

Chapter 4. Disturbance and seed inundation field experiment

“Invasion ... [is] ... simply a subset of the possible recolonization responses to disturbance”

R.J. Hobbs and L.F. Huenneke (1992 p. 332).

“... any disturbance that removes intact vegetation tends to promote the seedling establishment of grasses”

G. P. Cheplick (1998 p. 98).

Summary

The mechanisms that enable *N. neesiana* invasions are poorly understood, but well-managed grasslands with relatively intact cover of native grasses appear to be resistant to invasion. This chapter describes a field experiment in a *T. triandra* grassland to test the hypothesis that invasion is disturbance-driven, and to investigate the effects of a range of disturbances on *N. neesiana* recruitment. Replicated square metre experimental plots were treated by either killing all the existing dominant native tussock grasses with herbicide, half the dominant tussock grasses in a mosaic pattern or no tussock grasses, and applying either nutrients (N, P, N+P) or sugar (to immobilise nutrients), along with appropriate controls. Viable panicle seeds of *N. neesiana* were then applied at 500/m² to half the plots. Killing all the native tussocks enabled by far the greatest *N. neesiana* establishment, and produced much higher biomass and growth of *N. neesiana* plants to maturity in <1 year. Areas in which smaller gaps were created (‘half kill’ plots) had greatly reduced recruitment and much lesser biomass of new *N. neesiana* plants. Intact grassland control plots were highly resistant to invasion. Addition of fertilisers had no significant effect on *N. neesiana* recruitment or biomass production, but application of sugar significantly reduced establishment. Establishment and productivity of juvenile plants was probably facilitated by a nutrient pulse resulting from the decay of the killed vegetation. Once established, *N. neesiana* had a minor (but statistically detectable) suppressive impact on the biomass of native grasses and of other plant species as a group, despite achieving only low cover. The primary juvenile period of growth of *N. neesiana* was found to be less than one year for the most advanced 50% of plants in the cohort some *N. neesiana* plants were able to establish, grow and produce viable seed within this one year time frame.

Introduction

There is no general theory that explains the susceptibility of plant communities to invasion by exotic weeds, but propagule pressure and disturbance are generally recognised as key mechanisms (Eschtruth and Battles 2009). In Australia there is little quantitative information on the mechanisms that enable environmental weed invasions, and few studies have incorporated deliberate experimental manipulations to investigate the effects, but the same key factors appear centrally important (Adair and Groves 1998, Grice 2004a, Grice *et al.* 2004, Coutts-Smith and Downey 2006).

Propagule pressure is a complex function, based on age at reproductive maturity and fecundity, dependent on propagule dispersal mechanisms and the availability and incidence of dispersal agents, and ultimately determined by the ability of the propagules to find suitable habitat and establish new populations (Williamson and Fitter 1996). In situations where an invasive species is already present and reproducing, use of the term “propagule rain” may be preferable (Lockwood *et al.* 2009). Production and dispersal of *N. neesiana* seed is relatively well understood because of the significance of the weed in agriculture, and research that has investigated this (Gardener 1998, Grech 2007). Seeds appear to be dispersed mainly by human activities, primarily on mowing and slashing machinery and vehicles, and by livestock via external attachment, but also within the digestive tract of domestic animals, particularly sheep (Bedggood and Moerkerk 2002, Gardener *et al.* 2003a, Snell *et al.* 2007). No definitive published information appears to be available on the period required before young *N. neesiana* plants enter the reproductive phase for the first time. Benson and McDougall (2005) listed the primary juvenile periods of many grasses but failed to provide one for *N. neesiana*. According to Sethu Ramasamy (RMIT University, pers. comm. 13 June 2007) plants in their first year of growth do not flower. Large soil seed banks (>2500 seeds m⁻²) have been measured in infested agricultural grasslands (Bourdôt and Hurrell 1992, Hurrell *et al.* 1994, Gardener 1998, Gardener *et al.* 2003b) and substantially smaller ones (often <1000 viable seeds m⁻²) in conservation areas (Beames *et al.* 2005, Hocking 2005b). An abundance of uncontrolled infestations along carriageways around many temperate grassland remnants and within the remnants themselves ensures that a new flux of seed rain is produced each year.

The other processes that enable, or contribute to the capacity of, *N. neesiana* to actively invade have not been clearly determined. In general, areas with higher susceptibility to weed invasion either have strong, temporally-varying change (i.e. high levels of natural or artificial disturbance) that creates periods of abundant under-utilised resources (e.g. high nutrient levels, high soil water availability), or are subject to anthropogenic application of resources (Hobbs 1989, Davis *et al.* 2000, Cox 2004). Disturbance has generally been found to favour

exotic plant invasions over native vegetation re-establishment (Hobbs 1991, D'Antonio *et al.* 1999, Prieur-Richard and Lavorel 2000) although some authors (e.g. Carr 1993) argue that major invasions of some plants occur without such disturbance. The tenet that disturbance is a prerequisite for invasion is implicitly based on the notion that in undisturbed, successional mature vegetation, surplus resources are absent (Carr 1993), minimised or unobtainable by the existing flora. The fluctuating resources theory posits that a "plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources" (Davis *et al.* 2000). The community becomes more susceptible to invasion by a particular exotic plant if the particular resource that becomes available was previously limiting the growth or survival of that plant (Hobbs 1991). Continuity of the invasion requires that gains the invader makes are not lost when the supply of the resource contracts.

Conversely, resistance to invasion is provided by undisturbed vegetation that is utilising all of the available resources. A majority of plant diversity studies and experiments at small spatial scales (patch, plant association) indicate that greater resistance is provided when the vegetation is more species diverse (Prieur-Richard and Lavorel 2000, Symstad 2000, Dunstan and Johnson 2006). However even at the small scale (1 m²) contradictory results have been found, suggesting that environmental factors other than plant diversity may frequently be important in determining invasibility (Stohlgren 2007). For instance cover and abundance data from surveys at Derrimut Grassland Reserve, Victoria, suggest that *N. neesiana* seedling establishment is uncommon in areas of dense, low diversity stands of the dominant grass *T. triandra* (Lunt and Morgan 2000). A native-based community or one of its members may repel even a superior competitor simply by the priority effect that established residents have over invaders (Symstad 2000, Körner *et al.* 2008). In temperate native grasslands of south-eastern Australia, the biomass of the non-dominant inter-tussock species is generally exceeded by that of the dominant native grasses by at least one to two orders of magnitude, and in areas that remain unburnt and ungrazed, the dominant native grass may account for >90% of plant biomass (Lunt and Morgan 2002). *Themeda triandra*, the dominant native grass in temperate native grasslands of south-eastern Australia, is probably the main provider of biotic resistance to invasion, and appears to function as a keystone species (Prober and Lunt 2009).

Conditions suitable for grass seedling recruitment in perennial grasslands are generally rare or infrequent (Lauenroth and Aguilera 1998). Habitat disturbance is an important factor in the creation of suitable sites for the germination of grass seeds, and any disturbance that damages or kills the existing vegetation favours the establishment and survival of juvenile grass plants, whether native or exotic (Cheplick 1998). Numerous experimental studies have

demonstrated negative effects on grass recruitment due to the presence of established grasses (Lauenroth and Aguilera 1998), including effects from the roots and litter. An invader is favoured by disturbance if its competitors are recruitment-limited, or the invader is better able to exploit the resources made available by the disturbance (Seabloom *et al.* 2003). Recruitment limitation of native species has long been recognised as a problem in Australian temperate grasslands, mainly due to their small, ephemeral seed banks (Lunt 1990b 1995a, McIntyre 1993, Stuwe 1994, Morgan 1998a 1998b 1998c 2001). Because of this, disturbances are likely to favour exotic species that have larger seed banks if other resources for recruitment are available. The post-disturbance vegetation may have multiple stable states determined by which species arrives first (the ‘priority effect’) and the ability of that species to reduce the availability of limiting resources to potential competitors (Seabloom *et al.* 2003, Körner *et al.* 2008). Water may be the key resource in Australian temperate grasslands but nutrients are demonstrably important (Wijesuriya and Hocking 1999).

Nutrient enrichment is a major cause of alien grass invasion worldwide (Milton 2004) and can, in the long term, substantially alter the floristic composition of temperate grasslands (Morgan 2007). Nutrient increases and/or soil disturbances appear to be a critical cause of invasions by high biomass perennial exotic Poaceae in the *T. triandra* dominated grasslands of south-eastern Australia (Morgan 1998d). Exotic plants proliferate in these systems when there is soil disturbance that creates bare ground and releases nutrients normally locked up in the crown and roots of the dominant native grasses (Wijesuriya 1999, Wijesuriya and Hocking 1999). Invasion of high-biomass perennial exotic grasses appears to require intense pulses of N and possibly P in these grasslands (Morgan 1998d, Wedin 1999, Groves and Whalley 2002, Groves *et al.* 2003).

In unmanaged *Themeda triandra* Forssk. grasslands, senescence and partial or complete *T. triandra* death, leading to a similar process of nutrient enrichment, may permit or contribute to *N. neesiana* invasion (Lunt and Morgan 2000). Evidence and field observations indicate that a site occupied by a dominant native grass, that suffers no significant soil disturbance, remains resistant to invasion (Hocking 1998, Phillips 2000). This is probably largely the result of the very high levels of sequestration of system nutrients in the tissues of the dominant grass, and may be due in part to the ability of C₄ grasses such as *T. triandra* to use available soil N more efficiently than C₃ grasses such as *N. neesiana* (Wijesuriya 1999, Murphy and Bowman 2009). *Themeda triandra* swards produce low quality litter with a C:N ratio >30:1, which decays slowly because microbial decomposers are N-limited, resulting in little or no release of nitrate and ammonium into soil solution. Thus *T. triandra* appears able to perpetuate its competitive advantage by a variety of mechanisms that enable it to monopolise key nutrient resources (Wedin 1999, Groves and Whalley 2002).

Reduction of available soil nutrients to restore degraded grasslands can include biomass reduction such as burning, and application of labile C, which is believed to feed, or provide substrates for rapid growth of soil microbes that can temporarily ‘mop-up’ available soil N and other nutrients and decrease rates of N mineralisation and nitrification (Reever Morghan and Seastedt 1999, Wijesuriya and Hocking 1999, Eschen *et al.* 2007, Morgan 2007, Chigineva *et al.* 2009, Prober and Lunt 2009). Addition of sucrose to soil can rapidly stimulate microbial activity, probably mostly of Gram negative bacteria (Nottingham *et al.* 2009). Sugar application has frequently been used effectively to decrease above-ground biomass and reduce the competitive ability of invasive plants, which tend to proliferate in soils with high nutrient levels (Reever Morghan and Seastedt 1999, Prober *et al.* 2005, Eschen *et al.* 2007, Smallbone *et al.* 2008, Prober and Lunt 2009). The technique has sometimes been called ‘reverse fertilisation’. In Australia it has been demonstrated to be an effective technique for the restoration of *T. triandra* and some native forbs in degraded woodlands dominated by exotic annuals (Prober *et al.* 2005, Smallbone *et al.* 2007, Prober and Lunt 2009).

For the research reported here, an experiment was established to test an ‘invasion requires disturbance’ hypothesis for *N. neesiana* and assess whether intact grassland would provide resistance to invasion. The aim was to examine the types of disturbance required to enable invasion of a *T. triandra* grassland, and determine which of a range of disturbances result in higher rates of *N. neesiana* seedling establishment and survival. The experimental design is similar to pioneer work described by Hobbs (1989) in Australia. A range of disturbances involving killing of the existing dominant native grasses (‘pre-treatments’) followed by the application of N or P fertiliser or sugar (‘nutrient treatments’) was applied in replicated 1 m² plots in relatively intact native grassland. Propagule pressure (‘seed treatment’) was applied by spreading *N. neesiana* seeds on a subset of plots. A range of control plots was also integrated into the trial.

Methods

A distinction between seedlings and juvenile plants was not made in this study, due to the rapid growth of *N. neesiana* seedlings and difficulty in clearly distinguishing the stages of development in the field. *Nassella neesiana* plants that resulted from seeding are referred to as juvenile plants unless reproductive structures were present.

Study area

The experimental site was a native grassland at Iramoo Wildlife Reserve, Cairnlea, Victoria, 15 km west of Melbourne. The experimental area was located on the southern side of management zone M2, with the centre of the experimental area at 37°45.231’ 144°47.405’

(Figs. 4.1, 4.2). Choice of site was limited by the nature of the experiment. Managers of grasslands of high conservation value were unwilling to allow the deliberate spreading of seeds of a declared noxious weed, which is illegal under the Victorian *Catchment and Land Protection Act 1994*, in high quality remnants under their control. The compromise involved acceptance of a lower quality, more weed infested grassland already carrying severe infestations of *N. neesiana* in some areas, and with significant populations of the exotic *Nassella trichotoma* (Nees) Hack. ex Arechav.



