae													X							
Helictotrichon turgidulum (Stapf) Schweick.	Poaceae		X					X		X											
Hermannia sp.	Malvaceae	X																			
**Hermannia violacea* (Burch. ex DC.) K.Schum.	Malvaceae				X																
Heteropogon contortus (L.) Roem. & Schult.	Poaceae			X	X											X					
Hyparrhenia hirta (L.) Stapf	Poaceae				X	X						X									
Hypoxis argentea Harv. ex Baker var. *argentea*	Hypoxidaceae							X													

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Indigofera sessilifolia DC.	Fabaceae			X		X		X				X									
Jamesbrittenia pristisepala (Hiern) Hilliard	Scrophulariaceae											X									
Koeleria capensis (Steud.) Nees	Poaceae				X													X		X	
Lactuca inermis Forssk.	Asteraceae		X							X				X	X			X			X
Lepidium africanum (Burm.f.) DC. subsp. *africanum*	Brassicaceae	X																			
Leucosidea sericea Eckl. & Zeyh.	Rosaceae	X											X								X
Lobelia flaccida (C.Presl) A.DC. subsp. *flaccida*	Lobeliaceae															X					X
**Lycium oxycarpum* Dunal	Solanaceae	X				X	X														
Melinis nerviglumis (Franch.) Zizka	Poaceae													X	X						
Melolobium burchelli N.E.Br.	Fabaceae											X									
Tenaxia disticha (Nees) Conert	Poaceae	X	X					X	X		X	X									X
Miscanthus capensis (Nees) Andersson	Poaceae												X					X			

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Monechma divaricatum (Licht. ex Roem. & Schult.) C.B.Clarke	Acanthaceae	X																			
Nassella trichotoma (Nees) Hack. ex Arechav.	Poaceae	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nicandra physalodes (L.) Gaertn.	Solanaceae											X									
**Otholobium macradenium* (Harv.) C.H.Stirt.	Fabaceae						X				X										
Oxalis smithiana Eckl. & Zeyh.	Oxalidaceae	X			X			X	X	X		X	X					X			X
**Pelargonium sidoides* DC.	Geraniaceae							X				X						X			
Pennisetum macrourum Trin.	Poaceae		X			X				X					X						X
Pentaschistis airoides (Nees) Stapf subsp. *airoides*	Poaceae													X	X	X	X	X	X		
Pseudognaphalium luteoalbum (L.) Hilliard & B.L.Burtt	Asteraceae				X			X		X	X										
Radyera urens (L.f.) Bullock	Malvaceae	X																			
**Sarcocaulon camdeboense* Moffett	Geraniaceae			X																	

Species	Family	Site number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Schoenoxiphium sparteum (Wahlenb.) C.B.Clarke	Cyperaceae			X		X		X		X		X									
Selago sp.	Scrophulariaceae	X	X	X		X		X		X											
Senecio coronatus (Thunb.) Harv.	Asteraceae									X					X		X				X
Setaria pumila (Poir.) Roem. & Schult.	Poaceae															X		X			X
Sida ternata L.f.	Malvaceae	X		X								X									
Silene burchellii Otth ex DC.	Caryophyllaceae												X								
Sisymbrium capense Thunb.	Brassicaceae	X	X	X	X	X		X		X			X								
Solanum anguivi Lam.	Solanaceae													X							
Solanum sp.	Solanaceae						X				X										
Sporobolus pyramidalis P.Beauv.	Poaceae													X							
Stachys aethiopica L.	Lamiaceae						X	X	X				X								
Tagetes minuta L.	Asteraceae												X							X	
Teucrium africanum Thunb.	Lamiaceae							X			X	X		X							
Themeda triandra Forssk.	Poaceae	X	X	X		X		X	X	X				X	X	X	X	X	X	X	X
Thesium sp.	Santalaceae									X		X									
Trifolium africanum Ser.	Fabaceae															X	X		X	X	

		Site number																			
Species	Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Xanthium spinosum L.	Asteraceae						X				X										

Appendix D. Output of the univariate analysis for *Nassella trichotoma* plots, using the mvabund R-package.

The species names are indicated by the first three letters of the genus and the specific epithet joined by an underscore.