Figure 4.1. Iramoo Wildlife Reserve, Cairnlea, Victoria, showing the land management units and the location of the field experiment (small rectangle) on the southern side of M2.



Figure 4.2. Site of the disturbance experiment at Iramoo Grassland Reserve, Cairnlea, Victoria, 25 June 2007, looking SE before the commencement of treatment on 10 July. 'Tree line 2' in the distance at left and unburnt grassland of M3 in the midground at right.

A relatively uniform experimental area lacking obvious soil disturbance was selected, with a relatively uniform density of the dominant native grass *Themeda triandra* (Fig. 4.2). The site had been burnt for management purposes on 2 April 2007 and several times prior to this on a 3-4 year ecological burning cycle. Soil was a cracking clay. Small patches of *N. neesiana* existed within 2 m of the chosen areas and dense patches within 60 m. A few existing *N. neesiana* plants within and close to the edges of the buffer zone were killed with herbicide or removed by hand prior to initiation of the experiment. The possibility of recruitment resulting from long-distance dispersal was thus reduced. Other than occasional human visits the possible long distance seed vectors were restricted to foxes and birds. No rabbits were present within the reserve.

A permit was obtained from the Victorian Department of Primary Industries that allowed dispersal of *N. neesiana* seeds.

Plot layout

Ninety 1 m x 1 m plots were established in a rectangular block (5 x 18 plots) defined at each corner by short wooden stakes, with 1 m buffer zones between plots (Fig. 4.3).

Pre-existing vegetation

Initial condition of the vegetation was determined by counting the number of individual plants and estimating canopy cover of existing vegetation in all 90 one square metre plots over the period 26 June to 9 July in the following categories: *N. neesiana*, *T. triandra*, other native grasses, *N. trichotoma*, the exotic *Romulea rosea* (L.) Eckl. (Iridaceae), native forbs and other exotic forbs. Species were classed as native or exotic using Walsh and Stajsic (2007). The cover of plant litter, cryptogam crust, bare ground and rocks was similarly assessed. The identity of the other grasses and forbs present in each plot was determined.

The experimental area was dominated by native perennial grasses but *N. trichotoma* had a major presence. There was an average of 26.9 *T. triandra* tussocks m⁻², 9.6 *N. trichotoma* tussocks and 2.7 tussocks of other native grasses (mainly *Austrostipa bigeniculata* with some *Austrodanthonia* spp.). Due to recent burning, mean total projected foliar cover (aerial or canopy cover) of vascular species at commencement was only 12% (i.e. 88% bare ground including 0.1% rocks) with cryptogam crust cover of 0.2%. Proportions of the foliar cover represented by the major species were *T. triandra* 72.0%, *N. trichotoma* 17.1%, other native grasses 3.0% and *Romulea rosea* 5.0%. (Total = 97.1%). Proportions of above-ground biomass in untreated plots at the conclusion of the experiment in November 2008 represented by the main species were: *N. trichotoma* 33.5%, *T. triandra* 33.0%, *Austrostipa bigeniculata* 20.9%, *Romulea rosea* 11.2% and exotic annual grasses (mainly *Bromus hordeaceus* L.) 0.6% (= 98.6%).

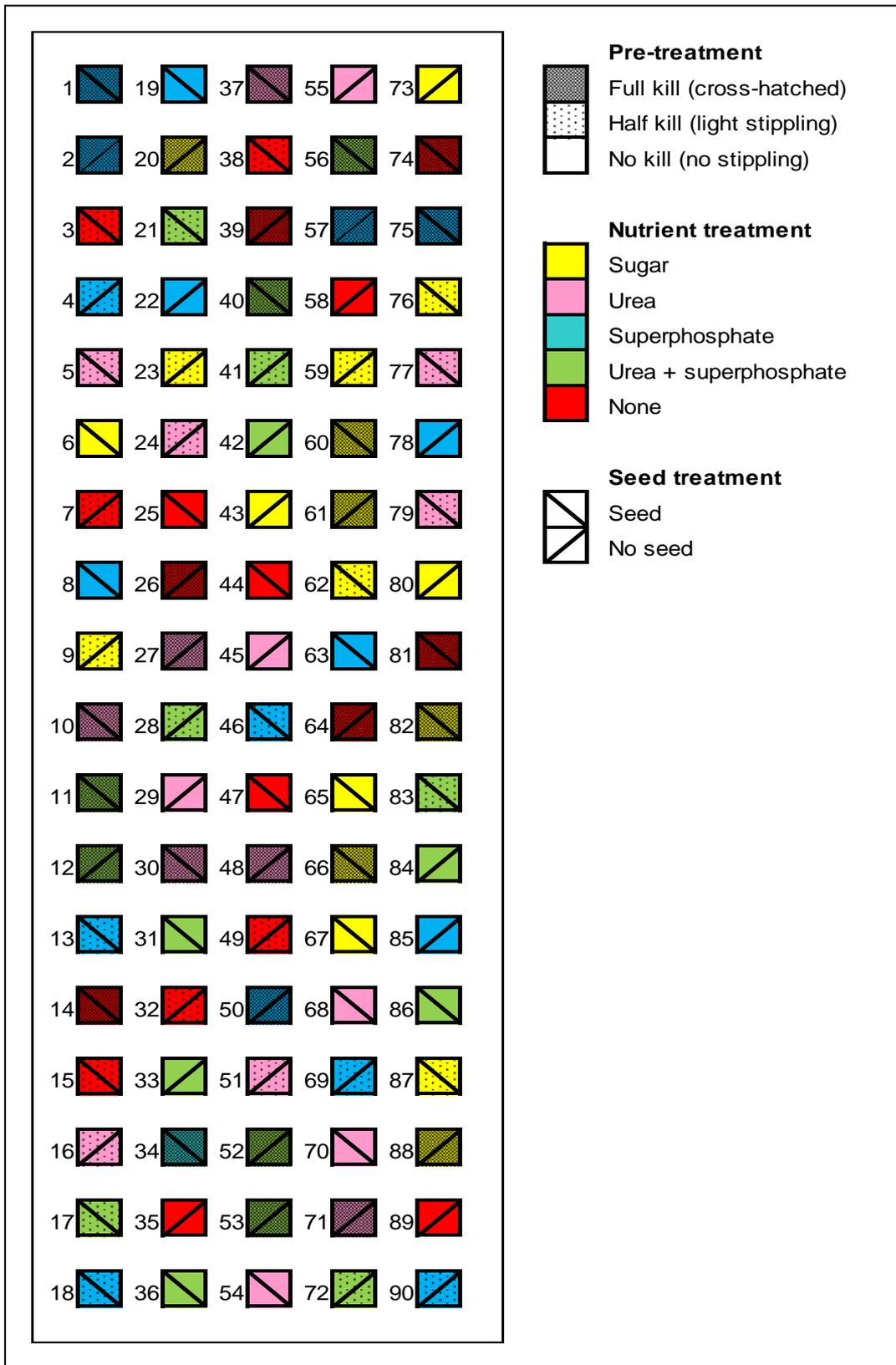


Figure 4.3. Plot layout and treatment regime in the disturbance experiment.

Nassella neesiana soil seed bank

To assess any pre-existing *N. neesiana* seed bank, two soil cores of 3.5 m diameter (9.6 cm²) and 5 cm depth were taken in the buffer zone close to the north and west edges of each plot on 6-10 July (a total of 180 cores). Preliminary tests, undertaken by mixing awned and de-awned *N. neesiana* panicle seeds with soil from the site, established that a 3.35 mm sieve would trap most *N. neesiana* seeds in a soil slurry and that the remaining seeds would be trapped in a 1.2 mm sieve. Cores were soaked in water for a few hours or overnight, then broken up in water with a spatula and agitated until the soil was reduced to fine particles. The soil slurry was washed through a 3.35 mm sieve on top of a 1.2 mm sieve. Larger debris including roots and stones were removed from the coarse sieve with forceps before picking out seeds. Seeds were similarly removed from the fine sieve. Fines that passed through both sieves were inspected for seeds after drainage.

The viability of seeds detected was determined by squeezing the seed with fine forceps under magnification to determine whether the seed was filled (i.e. containing an embryo and endosperm) or unfilled.

Previous studies at the site demonstrated that late spring burning removed all viable *N. neesiana* seeds on the soil surface and that neither early or late spring burning resulted in major recruitment (Hocking 2005b). The autumn burning before this experiment was expected to have a similar impact and to destroy most seed on or near the soil surface.

Soil nutrient status

The soil at the site was sampled on 11 July 2007 and analysed by the Nutrient Advantage laboratory of Incitec Pivot Ltd. (Werribee, Victoria). Thirty soil cores of 3.5 cm diameter and 10 cm depth were taken. Core samples were evenly spaced along the 6 rows in the plot layout, with 5 samples per row. Sampled soil was thoroughly mixed in a bucket and stored in a cool box with freezer blocks. A subsample of 500 g was delivered for analysis within 2 hours of sampling. Analyses were performed on soil dried at 40°C and ground to 2 mm particle size or less.

Pre-treatments

Three pre-treatments were applied on 10 July 2007: 1. spraying of all existing grass tussocks with glyphosate (glyphosate isopropylamine salt, 360 g/litre, “Glyphosate 360”) at 10 ml/L, the label rate for perennial grasses (= ‘full kill’); 2. spraying half the tussocks (= ‘half kill’) at the same application rate; 3. no artificial kill of existing vegetation. In ‘half kill’ plots every second tussock was sprayed in a mosaic pattern, rather than every tussock in half the plot. ‘Full kill’ pre-treatments are considered to have created large gaps (1 m²) in the native vegetation while ‘half kill’ pre-treatments created small gaps (10-30 cm).

Follow Up Treatments

Five treatments were applied on 13 July 2007 (week 0):

1. Nitrogen fertiliser at 10 kg N ha⁻¹ (2.17 g m⁻² of Incitec Pivot Granular Urea, 46.0% N by weight);
2. Phosphorus fertiliser at 10 kg P ha⁻¹ (4.83 g m⁻² of Incitec Pivot Triple Super, a granular preparation, 20.7% P by weight: 16.1% water soluble P, 4.0% citrate soluble P, 0.6% citrate insoluble P; 1.0% S as sulfate, 15.0% Ca as superphosphate).
3. Both nitrogen and phosphorus fertiliser – at the same rates as in 1 and 2;
4. Carbon (sucrose) at 0.22 kg C m⁻² (white cane sugar, 42% C by weight);
5. control - no fertiliser or sugar.

A second treatment at the same rates was applied on 19 September (week 9) in anticipation of a substantial rainfall event, however only 7 mm was recorded during the remainder of the month. A third and final treatment at the same rates was made on 22 November (week 17). Substantial rainfall was recorded on 21 November (17 mm) and 14 mm on 22 November. Fertilisers and sugar were applied by hand broadcasting, and were not deliberately washed into the soil. Total additions of N and P were thus 30 kg ha⁻¹, and the total addition of C was 6600 kg ha⁻¹.

Fertilisation treatments were similar to those used in previous studies. Wijesuriya (1999) applied 0.832 g m⁻² of both N and P per month for 3 months; that is c. 25 kg ha⁻¹ of each nutrient over 3 months. In south-east Australian temperate perennial pastures, superphosphate has generally been applied at the rate of 28 kg P ha⁻¹ at the time of sowing, followed by annual applications of 15 to 18.5 kg P ha⁻¹ (Moore 1993). Eschen *et al.* (2007) used 0.22 kg m⁻² of C as a mixture of sucrose and sawdust, with 3 applications over 3 months. Sugar was applied at a high rate and the plots appeared as if dusted by a light fall of snow. Sugar was gradually washed into the soil by precipitation and did not appear to be removed by ants or other macroscopic organisms. At the time of application of the second and third treatments no sugar from the previous treatments was apparent on treated plots. Sugar was expected to have only short term effects (Prober *et al.* 2005) on nutrient uptake by soil microbes and its impact was expected to dissipate rapidly due to decay of microbial biomass and release of plant-captured nutrients back into the soil. Short-term ‘immobilisation’ of nutrients via sugar application was expected to reduce establishment and survival of juvenile plants of *N. neesiana* and of all other vascular plants.

Seed treatment

Nassella neesiana panicle seed was collected on private grazing land at Greenvale, Victoria (north of Somerton Road, c. 1.4 km WNW of the intersection of Mickleham and Somerton Roads), on 24 December 2003 by Charles Grech and stored in paper bags in the laboratory. Petri dish germination tests (February-March 2007) demonstrated 84% seed viability. Seeds were counted in lots of 500 in the laboratory. ‘Unfilled’ seeds (lacking an embryo) were readily detected by their lighter weight and hollow lemma and were rejected. Seed was applied by broadcasting at a density of 500 seeds m⁻² on 13 July 2007 to three replicates of each of the disturbance-treatments. No seed was applied in the other set of replicates.

Seed, fertiliser and sugar were applied evenly by hand in still conditions. The 3 pre-treatments, 5 nutrient treatments and 2 seeding treatments gave a total of 30 treatments which were replicated 3 times (= 90 plots). Treatments were assigned randomly to plots (Fig. 4.3).

Rainfall and watering regime

Rainfall over the period of the experiment was recorded at the Iramoo Sustainable Community Centre, approximately 300 m to the north-east of the experimental area (Fig. 4.4). Data is approximate, particularly for the number of rain days, since the rain gauge was monitored only on week days. Because of ongoing dry conditions, deliberate watering was undertaken with watering cans in December 2007 in an attempt to facilitate *N. neesiana* establishment, using water from a lake on nearby Jones Creek. A ten litre aliquot was applied to each plot on 10 December 2007 and a further five litre aliquot on 19 December, equivalent to 10 mm and 5 mm of rainfall respectively. Water applied in December was 15 mm and rainfall 42 mm, giving a total of 57 mm of which 26% was supplemental water. Streamwatch data from the lake over several years had detected no significant N or P levels.

Assessment

The fate of seed applied was examined ten days after seed application. A search was made in the buffer zones around seeded plots and any seeds detected were counted. The ‘burial status’ of the first 20 seeds observed on each seeded plot was determined. Seeds were considered ‘buried’ if >50% of the lemma was concealed by soil.

All plots were photographed prior to disturbance treatment and photography was repeated on several occasions during the course of the experiment when other assessments were undertaken.

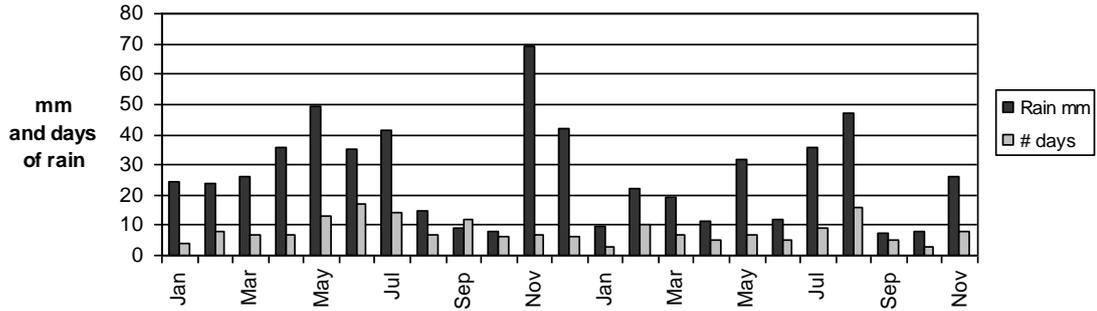


Figure 4.4. Monthly rainfall and number of rain days from January 2007 to November 2008, over the period of the experiment (July 2007 seed application to November 2008 biomass harvest) and the preceding six months. Supplemental watering totaling 15 mm was applied in December 2007.

N. neesiana plant counts

Establishment of *N. neesiana* was assessed by counting the number of plants in each plot at intervals over a period of 69 weeks.

Cover

The canopy cover of *N. neesiana* and major pre-existing species and species groups was assessed at intervals using a 1 m² quadrat subdivided by a 10 x 10 cm grid. The most critical cover estimates were undertaken prior to disturbance treatments and at 69 weeks (end of trial). Counts were also taken of *T. triandra* tussocks and other native and exotic tussock grasses. The percentage canopy cover of *N. neesiana*, *T. triandra*, other native grasses, other exotic grasses, native and exotic forbs, cryptogram crust and bare ground was determined on each occasion.

Biomass

At the end of the experiment the standing phytomass of each species in each plot was harvested by clipping all plants at close to ground level (<0.5 cm). Material of each species in each plot was harvested directly into separate paper bags. The biomass harvest was conducted over an 18 day period from 6 to 25 November 2008. *Nassella neesiana* plants were all harvested on 6-7 November before seed fall.

Harvested biomass was initially allowed to dry indoors under ambient conditions before being dried to constant weight in ovens at c. 90°C. Each sample was weighed immediately after removal from the oven to minimise subsequent atmospheric moisture uptake. Soil and other debris were removed before weighing.

N. neesiana plant production characters

Harvested *N. neesiana* plants were individually bagged at the time of harvest, and individually weighed. After drying and weighing the number of 'living' and dead leaves was counted or estimated for each plant. The reproductive characters of each plant were also determined, namely the number of panicles, emerged glume pairs, emerged awns and loose (released after harvest) seeds, in order to examine treatment effects on fecundity and assess the primary juvenile period of the grass. A range of reproductive characters was assessed because panicles may have few or many flowers, glumes are often sterile and seeds may be rapidly shed.

Post experiment

All detectable *N. neesiana* plants still surviving in the experimental area were removed, including their roots, after biomass harvest. The area was inspected 6 and 9 months later and any *N. neesiana* or other noxious weeds detected were killed with herbicide. This was one of the conditions of the permit allowing distribution of *N. neesiana* seeds on the site in the first instance.

Statistical analyses

Data on the number of *N. neesiana* plants that established under various treatments and on the biomass of *N. neesiana* and categories of other plants was analysed using general analysis of variance (ANOVA, F tests). Mean values per plot are reported, equivalent to means per square metre. Analysis was undertaken on the basis of a fully randomised treatment design and the following treatment structure:

Seed* Kill*(Sugar/(Nitrogen*Phosphorus))

The analyses undertaken are listed in Table 4.1.

Transformations were undertaken to achieve the best normalisations of the means. Transformations performed are reported in the results tables.

There were negligible numbers of *N. neesiana* plants in unseeded control plots, and at harvest there was negligible biomass of *N. neesiana* in unseeded plots, 'no kill' plots and 'half kill' plots, so analysis of treatment impacts was in many cases restricted to seeded plots or 'full kill' seeded plots. When there was a significant response to nitrogen or phosphorus, the sugar treated plots were compared to control plots, not with fertilised plots. When there was no significant response to fertilisers, sugar-treated plots were compared with both control (no nutrient treatment) and fertilised plots to improve precision of the analysis.

Data on *N. neesiana* production characters is reported as median values per square metre. Transformations were undertaken to achieve the best normalisation, as reported in the results

tables. Analysis of the effects of sugar, nitrogen and phosphorus treatments was undertaken using general ANOVA and the following treatment structure:

Sugar/(Nitrogen*Phosphorus)

Table 4.1. Analysis of variance used for all treatment effects. Many analyses were restricted to seeded plots or ‘full kill’ seeded plots, and therefore only used part of this analysis of variance (see the Statistical analyses section).

Effect	Degrees of freedom
Seed (none v seeded)	1
Kill (none v half v full)	2
Sugar (none v applied)	1
Seed by Kill interaction	2
Kill by Sugar interaction	2
Seed by Sugar interaction	1
Seed by Kill by Sugar interaction	2
Nitrogen within no sugar (none v applied)	1
Phosphorus within no sugar (none vs applied)	1
Nitrogen within no sugar by Phosphorus within no sugar interaction	1
Seed by Nitrogen within no sugar interaction	1
Seed by Phosphorus within no sugar interaction	1
Seed by Nitrogen within no sugar by Phosphorus within no sugar interaction	1
Kill by Nitrogen within no sugar interaction	2
Kill by Phosphorus within no sugar interaction	2
Kill by Nitrogen within no sugar by Phosphorus within no sugar interaction	2
Seed by Kill by Nitrogen within no sugar interaction	2
Seed by Kill by Phosphorus within no sugar interaction	2
Seed by Kill by Nitrogen within no sugar by Phosphorus within no sugar interaction	2
Residual	60

Results

Soil nutrient status

The soil at the site was brown (Munsell) medium clay with nutrient levels and other characteristics as shown in Table 4.2.

Table 4.2. Soil analysis for the experimental site, sampled on 12 July 2007. CEC = cation exchange capacity. Analysis by Incitec Pivot Limited.

	mg/kg		%		Meq/100g
Available Potassium	390	Potassium (CEC)	7.7	Potassium (Amm-acet.)	1.0
Nitrate Nitrogen	1.1	Calcium (CEC)	29	Calcium (Amm-acet.)	3.8
Ammonium Nitrogen (KCl)	5.1	Magnesium (CEC)	45	Magnesium (Amm-acet.)	5.9
Phosphorus (Colwell)	8.5	Organic Carbon	2.1	Sodium (Amm-acet.)	2.2
Phosphorus (Olsen)	3.84			Aluminium (KCl)	0.10
Sulfate Sulfur (KCL40)	5.8				
Chloride	30			K/Mg ratio	0.17
Copper (DTPA)	1.4			Ca/Mg ratio	0.64
Zinc (DTPA)	1.8			pH (1:5 water)	6.7
Manganese (DTPA)	31			pH(1:5 CaCl ₂)	5.3
Iron (DTPA)	140			Electrical conductivity (dS/m)	0.12
Boron (Hot CaCl ₂)	1.8			Cation exchange capacity (Meq/100 g)	13.0

Soil seed bank

No *N. neesiana* seeds were found in the soil cores sampled prior to application of *N. neesiana* seeds. *Themeda triandra* seeds, similar in size and shape to those of *N. neesiana*, comprised 89% of seeds detected, with a mean of 1.38 seeds per core (c. = 1400 seed m⁻²), of which 92% were deemed non-viable after visual inspection, and a high proportion were substantially decayed. Seeds of *Austrostipa bigeniculata*, *Austrodanthonia* spp., *Vulpia* sp., *Walwhalleya proluta* and unidentified grasses were also detected, but all with a mean abundance of <0.05 seeds per core. Many cores contained corms of *Romulea rosea*.

Seed burial and dispersal

Ten days after seed application (23 July 2007), 49% of the seeds examined had 'buried' themselves in the soil (>50% of the lemma concealed by soil). Overall, a high proportion of the seed had attached to the soil or burrowed into tussock bases, often only to a depth that concealed the lower half of the callus. Over 95% of seeds retained their awns. Inspections in the buffer zones revealed that very little seed had moved out of plots: 29 seeds were found off-plot, 0.13% of the seeds applied. No seeds were found beyond 11 cm from a plot edge.

A few awnless seeds were still apparent in plots 39 weeks after seed application (15-16 April 2008) but a high proportion of these appeared to have decayed and to be no longer viable.

Recruitment and growth

No seed germination was detected on 23 July 2007, 10 days after seed application and none by 2 November. Rainfall during this period was well below average (Fig. 4.4). The first juvenile plants were detected on 22 November, c. 18 weeks after seed application, when 16 were counted (Fig. 4.5).

Germination and establishment apparently occurred as a single prolonged event from late spring through to mid-summer, c. 17-27 weeks after seed was applied, but mostly between 18 and 22 weeks after seed application (Fig. 4.5). The first major rainfall event after seed application occurred on 5 November when 28 mm was recorded, preceded by 5.5 mm on 2 November (Fig. 4.4). Mass germination occurred in the subsequent four weeks to 19 December 2007 (Fig. 4.5). Recruitment was clearly associated with substantial rainfall in November and December (Fig. 4.4) assisted by supplementary watering. Peak plant counts occurred at 27 weeks, in mid-late December. A few plots continued to show net increases in the number of plants through to 17-21 January 2008 (week 27), but in most plots the number of plants declined, and thereafter there was continued net decline in most treatments (Fig. 4.5). Ungerminated seed, evidently from its distribution originating from the deliberate seed dispersal treatment, was still evident in most seeded plots during detailed assessment on 17-21 January 2008.

Plant numbers declined over late summer and autumn (weeks 35-39) corresponding with a very dry period. Losses were the result of two major factors: predation by grasshoppers, probably mostly the Yellow-winged Locust, *Gastrimargus musicus* (Fabricius), and soil moisture stress. Drought stress appeared to be more severe close to plot edges where competition for water and nutrients with pre-existing grasses in the buffer zones would have been more severe (Fig. 4.6).

By 26 September 2008 strong lateral shooting had commenced on larger plants, but no panicles had emerged. Elsewhere in the grassland only a single bolting plant was observed. By the time of biomass harvest (69 weeks) many plants had produced panicles, and numerous plants had set seed (Table 4.3), but there were also many small plants that showed no signs of entering the reproductive phase.