Analysis of Variance Table

Univariate Tests:

	aju_oph		alc_bic		ant_mon		arc_arc	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	2.441	1.000	0.171	1.000	2.204	1.000	2.388	1.000
treatment	2.136	0.894	0.19	1.000	3.469	0.133	1.054	1.000
site:treatment	0.044	1.000	0.024	1.000	0.047	1.000	0.248	1.000

	arg_can		ari_con		ari_dif		ber_bup	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.172	1.000	0.154	1.000	3.047	0.997	0.198	1.000
treatment	0.202	1.000	0.186	1.000	1.84	0.969	0.243	1.000
site:treatment	0.028	1.000	0.034	1.000	1.915	0.988	0.023	1.000

	ber_mul		bro_cat		bul_his		car_glo	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	3.407	0.966	2.25	1.000	3.243	0.989	0.385	1.000
treatment	0.362	1.000	0.694	1.000	1.903	0.967	1.022	1.000

site:treatment	1.522	0.988	0.056	1.000	0.772	0.999	0.277	1.000

	che_alb		chr_obl		cli_str		clu_imp	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	5.219	0.156	2.51	1.000	3.863	0.813	1.916	1.000
treatment	3.205	0.234	1.084	1.000	1.731	0.969	0.252	1.000
site:treatment	2.499	0.988	0.07	1.000	0.028	1.000	0.043	1.000

	com_afr		con_alb		con_pod		cot_his	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.167	1.000	0.163	1.000	2.05	1.000	3.018	0.997
treatment	0.189	1.000	0.188	1.000	1.737	0.969	0.502	1.000
site:treatment	0.027	1.000	0.029	1.000	1.586	0.988	1.342	0.988

	cyn_dac		cyn_his		cyn_inc		dat_str	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	5.335	0.133	0.892	1.000	3.426	0.961	0.175	1.000
treatment	1.947	0.958	0.934	1.000	1.326	0.999	0	1.000
site:treatment	3.639	0.647	0.04	1.000	0.248	1.000	0	1.000

	ehr_lon		ehr_ram		era_cap		era_cur	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	3.22	0.990	2.847	0.997	2.55	1.000	9.012	0.001
treatment	1.831	0.969	1.862	0.969	3.327	0.185	3.922	0.046
site:treatment	0.063	1.000	0.04	1.000	0.054	1.000	6.884	0.025

	eri_sp.		fel_fil		fel_mur		geu_cap	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	2.146	1.000	2.814	0.997	3.071	0.996	4.064	0.766
treatment	2.946	0.362	0.271	1.000	2.305	0.809	1.868	0.969
site:treatment	0.043	1.000	0.047	1.000	0.035	1.000	2.432	0.988

	gna_gri		gom_fru		hap_sca		hel_cym	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.713	1.000	2.749	0.997	2.124	1.000	4.497	0.543
treatment	0	1.000	3.604	0.097	2.237	0.842	2.161	0.894
site:treatment	0	1.000	1.933	0.988	0.064	1.000	2.491	0.988

	hel_odo		hel_psi		hel_rug		hel_spi	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.191	1.000	3.707	0.846	4.119	0.766	0.18	1.000
treatment	0.263	1.000	1.466	0.995	1.113	1.000	0.224	1.000
site:treatment	0.034	1.000	1.365	0.988	1.931	0.988	0.029	1.000

	hel_tur		her_sp.		her_vio		het_con	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	1.746	1.000	0.172	1.000	0.167	1.000	2.372	1.000
treatment	1.586	0.990	0.202	1.000	0.189	1.000	2.518	0.662
site:treatment	0.049	1.000	0.028	1.000	0.027	1.000	0.053	1.000

	hyp_arg		hyp_hir		ind_ses		jam_pri	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.175	1.000	1.739	1.000	0.821	1.000	0.172	1.000
treatment	0.951	1.000	0.286	1.000	1.518	0.994	0.202	1.000
site:treatment	0.007	1.000	0.047	1.000	0.041	1.000	0.028	1.000

	koe_cap		lac_ine		lep_afr		leu_ser	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	1.271	1.000	1.802	1.000	0.184	1.000	0.967	1.000
treatment	0.377	1.000	2.13	0.894	0	1.000	0.132	1.000
site:treatment	0.052	1.000	0.063	1.000	0	1.000	0.044	1.000

	lob_fla		lyc_oxy		mel_bur		mel_ner	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.149	1.000	1.41	1.000	0.18	1.000	0.219	1.000
treatment	0.184	1.000	0.234	1.000	0.224	1.000	0	1.000
site:treatment	0.037	1.000	0.041	1.000	0.029	1.000	0.04	1.000

	mer_dis		mis_cap		mon_div		nic_phy	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	3.984	0.813	3.266	0.989	0.158	1.000	0.167	1.000
treatment	5.511	0.003	0.362	1.000	0.168	1.000	0.189	1.000
site:treatment	0.06	1.000	0.046	1.000	0.026	1.000	0.027	1.000

	oth_mac		oxa_smi		pel_sid		pen_air	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.65	1.000	2.791	0.997	0.235	1.000	5.337	0.133
treatment	0.216	1.000	2.152	0.894	0.566	1.000	4.934	0.009
site:treatment	0.033	1.000	1.102	0.995	0.047	1.000	0.923	0.999