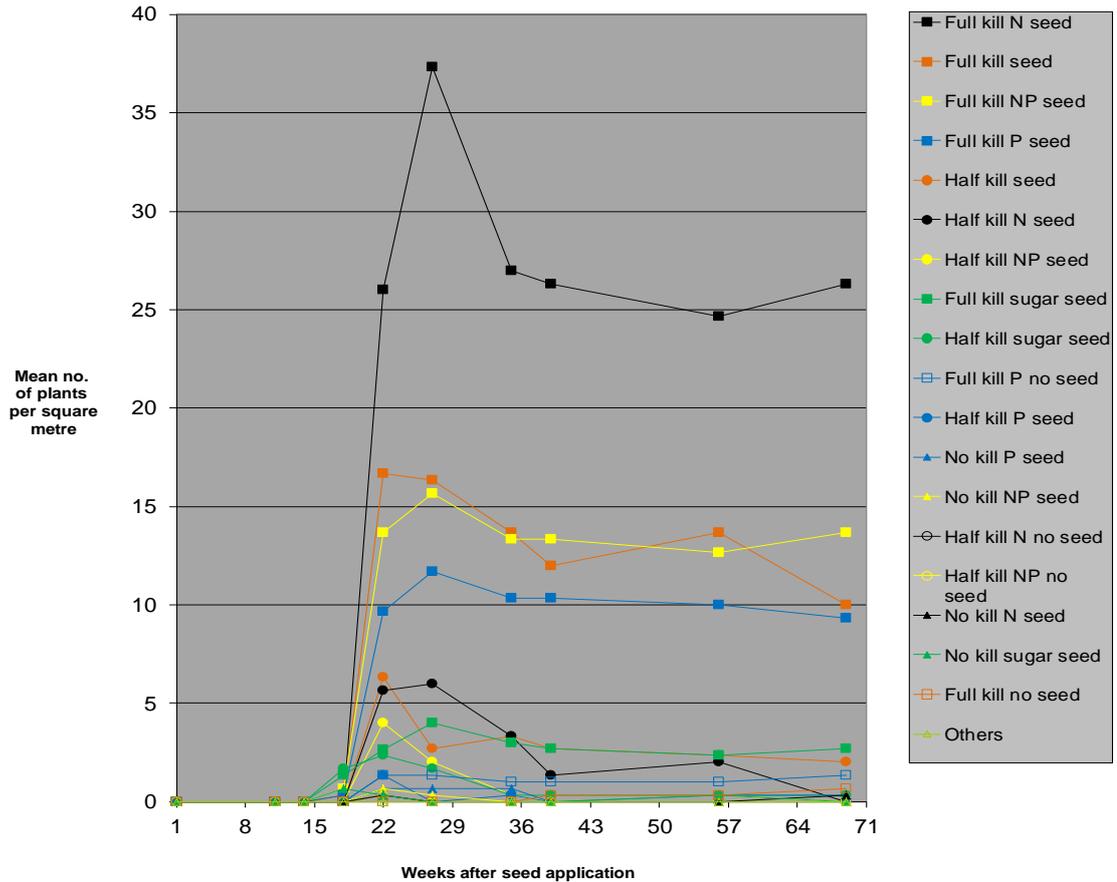


Figure 4.5. Time trend of the mean number of *N. neesiana* plants per plot (plants m⁻²) for each treatment over the course of the experiment. Seed was applied on 13 July 2007 (= week 0). The first major rainfall occurred at week 16. Plants were harvested at week 69. ‘Full kill’ treatments are indicated by squares, ‘half kill’ treatments by circles and ‘no kill’ treatments by triangles. Seeded treatments are indicated by solid symbols and unseeded treatments by outline symbols. Nitrogen treatments are indicated in black, phosphorus treatments in blue, N + P treatments in yellow and sugar treatments in green. Treatments without nutrient or sugar addition are in orange. “Other” treatments include almost all the no-seed treatments. A number of treatments that resulted in minimal establishment have coincident means and therefore overlap in the chart.

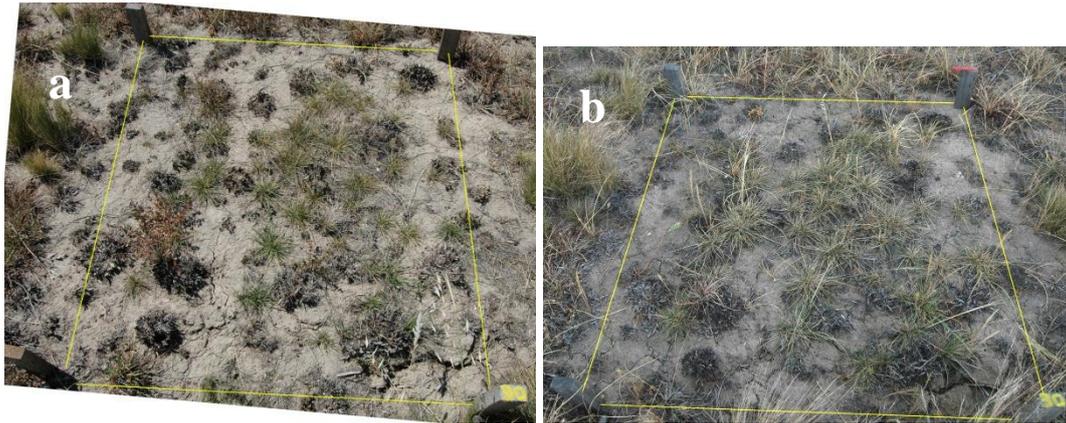


Figure 4.6. Recruitment pattern of *N. neesiana* in a ‘full kill’ plot with substantial establishment (Plot 30). a. 17 January 2008, 27 weeks after seed application; b. 3 November 2008, 69 weeks after seed application. New *N. neesiana* plants established away from the plot edges, suggesting that below-ground competition, probably mainly for water, had a strong influence on establishment. Establishment around the bases of killed tussocks is also evident.

Table 4.3. Median number of *N. neesiana* panicles m⁻², glume pairs m⁻², emerged awns m⁻², detached seeds m⁻² and leaves m⁻² for those plots with *N. neesiana* present.

	Seed	Kill		
		Full	Half	None
Median number of panicles m ⁻²	No	9.5	-	-
	Yes	16	0	0
Median number of glume pairs m ⁻²	No	204	-	-
	Yes	277	0	0
Median number of emerged awns m ⁻²	No	136.5	-	-
	Yes	80	0	0
Median number of detached seeds m ⁻²	No	84	-	-
	Yes	116.5	0	0
Median number of leaves m ⁻²	No	188.5	-	-
	Yes	842.5	63	34

Effectiveness of kill treatments

Visual inspection 15 weeks after herbicide treatment indicated that the ‘full kill’ and ‘half kill’ treatments were highly effective (Fig. 4.7). All the grass tussocks and almost all the standing vegetation was killed in ‘full kill’ plots and approximately half the pre-existing vegetation was killed in ‘half kill’ plots.

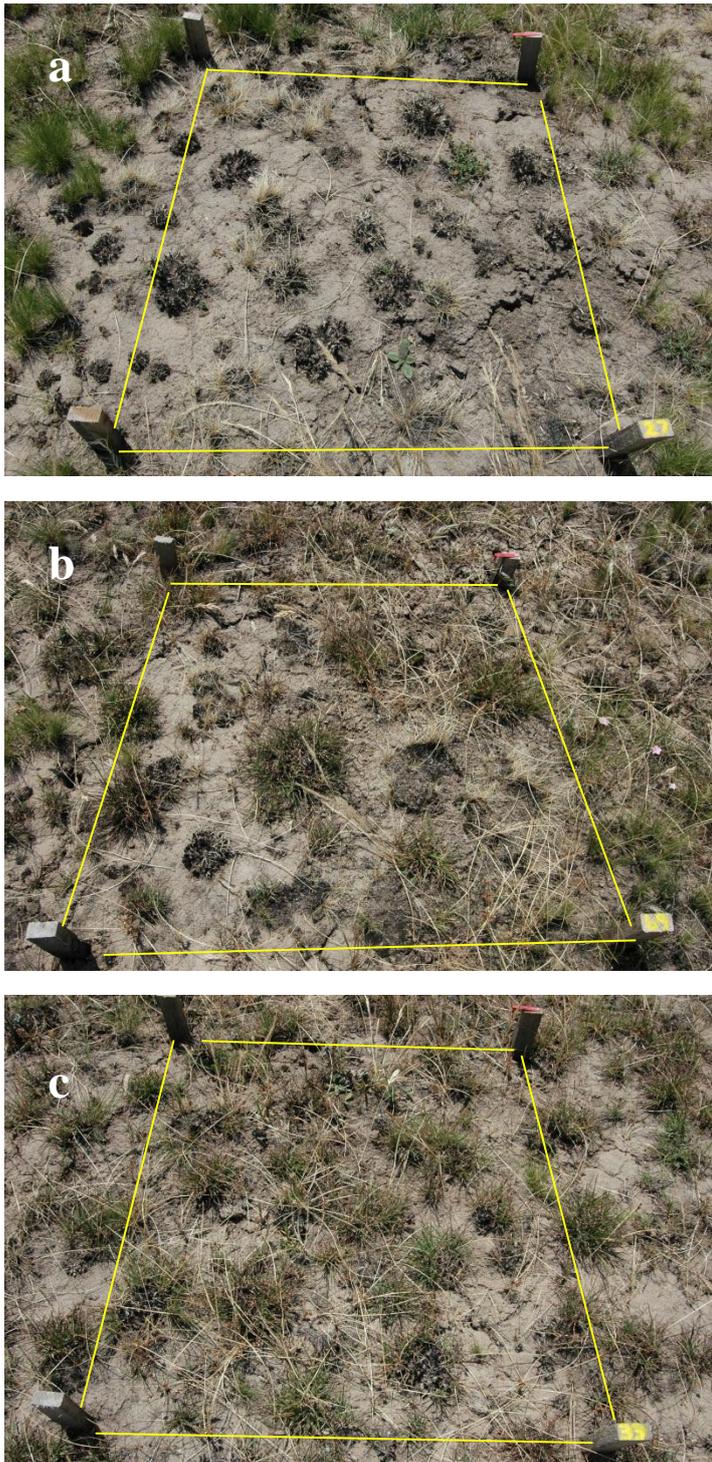


Figure 4.7. Effects of (a) ‘full kill’ (plot 27), (b) ‘half kill’ (plot 33) and (c) ‘no kill’ (plot 69) treatments in the disturbance experiment as at 8 November 2007, 15 weeks after herbicide treatment, indicating the effectiveness of the herbicide kill on the pre-existing vegetation.

The efficacy of the kill treatments was confirmed at the time of biomass harvest, c. 70 weeks after the kill treatments were applied. The kill and ‘half kill’ treatments had significant impacts on above-ground biomass of the dominant grasses (Table 4.4). ‘Full kill’ treatments

carried mean dry phytomass of 58.5 g m⁻², ‘half kill’ plots mean phytomass of 133.5 g m⁻² and ‘no kill’ plots 171.5 g m⁻². The ‘full kill’ treatments carried an average 34% of the biomass of ‘no kill’ treatments, while ‘half kill’ treatments carried on average 78% of the biomass of ‘no kill’ treatments. ‘Half kill’ plots had recovered a major portion of their cover, mainly from regrowth from tussocks that were not killed, but they still contained considerably sparser cover and much less biomass than plots that were not defoliated (Table 4.4, Fig. 4.8). A weaker impact on native grass biomass as compared to other exotic perennial grasses (mainly *Nassella trichotoma*) in ‘half kill’ plots reflects preferential targeting of exotic species with herbicide at the time of treatment (Table 4.4). The kill treatments also significantly boosted annual grass biomass.

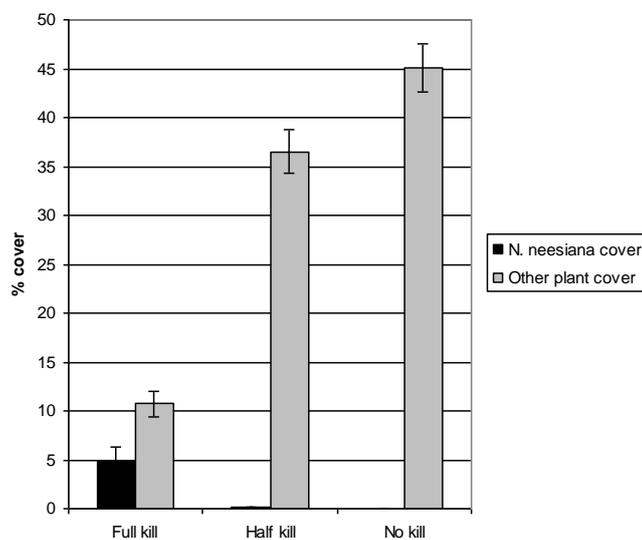


Figure 4.8. Mean projective foliar cover (%) of *N. neesiana* and all other plants combined, by kill treatment in seeded plots at the time of biomass harvest, c. 69 weeks after seed application. Error bars show standard errors.

Of equal significance was the result that ‘no kill’ plots to which *N. neesiana* seed had been added produced very few *N. neesiana* seedlings, and of those that did establish, even fewer survived beyond the juvenile stage (Fig. 4.5). This was the outcome, regardless of the nutrient treatment. ‘No kill’ plots to which seed was added had low overall percentage cover of vegetation at both the time of seed addition, and over the experimental period so suppression of *N. neesiana* plant establishment by above-ground competition for light is unlikely to have been a significant factor, especially given the extent to which *N. neesiana* plant establishment did not occur. This is strong evidence that native grasslands with intact vegetation are resistant to invasion by *N. neesiana*, even under high seed rain, and that the basis of this resistance is linked to below-ground competition for resources.

Table 4.4. Effects of kill treatments and *N. neesiana* seed application on biomass of vascular plant species and groups 69 weeks after *N. neesiana* seed application. ‘Exotic perennial grasses excluding *N. neesiana*’ = *Nassella trichotoma* + *Nassella hyalina*. Significant P values in bold. Back transformed values g m⁻². P values for *N. neesiana* were calculated using permutation tests of the analysis of variance.

Kill treatment	<i>Nassella neesiana</i> seed	<i>Nassella neesiana</i>	Exotic perennial grasses excluding <i>N. neesiana</i>	Native grasses				Exotic annual grasses	Native forbs	Exotic forbs	Total excl. <i>N. neesiana</i>	Total
				Total	<i>Themeda triandra</i>	<i>Austrostipa bigeniculata</i>	<i>Austro-danthonia</i> spp.					
Transformation		log ₁₀ (y+10)	log ₁₀ (y+0.5)	log ₁₀ (y+10)	log ₁₀ (y+10)	log ₁₀ (y+1)	log ₁₀ (y+0.1)	log ₁₀ (y+1)	-1/(y+0.1)	log ₁₀ (y)	√(y)	√(y)
sed		0.057	0.171	0.048	0.060	0.159	0.247	0.119	1.319	0.108	0.562	0.608
P values												
Kill main effect		<0.001	7.1×10⁻¹⁹	7.7×10⁻³⁰	1.7×10⁻²¹	7.9×10⁻¹¹	0.13	0.0087	0.169	0.089	6.4 x 10⁻²⁴	2.8 x 10⁻¹⁸
Seed main effect		<0.001	0.62	0.072	0.67	0.11	0.24	0.716	0.235	0.227	0.085	0.74
Kill by seed interaction		<0.001	0.57	0.013	0.18	0.039	0.045	0.476	0.498	0.495	0.551	0.25
Back transformed means (g m⁻²)												
Full kill	No	1	0	16	10	1.8	0.7	6.8	0.1	14.1	50	51
	Yes	23	1	8	6	0.5	0.3	7.7	0.0	12.6	36	66
Half kill	No	0	12	81	50	15.0	0.6	5.8	0.1	19.5	137	137
	Yes	1	9	77	58	5.7	1.1	4.5	0.1	19.5	129	130
No kill	No	0	42	88	59	12.2	0.6	2.9	0.1	20.9	175	175
	Yes	0	30	95	61	19.0	2.5	4.0	0.1	14.1	168	168

Two other grasses on site related to *N. neesiana*, the exotic perennials *Nassella trichotoma* and *N. hyalina* (= ‘exotic perennial grasses excluding *N. neesiana*’) did not regrow in any ‘full kill’ plots and their biomass remained strongly depressed at the time of biomass harvest (Table 4.4). Biomass of other plant categories in ‘full kill’ plots recovered to a greater extent. Exotic annual grasses returned with greater biomass than in untreated areas (Table 4.4).

Impact of disturbance treatments on *N. neesiana* recruitment and biomass

Strong establishment of *N. neesiana* had occurred by 19 December 2007, 22 weeks after seed application. The effect of kill treatments on recruitment at this time was highly significant, with nearly four times as many juvenile plants established on ‘full kill’ plots (large gaps) as on ‘half kill’ plots (small gaps), and ten times as many on ‘half kill’ plots as on ‘no kill’ plots (‘no’ gaps) (Table 4.5). After 27 weeks, a very similar pattern was apparent (Fig. 4.9). Sugar had a strongly suppressive effect on establishment, reducing it by 87% in ‘full kill’ plots, and by 53% in ‘half kill’ plots after 22 weeks (Table 4.5). These patterns in the number of plants recruited remained very similar after 69 weeks, when the experiment was terminated.

Although a small number of plants did establish on unseeded plots and ‘half kill’ seeded plots, the only plots with major *N. neesiana* presence were ‘full kill’ seeded plots (Table 4.6).

Table 4.5. Effects of kill and sugar treatments on the mean number of *N. neesiana* plants on 19 December 2007, 22 weeks after seed application. Transformed $\log_{10}(y + 1)$ where y = mean number of *N. neesiana* plants m^{-2} .

Sugar	Back transformed		
	Full kill	Half kill	No kill
No	11.6	3.0	0.3
Yes	1.5	1.4	0.3

SED within column 0.222 SED within first row 0.141
 SED within second row 0.281
 P value kill main effect 8.2×10^{-7} (highly significant)
 P value sugar main effect **0.022**
 P value sugar x kill interaction 0.096

Effects of kill and sugar treatments on biomass at time of harvest were also significant (Table 4.7). ‘No kill’ plots to which seed had been added had zero *N. neesiana* biomass and ‘half kill’ plots had minor amounts. ‘Full kill’ plots had approximately 75 times as much *N. neesiana* biomass as ‘half kill’ plots. Sugar treatment resulted in an approximately 10 fold decrease in biomass in ‘full kill’ plots and a roughly 3 fold decrease in ‘half kill’ plots.

Table 4.6. Proportion of plots (out of 15) in which *N. neesiana* was present, 69 weeks after seed application, by seed and kill treatments. There were 90 plots in the experiment, of which 45 were seeded and 45 not seeded. ‘Full kill’, ‘half kill’ and ‘no kill’ treatments were each applied to one third (15) of the plots in each seed treatment. Exact 95% binomial confidence intervals in parentheses. This analysis is a test of the hypothesis that the proportion in comparable replicates = 0.5. The only plots with major *N. neesiana* presence were ‘full kill’ seeded plots.

Seed	Full kill	Half kill	No kill
No	0.13 (0.02, 0.40)	0 (0, 0.22)	0 (0, 0.22)
Yes	0.8 (0.52, 0.96)	0.2 (0.04, 0.48)	0.13 (0.02, 0.40)

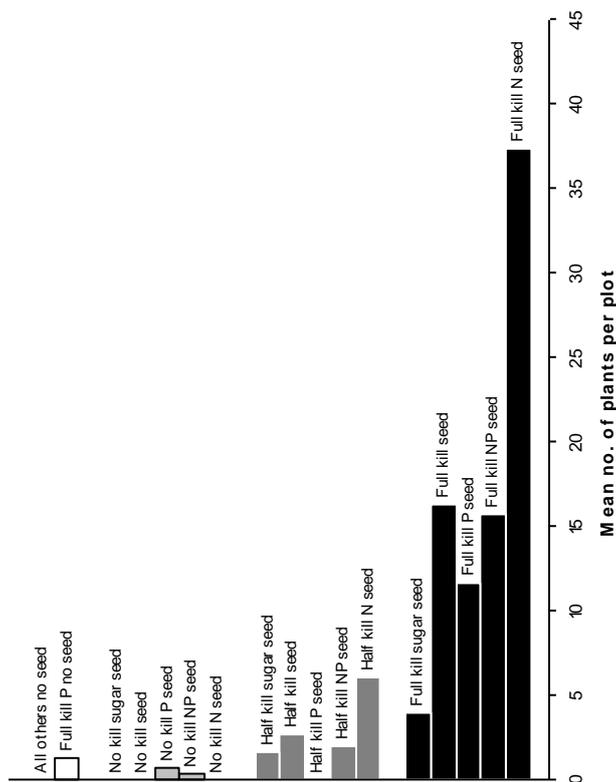


Figure 4.9. Mean number of juvenile *N. neesiana* per plot (= plants m⁻²) by treatment, 27 weeks after seed application. Treatments consisted of combinations of full, half or no kill of existing vegetation; nitrogen fertilisation (N), phosphorus fertilisation (P), both nitrogen and phosphorus (NP), no fertiliser, or sugar; and seed or no seed.

After 69 weeks the biomass of *N. neesiana* on the plot with the highest establishment (Plot 11, ‘full kill’, N+P, seed) was 127 g, 62% of the mean total plant biomass in untreated plots, and the total biomass for that plot was 169 g, 82% of the mean total biomass in untreated plots. The mean biomass of *N. neesiana* on ‘full kill’ seeded plots was 32 g m⁻² (Table 4.8).

Nutrient treatments had no significant impact on the mean biomass of *N. neesiana* plants that established (Table 4.9). Too few plants established in ‘half kill’ and ‘no kill’ plots to evaluate the

effect of kill treatments on biomass per plant, except to note that addition of nutrients did not relieve suppression of *N. neesiana* plant establishment on ‘no kill’ plots. The nutrient treatments also had no significant effect on the biomass of *N. neesiana* within ‘full kill’ seeded plots at the time of harvest (Table 4.10). Nutrient addition (N and P) treatments also had no detectable impact on the numbers of *N. neesiana* that established (Table 4.11) or on the biomass per unit area (Table 4.12).

Table 4.7. Effect of kill and sugar treatment on *N. neesiana* mean above-ground biomass m⁻² within seeded plots, 69 weeks after seed application. Transformed log₁₀(y+1), where y = *N. neesiana* biomass. Back transformed means (g m⁻²).

	Sugar	Full kill	Half kill	No kill
Transformed	No	1.37	0.12	0.02
	Yes	0.5	0.06	0.00
Back transformed	No	22.7	0.3	0.0
	Yes	2.2	0.1	0.0

seds	Full	Kill	Full kill	Half Kill	Half Kill	No Kill	No Kill
	No Sugar	Sugar	Sugar	No Sugar	Sugar	No Sugar	Sugar
Full Kill No Sugar	-						
Full kill Sugar	0.411	-					
Half Kill No Sugar	0.202	0.377	-				
Half Kill Sugar	0.247	0.404	0.185	-			
No Kill No Sugar	0.185	0.368	0.084	0.166	-		
No Kill Sugar	0.186	0.369	0.087	0.167	0.031	-	

P value kill main effect **8.5 × 10⁻⁹** (highly significant)
P value sugar main effect **0.050**
P value sugar x kill interaction 0.058

Table 4.8. Median *N. neesiana* biomass (g m⁻²), for those plots with *N. neesiana* present, 69 weeks after seed application.

Seed	Full kill	Half kill	No kill
No	12	-	-
Yes	32	5	0

Table 4.9. Effects of nutrient treatments on the mean biomass per *N. neesiana* plant, 69 weeks after seed application. Transformed log₁₀(y), where y = biomass. Back transformed means (g).

	Back transformed		sed	P value
	No	Yes		
Sugar	2.7	3.9	0.206	0.49
Nitrogen	2.7	2.7	0.225	0.97
Phosphorus	2.3	3.3	0.225	0.39

Table 4.10. Effects of sugar, and nitrogen and phosphorus fertilisation on *N. neesiana* biomass 69 weeks after seed application, within ‘full kill’ and seeded plots. Transformed $\log_{10}(y+10)$. Total back transformed mean (g m^{-2}). *N. neesiana* biomass showed no significant response to sugar, N or P treatments.

	Back transformed			P value
	No	Yes	sed	
Sugar	29	6	0.230	0.13
Nitrogen	21	48	0.206	0.35
Phosphorus	36	41	0.206	0.82

Table 4.11. Effects of nitrogen and phosphorus treatments on the mean number of *N. neesiana* plants m^{-2} on 19 December 2007, 22 weeks after seed application. Transformed $\log_{10}(y + 1)$ where y = mean number of *N. neesiana* plants m^{-2} .

	Back transformed			P value
	No	Yes	SED	
Nitrogen	2.6	3.5	0.115	0.42
Phosphorus	3.5	2.6	0.115	0.38

Table 4.12. Effects of nitrogen and phosphorus fertilisation on mean biomass m^{-2} of *N. neesiana* within ‘full kill’ plots 69 weeks after seed application. Transformed $\log_{10}(y+1)$. Total back transformed mean (g m^{-2}). There was no interaction between N and P.

	Back transformed			P value
	No	Yes	sed	
Nitrogen	2.0	2.4	0.171	0.72
Phosphorus	2.5	1.9	0.171	0.55

Treatment effects on *N. neesiana* characteristics at harvest

At the time of biomass harvest almost all *N. neesiana* plants were <1 year old. The mean number of leaves per plant was 80 and the range 9-394. Ninety five of 204 plants (47%) had produced panicles within this first year of growth, of which 4 had only unemerged panicles (covered by the leaf sheath). The average number of panicles on reproductive plants was 3, the maximum was 16, and the maximum number of glume pairs on a single plant was 337. Nearly half the plants at the time of biomass harvest had produced panicles, mostly with set seed, none were older than 53 weeks and a high proportion were <1 year old. The primary juvenile period can evidently be less than 1 year.

Few *N. neesiana* plants established on seeded ‘no kill’ and ‘half kill’ plots and the median number of leaves m^{-2} on those ‘no kill’ plots where they did establish was only 34 and for ‘half kill’ plots 63, while on ‘full kill’ seeded plots it was 843 (Table 4.3). Even in plots where there was good establishment, little cover had developed by the time of harvest (Fig. 4.10, *N. neesiana* with total across all plants of c. 800 leaves m^{-2}). Mean *N. neesiana* cover on ‘full kill’ seeded plots was

approximately 5% at the time of biomass harvest, less than half that of other species combined (Fig. 4.8). The maximum *N. neesiana* cover in a single plot was 17%.