	pen_mac		pse_lut		rad_ure		sar_cam	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	5.017	0.191	0.774	1.000	0.167	1.000	0.158	1.000
treatment	0.296	1.000	1.24	1.000	0.189	1.000	0.168	1.000
site:treatment	1.788	0.988	0.052	1.000	0.027	1.000	0.026	1.000

	sch_spa		sel_sp.		sen_cor		set_pum	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.923	1.000	3.113	0.995	1.229	1.000	2.646	0.997
treatment	1.743	0.969	1.018	1.000	1.346	0.998	0.307	1.000
site:treatment	0.058	1.000	2.152	0.988	0.041	1.000	0.049	1.000

	sid_ter		sil_bur		sis_cap		sol_ang	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.15	1.000	0.171	1.000	3.482	0.942	0.158	1.000
treatment	0.194	1.000	0.19	1.000	3.04	0.308	0.168	1.000
site:treatment	0.04	1.000	0.024	1.000	0.071	1.000	0.026	1.000

	sol_sp.		spo_pyr		sta_aet		tag_min	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	0.516	1.000	0.188	1.000	3.974	0.813	0.996	1.000
treatment	0	1.000	0.244	1.000	1.386	0.998	0.566	1.000
site:treatment	0.033	1.000	0.03	1.000	4.043	0.424	0.03	1.000

	teu_afr		the_sp.		the_tri		tri_afr	
	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)	wald	Pr(>wald)
(Intercept)								
site	3.061	0.996	0.154	1.000	7.55	0.006	2.145	1.000
treatment	0.261	1.000	0.186	1.000	9.284	0.001	1.837	0.969
site:treatment	0.047	1.000	0.034	1.000	2.396	0.988	0.058	1.000

	xan_spi	
	wald	Pr(>wald)
(Intercept)		
Site	0.516	1.000
Treatment	0.187	1.000
site:treatment	0.031	1.000

Arguments:

Test statistics calculated assuming uncorrelated response (for faster computation)

P-value calculated using 999 iterations via PIT-trap resampling.

Appendix E. A checklist of species recorded in the field Harrismith during the study of *Nassella neesiana* in March and November 2021

		Site number						
Species	Family	1	2	3	4	5	6	7
Aristida adscensionis L.	Poaceae		X					
Aristida junciformis Trin. & Rupr. subsp. *junciformis*	Poaceae		X	X		X	X	
Bidens pilosa L.	Asteraceae	X	X	X	X			X
Bidens schimperi Sch.Bip. ex Walp.	Asteraceae				X		X	
Brachiaria eruciformis (Sm.) Griseb.	Poaceae		X	X			X	
Bromus catharticus Vahl	Poaceae	X						
Chenopodium album L.	Amaranthaceae	X						
Commelina africana L. var. africana	Commelinaceae	X	X					
Conyza albida Willd. ex Spreng.	Asteraceae							X
Cynodon aethiopicus Clayton & Harlan	Poaceae	X		X	X		X	X
Cynodon dactylon (L.) Pers.	Poaceae		X				X	X
Cyperus schinzii Boeck.	Cyperaceae	X						
Digitaria sanguinalis (L.) Scop.	Poaceae	X						
Eragrostis capensis (Thunb.) Trin.	Poaceae		X					
Eragrostis curvula (Schrad.) Nees	Poaceae	X	X		X	X		X
Euphorbia indica Lam.	Euphorbiaceae		X	X		X	X	X
Helichrysum odoratissimum (L.) Sweet var. *odoratissimum*	Asteraceae		X	X				

		Site number						
Species	Family	1	2	3	4	5	6	7
*Hermannia d*epressa N.E.Br.	Malvaceae		X	X				X
Hibiscus trionum L.	Malvaceae					X		
Hyparrhenia hirta (L.) Stapf	Poaceae	X	X	X	X			
Hypochaeris radicata L.	Asteraceae			X		X		
Lepidium virginicum L.	Brassicaceae	X						
Nassella neesiana (Trin. & Rupr.) Barkworth	Poaceae	X	X	X	X	X	X	X
Paspalum scrobiculatum L.	Poaceae	X	X				X	
Physalis viscosa L.	Solanaceae	X	X	X	X	X		X
Plumbago zeylanica L.	Plumbaginaceae		X	X				X
Pycreus nitidus (Lam.) J.Raynal	Cyperaceae		X				X	
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Asteraceae	X			X		X	X
Setaria pumila (Poir.) Roem. & Schult.	Poaceae	X	X		X			
Sida rhombifolia L. subsp. rhombifolia	Malvaceae	X						
Sporobolus pyramidalis P.Beauv.	Poaceae	X	X	X		X		X
Tagetes minuta L.	Asteraceae	X		X	X			
Themeda triandra Forssk.	Poaceae					X		X
Trifolium africanum Ser. var. *africanum*	Fabaceae					X		
Verbena brasiliensis Vell.	Verbenaceae	X	X					X