Reproductive outputs per square metre were relatively small compared with long established swards, but the populations of young plants produced in excess of 200 seeds m⁻² from around 10 or more panicles m⁻² (Table 4.3). ‘No kill’ plots had zero reproductive output, and in ‘half kill’ plots the recorded reproductive attributes were close to zero, with only five plants producing a total of six panicles. Similarly only 5 plants reached the reproductive stage on unseeded plots, producing a total of 19 panicles.

No significant differences ($P = 0.05$) were found in the effects of treatment with sugar or fertilisation with N or P on the mean number of *N. neesiana* panicles m⁻², glume pairs m⁻², emerged awns m⁻², detached seeds m⁻² and leaves m⁻² within ‘full kill’ and seeded plots (Tables 4.13-4.15). However a significant negative effect of sugar ($P = 0.075$) on the number of leaves at the 10% level was apparent and all the reproductive outputs were lower where sugar treatment had been applied (Table 4.15). Similarly all the plant production attributes were higher where urea had been applied than where it had not, suggesting that a larger experiment with more replicates would have detected significant positive effect of N fertilisation on *N. neesiana* (Table 4.13, back transformed values). Superphosphate treatment produced no similar consistent trend (Table 4.14).



Figure 4.10. One of the largest plants, fruiting for the first time, just prior to biomass harvest, 69 weeks after seed application (plot 11). This plant germinated sometime between 22 November and 19 December 2007 so was between 47 and 50 weeks old.

Table 4.13. Effect of nitrogen on the median number of *N. neesiana* panicles m⁻², glume pairs m⁻², emerged awns m⁻², detached seeds m⁻² and leaves m⁻² for those plots with *N. neesiana* present within ‘full kill’ and seeded plots.

	Nitrogen	SED	P value	Back transformed
Median number of panicles m⁻² (transformed log (y+1))	No	0.350	0.48	9
	Yes			16
Median number of glume pairs m⁻² (transformed log (y+10))	No	0.402	0.64	125
	Yes			204
Median number of emerged awns m⁻² (transformed log (y+10))	No	0.348	0.59	58
	Yes			95
Median number of detached seeds m⁻² (transformed log (y+10))	No	0.332	0.90	69
	Yes			77
Median number of leaves m⁻² (transformed log (y+100))	No	0.274	0.43	550
	Yes			970

Table 4.14. Effect of phosphorus on the median number of *N. neesiana* panicles m⁻², glume pairs m⁻², emerged awns m⁻², detached seeds m⁻² and leaves m⁻² for those plots with *N. neesiana* present within ‘full kill’ and seeded plots.

	Phosphorus	SED	P value	Back transformed
Median number of panicles m⁻² (transformed log (y+1))	No	0.350	0.62	9
	Yes			15
Median number of glume pairs m⁻² (transformed log (y+10))	No	0.402	0.71	131
	Yes			190
Median number of emerged awns m⁻² (transformed log (y+10))	No	0.348	0.73	88
	Yes			62
Median number of detached seeds m⁻² (transformed log (y+10))	No	0.332	0.34	46
	Yes			110
Median number of leaves m⁻² (transformed log (y+100))	No	0.274	0.79	810
	Yes			660

Table 4.15. Effect of sugar on the median number of *N. neesiana* panicles m⁻², glume pairs m⁻², emerged awns m⁻², detached seeds m⁻² and leaves m⁻² for those plots with *N. neesiana* present within ‘full kill’ and seeded plots.

	Sugar	SED	P value	Back transformed
Median number of panicles m⁻² (transformed log (y+1))	No	0.392	0.11	12
	Yes			2
Median number of glume pairs m⁻² (transformed log (y+10))	No	0.450	0.16	160
	Yes			25
Median number of emerged awns m⁻² (transformed log (y+10))	No	0.389	0.24	73
	Yes			18
Median number of detached seeds m⁻² (transformed log (y+10))	No	0.371	0.25	73
	Yes			20
Median number of leaves m⁻² (transformed log (y+100))	No	0.307	0.075	730
	Yes			100

Impact of *N. neesiana* on other plants

While virtually all of the native *T. triandra* was killed on ‘full kill’ plots, there was minor regrowth and subsequent growth of a number of other plant species including the native grasses *Austrostipa bigeniculata* and *Austrodanthonia* spp. At the conclusion of the experiment, after the growth of *N. neesiana* plants and regrowth of other species, it was possible to test whether the *N. neesiana* that grew had impacted on the biomass of these other species. Substantial impact on other plant categories resulting from seeding with *N. neesiana* occurred only with native grass biomass in ‘full kill’ plots, the mean native grass biomass being reduced by half, but the overall impact across treatments was only significant at the 10% level ($P = 0.072$, Table 4.4). Suppression of native grasses by *N. neesiana* in the ‘full kill’ seeded plots contributed to a near-significant ($P = 0.085$) negative impact of seed treatment on total biomass excluding *N. neesiana*.

A significant kill x seed interaction in the statistical analysis (Table 4.4) indicates that where *N. neesiana* was present, it strongly displaced and out-competed native grasses. The effect was not apparent in ‘half kill’ plots, where the number of *N. neesiana* plants was very limited. *Austrostipa bigeniculata* and *Austrodanthonia* spp. were significantly affected. Regrowth of *T. triandra* was probably affected to a similar extent, there being a marked suppression of biomass recovery in seeded plots, but the kill x seed interaction was not significant under these experimental conditions. There was no significant kill x seed interaction effect with any other category of plants. Other exotic perennial grasses (mainly *Nassella trichotoma*) were not affected, nor were exotic annual grasses.

It is clear that *N. neesiana* needs an ecological opening of sufficient size to establish, so only prospered in the ‘full kill’ plots. There is some evidence from these trials that native grasses come back if the space is not occupied by *N. neesiana*. However native species had established poorly on kill plots at the time of biomass harvest, indicating that even the dominant native grasses had either negligible soil seed banks, or conditions for recruitment were unsuitable, or both.

In summary, once established, *N. neesiana* had a suppressive effect on the growth of native grasses, exotic annual grasses, and the other *Nassella* species present. It had a small suppressive effect on native grasses and was able to hold its place in competition with other species.

Effect of sugar treatments on other plants

Although the experiment was not designed to investigate the effects of sugar treatment on plant species other than *N. neesiana*, analysis revealed some significant effects on their biomass (Table 4.16). At the time of biomass harvest (c. 69 weeks after the first and 52 weeks after the last sugar treatments), the average above-ground biomass of *N. neesiana* within sugar-treated, seeded and ‘full kill’ plots was greatly reduced (-79%) but the effect was not significant (Table 4.16). Sugar addition reduced *N. neesiana* establishment, but after the dissipation of the sugar effect there was compensatory growth of the plants which did establish. Sugar addition significantly reduced above-ground biomass of all plant species combined (-32%), all species excluding *N. neesiana* (-29%),

exotic forbs (-53%), exotic annual grasses (-56%), exotic perennial grasses excluding *N. neesiana* (-50%), and native perennial grasses (-18%) with all the main native grass species present contributing to the effect. Native forbs were the only plant group for which no response was measured, but these were present with very low biomass throughout the trial.

Effect of fertiliser treatments on other plants

The experiment was also not designed to investigate impacts of fertilisation on the biomass of species other than *N. neesiana*, but again analysis revealed some significant effects (Tables 4.17, 4.18). Nitrogen was applied by surface broadcasting of pelletised urea. Pellets slowly reduced in size over the experimental period, and some small pellet remnants were still apparent on the soil surface at the time of biomass harvest. In contrast to sugar, the effects of nitrogen addition thus extended over the whole experimental period. Addition of urea more than doubled the resulting average above-ground biomass of *N. neesiana* within seeded and 'full kill' plots, but the effect was not significant (Table 4.17). Nitrogen fertilisation had no effect on above-ground biomass of all species combined, but significantly reduced biomass of *Themeda triandra* (33% reduction) and significantly increased biomass of exotic annual grasses (48% increase) and *Austrodanthonia* spp. (171%).

Superphosphate was applied as pellets, which broke down more rapidly than those of urea but took many months to disappear. The impact of phosphorus addition thus probably extended over the whole of the experiment. Addition of superphosphate had no significant effect on the resulting above-ground biomass of *N. neesiana*. However average above-ground biomass of all plant species combined was increased by approximately one third, mainly due to a highly significant increase in biomass of exotic annual grasses (379%) (Table 4.18). Phosphorus fertilisation also significantly increased the biomass of *Austrodanthonia* spp. (158%). Biomass of exotic perennial grasses excluding *N. neesiana* was increased by c. 50% but the effect was not significant. Except for native forbs and *Themeda triandra*, application of P increased the biomass of the other plant groups by 12-16%.

Off-plot seed movement

At the time of biomass harvest a total of 204 *N. neesiana* individuals were found within plots, and 2 plants within the buffer zones outside plots. A total of six plants established in two of the 45 unseeded plots, indicating that a small amount of seed movement occurred after broadcasting. Plots in the experiment had either 3, 5 or 8 neighbour plots depending on whether they were located at corners, sides or centrally within the experimental area (Fig. 4.3). The random arrangement of the treatments resulted in unseeded plots having a range of 0-87.5% of their neighbour plots that were seeded. The average proportion of neighbour plots that were seeded was 51.2% for the 43 unseeded plots in which *N. neesiana* did not establish. The two unseeded plots in which establishment did occur had respectively 80% and 50% of their neighbour plots seeded; thus one was more likely to be contaminated with seed from neighbours than the unseeded plots in which no establishment occurred, and one had approximately the average likelihood of contamination. .

Table 4.16. Effect of sugar treatments on above-ground biomass of vascular plant species and groups 69 weeks after *N. neesiana* seed application. When there was a significant response to nitrogen or phosphorus (see Tables 4.17 and 4.18) the sugar treated plots were compared to control plots, not with fertilised plots. When there was no significant response to fertilisers, sugar-treated plots were compared with both control (no nutrient treatment) and fertilised plots (N, P and N+P) to improve precision of the analysis. Thus the data for ‘*Austrodanthonia* spp.’ and ‘exotic annual grasses’ are only for 0 nitrogen and 0 phosphorus treatments; for ‘*Themeda triandra*’ only for the 0 nitrogen treatments, and for ‘total biomass excluding *N. neesiana*’ and ‘total biomass’ only for 0 phosphorus. Data for *N. neesiana* was analysed only for ‘full kill’ seeded plots. ‘Exotic perennial grasses excluding *N. neesiana*’ = *Nassella trichotoma* + *Nassella hyalina*. Significant P values in bold. Back transformed means g m⁻².

Treatment	<i>Nassella neesiana</i>	Exotic perennial grasses excluding <i>N. neesiana</i>	Native grasses Total	<i>Themeda triandra</i>	<i>Austrostipa bigeniculata</i>	<i>Austrodanthonia</i> spp.	Exotic annual grasses	Native forbs	Exotic forbs	Total excluding <i>N. neesiana</i>	Total
Transformation	log ₁₀ (y+10)	log ₁₀ (y+0.5)	log ₁₀ (y+10)	log ₁₀ (y+10)	log ₁₀ (y+1)	log ₁₀ (y+0.1)	log ₁₀ (y+1)	-1/(y+0.1)	log ₁₀ (y)	√(y)	√(y)
sed	0.230	0.136	0.034	0.048	0.115	0.225	0.109	0.95	0.078	0.45	0.48
P value	0.13	0.045	0.043	0.35	0.088	0.12	0.045	0.11	0.00018	0.00075	0.00036
Back transformed means (g m⁻²)											
No sugar	29	6.1	50	40	6.8	0.4	2.7	0.1	19	102	110
Sugar	6	4.0	41	35	3.9	0.1	1.2	0.1	9	72	76

Table 4.17. Effect of nitrogen fertilisation on above-ground biomass of vascular plant species and groups 69 weeks after *N. neesiana* seed application. Data for *N. neesiana* was analysed only for ‘full kill’ seeded plots. Significant figures in bold. ‘Exotic perennial grasses excluding *N. neesiana*’ = *Nassella trichotoma* + *Nassella hyalina*. Significant P values in bold. Back transformed values g m⁻².

Treatment	<i>Nassella neesiana</i>	Exotic perennial grasses excluding <i>N. neesiana</i>	Native grasses			Exotic annual grasses	Native forbs	Exotic forbs	Total excluding <i>N. neesiana</i>	Total	
			Total	<i>Themeda triandra</i>	<i>Austrostipa bigeniculata</i>	<i>Austrodanthonia</i> spp.					
Transformation	log ₁₀ (y+10)	log ₁₀ (y+0.5)	log ₁₀ (y+10)	log ₁₀ (y+10)	log ₁₀ (y+1)	log ₁₀ (y+0.1)	log ₁₀ (y+1)	-1/(y+0.1)	log ₁₀ (y)	√(y)	√(y)
sed	0.206	0.111	0.031	0.039	0.103	0.159	0.077	0.85	0.070	0.35	0.39
P value	0.35	0.41	0.29	0.0021	0.12	0.011	0.036	0.57	0.42	0.60	0.43
Back transformed means (g m⁻²)											
No nitrogen	21	6.7	52	40	5.3	0.7	5.4	0.1	18	117	123
Nitrogen	48	8.4	48	27	8.3	1.9	8.3	0.1	20	121	130

Table 4.18. Effect of phosphorus fertilisation on above-ground biomass of vascular plant species and groups 69 weeks after *N. neesiana* seed application. Data for *N. neesiana* was analysed only for ‘full kill’ seeded plots. Significant figures in bold. ‘Exotic perennial grasses excluding *N. neesiana*’ = *Nassella trichotoma* + *Nassella hyalina*. Significant P values in bold. Back transformed values g m⁻².

Treatment	<i>Nassella neesiana</i>	Exotic perennial grasses excluding <i>N. neesiana</i>	Native grasses				Exotic annual grasses	Native forbs	Exotic forbs	Total excluding <i>N. neesiana</i>	Total
			Total	<i>Themeda triandra</i>	<i>Austrostipa bigeniculata</i>	<i>Austrodanthonia</i> spp.					
Transformation	log ₁₀ (y+10)	log ₁₀ (y+0.5)	log ₁₀ (y+10)	log ₁₀ (y+10)	log ₁₀ (y+1)	log ₁₀ (y+0.1)	log ₁₀ (y+1)	-1/(y+0.1)	log ₁₀ (y)	√(y)	√(y)
No phosphorus	1.56	0.82	1.75	1.64	0.83	-0.10	0.62	-5.2	1.24	10.1	10.5
Phosphorus	1.61	1.00	1.80	1.63	0.94	0.28	1.16	-4.2	1.32	11.6	12.1
sed	0.206	0.111	0.031	0.039	0.103	0.159	0.077	0.85	0.070	0.36	0.39
P value	0.82	0.10	0.13	0.66	0.28	0.022	2.4 x 10⁻⁹	0.25	0.23	0.00010	0.00015
Back transformed means (g m⁻²)											
No phosphorus	36	6.1	46	34	5.8	0.7	3.2	0.1	17	102	110
Phosphorus	41	9.5	53	33	7.7	1.8	13.5	0.1	21	135	146

The proportion of unseeded plots in which *N. neesiana* established was zero in ‘half kill’ and ‘no kill’ treatments and 0.13 for ‘full kill’ plots (Table 4.6). Major *N. neesiana* presence occurred only in ‘full kill’ seeded plots, so any off-plot seed movement had no effect on the experimental findings.

Discussion

The hypotheses that invasion by *N. neesiana* requires disturbance and that intact native grassland is resistant to invasion were tested and substantially confirmed by application of panicle seed to native grassland plots that were either left intact, partially killed, or fully killed, and to which fertiliser treatments or ‘inverse fertilisation’ using sugar were applied. Significant *N. neesiana* establishment, as measured by the number of plants that recruited and their biomass, occurred only where the native vegetation was killed, and much greater establishment occurred in ‘full kill’ plots, where the ecological ‘gaps’ created were large. Intact native grassland was found to be resistant to invasion. Fertiliser additions had no significant effect on recruitment or resulting biomass, but sugar application significantly reduced establishment in ‘full kill’ plots, and probably ‘half kill plots’ (although differences in the latter were not significant under the experimental conditions used) indicating that minimisation of the levels of plant-available soil nutrients at the time of *N. neesiana* germination and recruitment will reduce establishment, and that disturbances that result in increased soil nutrient availability are likely to assist *N. neesiana* invasion. The analysis compared sugar effects against pooled fertilised plots and control plots. Positive fertiliser effects on *N. neesiana* may nevertheless have occurred, being non-significant due to the low number of replicates in the experiment, so the approach has potential to exaggerate the real effects of sugar treatments.

Germination and establishment

Unnatural starting conditions are a general problem in experiments with plant communities (Körner *et al.* 2008). The timing of seed application created similar conditions to those that would normally occur, but may have enabled a higher proportion of the seed to remain on site (rather than decay or be removed by predators) until conditions became suitable for germination. *Nassella neesiana* panicle seed germinates mostly in spring (Snell *et al.* 2007). Panicle seed is generally shed in December and January, and according to Gardener *et al.* (2003b) has an after-ripening requirement of 3-12 months, so the earliest germination from the most recent seed crop could occur in autumn, but is more likely in spring. The seed used here was fully after-ripened, being over 3 years old, and was applied in mid-winter and germinated in spring. The rainfall and supplementary watering during the experiment provided conditions suitable for plant recruitment. Natural recruitment events in perennial

grasslands are generally rare to infrequent, and require a particular set of microenvironmental conditions, the most important of which is adequate rainfall at a suitable time (Lauenroth and Aguilera 1998). Rainfall that soaks the soil and keeps it moist for several days is required for germination of many grasses (Baskin and Baskin 1998). Most seedlings in this experiment appeared in the first wet month after seed was applied, and the recruitment that occurred appeared to result almost entirely from this one event. Once established, young grass plants are able to resist desiccation for relatively long periods (Lauenroth and Aguilera 1998), and this occurred with *N. neesiana* juvenile plants over a dry summer, in which drought stress caused relatively minor attrition of the population.

The extent to which the experiment approximated the range of real disturbances that occur in temperate native grasslands is debatable. Previous work at the Iramoo site (Mason 2005) demonstrated that *N. neesiana* established from the soil seed bank at mean densities of $7.4 (\pm 2.1) \text{ m}^{-2}$, ten months after herbicidal spraying of mixed, dense *N. neesiana*/*N. trichotoma* infestations (total mean tussock density of c. $18 \text{ plants } (\pm 4) \text{ m}^{-2}$), followed by tilling to 5 cm depth and application of *T. triandra* thatch, whereas seedling density in unsprayed plots where the existing *Nassella* cover was left intact was only $0.75 \text{ plants } (\pm 0.31) \text{ m}^{-2}$. The similarities between the Mason (2005) data set and the experimental results reported here are plain: recruitment occurs in disturbed ground, but in low numbers, and there is no or negligible recruitment where existing dominant tussock plant cover is not killed.

Peart (1979) tested a range of awned grass diaspores and generally found the highest germination rates when seeds were buried horizontally at a depth of 5 mm. However the native grass seed most similar to *N. neesiana*, that of *Auroloma verticillata* (Nees ex Spreng.) S.W.L. Jacobs & J. Everett germinated at a high rate (c. 45%) when the seed was lodged vertically (callus down) and half buried, and at much lower rates when lying flat on the soil surface or with just the callus buried. This corresponds with the observations of *N. neesiana* germinations reported here. 'Safe sites' for germination (Baskin and Baskin 1998) appeared to include areas where increased levels of soil water may have been expected, e.g. soil hollows and cracks, but these were also areas where penetration of the callus into the soil was more likely.

Large numbers of seedlings may have emerged and died between monitoring events, and may have been unrecorded. Most plant mortality occurs before seedling emergence, and in general it is not known what proportion occurs before germination, nor is the proportion of seeds that germinate, but fail to emerge or survive, easy to determine (Fenner 1987).

The rate of seed application in the experiment was limited by the availability of *N. neesiana* seed and the practicality of counting out large seed lots. Five hundred seeds m^{-2} may be considered to be towards the lower end of what might be expected under natural conditions.

This seeding density was approximately one third of the estimated maximum panicle seed production m^{-2} found by Gardener (1998) in an agricultural grassland on the New England Tablelands in the least productive of the three years he investigated, and a potential annual seed yield of c. 30,000 m^{-2} has been estimated (Slay 2001). However seed bank studies in southern Victoria native grasslands (Hocking 2005b) indicate that 500 seeds m^{-2} is a more realistic representation of actual seed banks than 1000 seeds m^{-2} . In addition, it is likely that a significant proportion of panicle seeds in the seedbank are not germinable.

Only *N. neesiana* seed was deliberately added, so other grasses present either had to have an existing seed bank, or to set and disperse seed to the defoliated plots within the timeframe of the experiment – most likely in mid to late spring. The results obtained (dominance of *N. neesiana* in some seeded plots) can thus be interpreted as a priority effect, resulting from earlier arrival of *N. neesiana*. Differences in arrival time of propagules of as little as 3 weeks can have profound effects on the composition of the subsequent vegetation, with first arriving species dominating the resulting biomass, and first arriving species of one functional group (e.g. grasses) greatly suppressing the growth of later arriving functional groups (Körner, *et al.* 2008). Pre-existing soil seed banks appeared to be low, so significant recolonisation of the bared areas by natives required a new season of seed production, 6 months into the experiment and after the main *N. neesiana* recruitment event.

Fluctuation in mean plant numbers in each treatment over the latter half of the experiment (Fig. 4.5) appeared to be the net result of limited additional germination, coalescence of plants growing very close together, division of some plants, possible regrowth of defoliated or apparently dead seedlings from concealed plant parts, and possible miscounting due to confusion with young *Austrostipa bigeniculata*. Continued presence of small juvenile plants (<10 leaves) 39-56 weeks after seed application suggests that seed germination continued at a low rate over a long period, but that plant mortality after the main germination outnumbered new recruitment.

Increased plant numbers at harvest (week 69), compared with the previous assessment (week 67) were in part due to the greater ease in distinguishing individual plants when they were removed from the ground, but may have been partly the result of inadvertent subdivision of tiller assemblages.

Effects of vegetation kill treatments

The experiment confirms existing understanding that *N. neesiana*, at least in part, recruits from panicle seeds and prospers in situations where competition from other plants is greatly reduced. Established grasses in general have strong negative effects on the recruitment of new grass seedlings and the growth and survival of juvenile plants (Lauenroth and Aguilera 1998). Competitive effects are mediated more through underground structures than above-ground

parts; the intensive adventitious root systems enable very high capabilities for resource absorption in the soil occupied (Lauenroth and Aguilera 1998), so areas that appear unoccupied above ground ('intertussock gaps') in a long established grassland are nevertheless probably more or less fully occupied in ecological terms below-ground. Lesser establishment in 'half kill' plots than 'full kill' plots reflects this fact. *Nassella neesiana* panicle seeds reportedly germinate only in gaps and bare areas (Gardener *et al.* 1996a, Gardener *et al.* 1999) and various studies have likewise shown that seedlings readily establish when there is reduced or no competition from existing grasses. Where there is a seed bank of *N. neesiana*, areas bared with herbicide "generally produce a large germination ... within 12 months" (Duncan 1993) and are 'quickly reinvaded' (Bourdôt and Ryde 1986). Cover and abundance data from surveys at Derrimut Grassland Reserve, Victoria, suggested that seedling establishment of *N. neesiana* was uncommon in areas of dense *T. triandra* (Lunt and Morgan 2000), a relationship confirmed by experiment in the Iramoo manipulations reported here. In experimentally bared ground (by glyphosate application) seedling emergence ceased after the regrowth of surrounding vegetation (Gardener *et al.* 2003b), suggesting, according to Gardener *et al.* (1996a) that sunlight and disturbance that creates bare ground are 'germination triggers'. Mason (2005) attributed the ability of *N. neesiana* seedlings to establish well on areas cleared by herbicide application and tilled, to increased levels of available resources both above and below ground.

In the trial reported here, kill treatments created bare ground, and such open areas encourage the activities of some ants species - conversely ant diversity and activity is reduced when a dense grass sward shades the soil surface (Greenslade 1979). Ants were observed harvesting experimentally deposited *N. neesiana* seeds during the course of the experiment. It is possible that increased ant activity including seed harvesting may have occurred in 'full kill' plots because of their greater openness. However 'no kill' plots did not carry dense vegetation (e.g. Fig. 4.7c). The maximum standing phytomass in a 'no kill' plot was 250 g m⁻² (2500 kg ha⁻¹) and the average for such plots was 175g m⁻², with maximum cover of 70% and average cover 46%. This compares for example to 6900-8700 kg ha⁻¹ in *T. triandra* grassland unburned for several years in southern Victoria measured by Stoner *et al.* (2004) and 3000 and 6000 kg ha⁻¹ in *T. triandra/Poa labillardierei* grassland with 50% *T. triandra* cover found by Dunin and Reyenga (1978) in New South Wales. 'No kill' areas seemed to be similarly inhabited by seed-harvesting species and provided other resources (nectar, plant exudates, invertebrate prey, seeds of other plant species) for ants that may well have made them considerably more attractive for ant foragers than the more open 'full kill' plots.