Appendix F. R-code for predicting and projecting the distribution of *Nassella neesiana* in South Africa and Lesotho.

```
#####################################################################################
# Required packages
#####################################################################################

library(sdm)
library(dplyr)
library(dismo)
library(tidyr)
library(mapview)
library(usdm)
library(raster)

#####################################################################################
# Check and fix multicollinearity
#####################################################################################
library(usdm)
# The raster file (with environmental variables) is called bio, and the species distribution file is called spg.
# Extract the raster values over species points
ex<-raster::extract(bio,spg)

# check for multi-collinearity using vifstep and at a threshold of 5
```

```
v<- vifstep(ex, th=5)

# Excluding the suggested variables, from model
bio<-exclude(bio,v)

##############################################################################################
# Generate the background data
##############################################################################################

# Using generated background data (bgdata) to produce the sdmData object
d<-sdmData(species~.,spgc,predictors=bioc, bg=list(bgdata)) # build sdmData object

##############################################################################################
# Run species distribution model for the current climatic conditions
##############################################################################################
library(sdm)

# Run the sdm function to fit the model with the following models:
# 'rf','svm','gam','mars','cart','brt'
m<-sdm(species~.,d, methods=c('rf','svm','gam','mars','cart','brt')
      , replication=c('boot'), test.p=30,n=10, parallelsettings=list(ncore=2,method='parallel'))
m
```

```
# Check the mean performance of each model
getEvaluation(m,opt=2)
getEvaluation(m,stat=c("Kappa","TSS"), opt=5)

# Getting variable importance
getVarImp(m)

# Getting the response curves with a confidence interval of 5%
rcurve(m,id=1:60, confidence=0.05)
getResponseCurve(m)

roc(m,smooth=T) # receiver operating characteristics averaged
##############################################################
# Use distribution in South America to see if model is a good fit
#
pnsa<- predict(m,bionsa, filename='')
ennsa<-ensemble(m,pnsa,filename='',setting=list(method='weighted',stat='tss',opt=2))
plot(ennsa)
pointnsa<-points(spgnsa,col='black')

###################################
# predict the current suitable areas in SA
```

```
# biosa is the name of the clipped raster for SA
# spgsa, is the clipped distribution points for SA
psac<- predict(m,biosa, filename='')
ensac<-ensemble(m,psac,filename='current_SA_neesiana',overwrite=TRUE,setting=list(method='weighted',stat='tss',opt=2))
plot(ensac)
pointsa<-points(spgsa,col='black')

#############################################################################################
# Run sdm to get distribution under future climate change - CMIP6-MIROC6 - 126
#############################################################################################
# Load the future variables.
# For ssp126 under MIROC6
bio126<-stack("./wc2.1_2.5m_bioc_MIROC6_ssp126_2081-2100/MIROC6/ssp126/wc2.1_2.5m_bioc_MIROC6_ssp126_2081-
2100.tif")

# Future variables should have the same variable names as current variables
names(bio126)<-names(bios) # renaming all variables in bio126 to names in bios
bio126<-exclude(bio126,v)   # removing variables with the collinearity problem
#######################################################
# Forecast the future distribution for South Africa
# Clip the area to South Africa
biosa126<-crop(bio126,biosa) # Crop the future raster
```

```
ensa126<-ensemble(m,biosa126,filename='future126_SA_neesiana2100',overwrite = True,
                  setting=list(method='weighted',stat='tss',opt=2))
plot(ensa126)

############################################################################################
# The minimum predicted value for the training sites
############################################################################################
library(raster)

# Load points and suitability plots
points <- spgsa
models_suitaiblity <- ensac

# Convert table to spPoints
coordinates(points) <- ~longitude+latitude
# Extract suitability values for each point
values_suitability <- raster::extract(ensac,spgsa)
thresh <- min(values_suitability)

# Reclass the map into 0-1 based on the “lowest presence threshold”
pre1 <- reclassify(ensac, c(-Inf,thresh,0, thresh,Inf,1))
plot(pre1)
```

```
pre126 <- reclassify(ensa126, c(-Inf,thresh,0, thresh,Inf,1))
plot(pre126)

##############################################################################################
# Calculating the change in invasion
##############################################################################################
ch126<- pre126-pre1
plot(ch126, col=c('yellow','gray','red'))
# red means potential expansion, gray no change, yellow decline/extinction

##############################################################################################
# Calculating the area
##############################################################################################

areacurrent <- round(raster::area(pre1, na.rm=T, weights=F),digits=2) # create raster with cell area
area126 <- round(raster::area(pre126, na.rm=T, weights=F),digits=2) # create raster with cell area

## Calculate areas in square kilometers
sum(values(pre126*area126), na.rm=T)     # Sum values
```