Effects of fertilisation

The soil P levels determined as part of soil nutrient analysis were very low (<5 mg/kg Olsen) compared to disturbed agricultural soils (Garden *et al.* 2003, Johnston *et al.* 2003, Charles Grech pers. comm.), but within the ranges determined for similar grasslands at Derrimut and Laverton North by Wijesuriya (1999). Available P levels below 25 mg/kg Colwell are considered “marginal” for pasture production in native pastures (Roberts *et al.* 2006), although ‘whole system’ nutrient assessments that includes the amount of P locked up in native vegetation and not ‘available’ in the soil is a more pertinent measure of nutrient status. O’Dwyer (1999) found that concentrations of available soil P above 14µg/g in *Austrodanthonia* grasslands were associated with weedy sites. Combined nitrate and ammonium N was slightly higher than the maxima of extractable N determined at Derrimut and Laverton North by Wijesuriya (1999). Potassium levels were markedly higher than, and organic carbon levels were about half those measured at Derrimut and Laverton North by Wijesuriya (1999).

Low available soil nutrient levels are typical in *T. triandra* grasslands: most of the nutrients are incorporated within the biomass of the dominant grass, and these are released when the tussock grasses are killed (Wijesuriya 1999, Wijesuriya and Hocking 1999, Hocking and Mason 2001).

The fertiliser pellets applied were not deliberately incorporated into the soil, took many months to disappear, and in the case of urea had not entirely disappeared by the time of biomass harvest. Soil nutrient measurements were not undertaken during the course of the experiment so it is unclear to what extent the applied nutrients were available for plant growth during particular phases of *N. neesiana* establishment. The estimation of available nutrients is an involved process (e.g. see Wijesuriya 1999) and was beyond the scope of this thesis.

Establishment of *N. neesiana* at the experimental site was not limited by available nutrients when the pre-existing vegetation had been killed: fertilisation with N, P, or both nutrients had no significant effect on the number of plants that established, the total biomass of *N. neesiana* per plot, or the mean biomass of plants that established. *Nassella neesiana* is common in the Southern Pampa and Flooding Pampa of Argentina (Soriano *et al.* 1992, Perelman *et al.* 2001, Honaine *et al.* 2006) where soils are notably deficient in P (Soriano *et al.* 1992), so *N. neesiana* may be somewhat ‘pre-adapted’ to Australian conditions where P is often deficient. However Grech (2007) found that *N. neesiana* juvenile plants responded strongly to P addition in pot trials in soils with an Olsen P of 6 mg kg⁻¹ (P “deficient”) by increasing leaf area, shoot weight, root length and weight, and root to shoot ratio, and mature plants also displayed a growth response. The responses in the Iramoo experiment suggest that P was not limiting under the prevailing conditions.

Lack of response of *N. neesiana* establishment or biomass to added N and P may not be particularly surprising. The great majority of plants established in 'full kill' plots which would have been enriched by the high levels of nutrients resulting from the breakdown of the vegetation that was killed (Wijesuriya and Hocking 1999). At the time of biomass harvest all *N. neesiana* plants were small, with a small absolute nutrient requirement that was evidently readily met from the soils enriched by plant decay, in the absence of substantial competition. Plants recruited almost entirely in 'full kill' plots where the deliberately applied fertilisers probably merely represented surplus nutrient resources. However the lack of an establishment response in 'half kill' and 'no kill' plots indicates that competition for resources other than soil nutrients was the controlling factor. Plenty of unshaded ground was available (77% bare ground before treatment) so competition for light was likely to be of no importance. Establishment patterns – concentrated away from plot boundaries (e.g. Fig.4.7) – and loss of juvenile plants due to drought suggest that competition for water may have been a dominant limiting factor for establishment and growth. This would explain why 'half kill' plots did not show and increase in *N. neesiana* plant establishment when N + P were added.

The negative impact of N fertilisation on *T. triandra* biomass would advantage *N. neesiana* when the two species co-occur under conditions of N enrichment. *Themeda triandra* is supposedly adapted to a low N environment and so would be disadvantaged when growing in competition with species that respond to N enrichment (Garden *et al.* 2003). Smallbone *et al.* (2008) found in pot experiments that *T. triandra* exhibited no growth response to increasing N enrichment of the soil, while the exotic annual grass they tested responded strongly. Wedin (1999) found that increased N levels favoured C₃ species over C₄ species. Prober *et al.* (2005) demonstrated that sugar applications that reduced soil nitrate levels enhanced the establishment and abundance of *T. triandra*. Badgery *et al.* (2005) found that of four Australian native grass species tested (*T. triandra*, *Bothriochloa macra* (Steud.) S.T. Blake, *Austrodanthonia racemosa* (R.Br.) H.P. Linder and *Microlaena stipoides* (Labill.) R.Br.), only the C₃ *M. stipoides* was competitive with the C₃ *Nassella trichotoma* (Nees) Hack ex Arechav. at high N levels, while all of the native grass species were more competitive than *N. trichotoma* at low soil fertility. Morgan (2007) found that responses to added N in *T. triandra* grasslands in western Victoria that lacked any substantial exotic flora were dependent on fire history, with annually burnt grasslands showing major increases in living biomass in response to N addition, and infrequently burnt (>4 years) areas showing declines of c. 20%. N appeared to be a limiting resource under conditions of frequent fire, and added N may have been immobilised in litter in the unburnt grasslands. It is also possible that native grasses growing vigorously under frequent fire regimes may deplete soil nutrients to a greater extent than plants growing more slowly when fire is less frequent. The grass and forb components reacted

similarly to N addition, in contrast to the results of the Iramoo experiment where only grasses exhibited significant biomass changes. However forbs at Iramoo were a very minor component of the biomass and a measurable response probably could not have been expected. Nutrient addition is believed to increase the fecundity of exotic grasses in annually burned *T. triandra* grassland (Morgan 2007) but there was no evidence in the Iramoo experiment that *N. neesiana* reproductive outputs were increased by the application of N and P fertilisers.

Lack of a strong growth response of native grasses to phosphorus in the experiment (except for *Austrodanthonia* spp.) despite the very low prior Olsen P values, may reflect that they are adapted to low P soils, so do not respond when superphosphate is applied. It is also possible, that under these particular experimental conditions, water was a greater limiting factor than soil nutrients, for the native grasses. Increased growth from *Austrodanthonia* spp. under P fertilisation would improve its competitiveness with *N. neesiana*. However Garden *et al.* (2003) reported progressive declines in biomass of *Austrodanthonia* spp. (mainly *A. duttoniana* (Cashmore) H.P. Linder) in “low” P pasture soils at Yass, NSW under increasing levels of P fertilisation (5.5, 11, 22 kg P ha⁻¹) that were not explained by responses in other pasture species, and at Harrogate, SA, slight increases in unidentified *Austrodanthonia* spp. from very low levels. Effects of added P on *Austrodanthonia* spp. may be species specific or dependent on small variations in P levels. These authors also reported slightly increased biomass of *T. triandra* in pasture at Bendigo, Victoria, with increasing P levels.

In pot experiments Groves *et al.* (1973) recorded a significant positive response of *T. triandra* to N and P when applied together but not to either nutrient when applied alone. However no such positive interaction was found in this experiment. Groves *et al.* (2003) found that *T. triandra* and *Austrodanthonia carphoides* became less competitive than four common introduced annual and perennial grasses as complete nutrient levels were increased. Grown alone *T. triandra* and *A. carphoides* produced maximum biomass per unit area at nutrient levels double the normal, and decreased biomass production at higher nutrient levels. Some seedlings of these native species died at high nutrient levels, which were “presumably” toxic. The exotic C₃ pasture grasses reached biomass production maxima at quadruple nutrient levels. *Themeda triandra* was the most productive species at the lowest nutrient level and its productivity declined steadily with increasing nutrient levels relative to the most productive species.

Effects of sugar

Wijesuriya (1999) demonstrated that addition of sucrose to deliberately dug and homogenised *T. triandra* grassland soil resulted in rapid, near complete exhaustion of soil nitrates, presumably due to increased microbial activity. In these soil-disturbed plots, a high biomass of exotic annual grasses and exotic Asteraceae weeds developed, but when sucrose was added

the biomass produced was much lower. Prober *et al.* (2005) demonstrated that sucrose addition in woodlands reduced soil nitrate to low levels and resulted in major reduction in the growth of exotic annuals and enhanced establishment and abundance of *T. triandra*. Reduction of plant-available nutrients via this mechanism presumably explains the strong effect of sugar on *N. neesiana* establishment in the experiment reported in this chapter. However this is mainly inference, since no evidence was gathered that sugar altered the levels of available nutrients, or that increased microbial nutrient uptake was responsible for the decreased plant growth. Perhaps the strongest circumstantial evidence that nutrient immobilisation occurred is from the findings of Wijesuriya (1999), who measured major reductions in N and P levels in the same types of soils, and in the immediate region in which the Iramoo experiments were carried out, at two separate sites. Although numerous previous studies have demonstrated that such nutrient reducing effects of sugar are usual, much remains unknown about the exact mechanism of how sugar applied to soils reduces nutrients.

Addition of simple carbohydrates to the soil often increases rates of decomposition of 'recalcitrant' C compounds in the litter and humus and increases the non-microbial pool of mineralised soil C (termed the 'priming effect'), but may cause decreases, and this appears to be dependent on the particular composition and structure of the saprotrophic soil community, the nature of the C inputs and other abiotic factors (Chigineva *et al.* 2009, Nottingham *et al.* 2009). R-strategists (primary decomposers) are usually increased, while secondary decomposers (k strategists) that decompose recalcitrant materials may be suppressed when populations of primary decomposers are stimulated (Chigineva *et al.* 2009). Thus sugar application may either increase the supply of plant-available nutrients by increasing rates of organic decay, or decrease the supply by providing a substrate for rapid population growth of a fraction of the soil microbe community, or both effects at the same time. The priming effect can increase C losses to the atmosphere as CO₂ that exceeds the amount of added C (Nottingham *et al.* 2009), or may decrease C losses (Hoyle *et al.* 2008). Carbon responses of soil microbial communities to added labile C are not consistent across soil types and ecosystems (Hoyle *et al.* 2008); therefore it appears likely that current consensus understanding about nutrient fluxes resulting from C addition (i.e. 'add C to mop up nutrients') may also be simplistic.

Application of sucrose to soil might have reduced *N. neesiana* establishment by affecting the macrobiota. Addition of labile carbon has been found to greatly alter populations of soil animals. Chigineva *et al.* (2009) recorded the near doubling of Collembola populations and a five-fold increase in earthworm biomass resulting from sucrose additions at the rate of 100 g C m⁻² month⁻¹. These animals may attack seeds or seedlings. Sugar might attract ants, which, if they are seed-harvesting species, might then have removed more seeds from the sugar-

treated plots. The brief assessments of the amount of seed apparent on the surface during the experiment provide no support for this proposition. Ants were observed removing *N. neesiana* seeds, but they were never observed removing sugar; neither was increased ant activity observed on sugar-treated plots.

Alpert and Maron (2000) found that use of carbon-based soil additives to decrease plant-available N had a differential negative impact upon exotic plants in a California coastal grassland, and suggested the use of such techniques to counter invasion. Prober *et al.* (2005) also found that C addition (sugar treatment) strongly reduced the growth of exotics, but the exotics did not include perennial grasses. However Reeve Morghan and Seastedt (1999) found that C addition similarly suppressed biomass production of both native and invasive plants. In the experiment reported here, the C treatment significantly reduced the biomass of all groups of exotic species and had no significant effect on any of the native species groups, supporting existing findings (Prober *et al.* 2005, Smallbone *et al.* 2007, Prober and Lunt 2009) that the technique has great potential for use in rehabilitation of Australian temperate grassy ecosystems.

Sugar and water stress

Sucrose may have had effects on soil chemistry and the plant-soil-microbe system that affected the outcome of the experiment by mechanisms other than the reduction of plant-available nutrients. Application of sugar may have disrupted water uptake by roots. Normally a water potential gradient exists from the soil through the plant to the atmosphere. Increasing the concentration of the soil solution via dissolved sugar results in decreased solute potential (osmotic potential). If soil water potential is less than that inside the roots, water may diffuse out by osmosis. Both plants and soil microbes incur energetic costs of osmoregulation, so dissolved sucrose in the soil water may result in water stress and increased costs to plants in maintaining turgor. However other studies have generally not voiced concerns that the reverse fertilisation effects of sugar application may be outweighed by effects on water relations in the soil.

Off-plot seed movement

The panicle seeds of *N. neesiana* are classed as creeping diaspores (Davidse 1986, Connor *et al.* 1993) that are able to move along the ground and position themselves in microsites favourable for germination (Gardener and Sindel 1998, Sinclair 2002). Creeping diaspores of grasses generally result in little actual dispersal via ‘creeping’, this adaptation being more important in enabling microsite lodgement (Peart 1979, Davidse 1986). Their movement capabilities on rough, dry ground appear to be very limited and insufficient to cross the 1 m buffer zones between plots in the experiment. Either a pre-existing soil seed bank or other dispersal factors must be invoked to explain establishment in unseeded plots.

Seed may have been moved on to unseeded plots by extreme wind events or by the activity of animals. Seed-harvesting ants (*Pheidole* sp.) were observed carrying *N. neesiana* seeds from plots to their nests and excavating some seeds that were firmly anchored in the ground by their calluses, and may have been responsible for some seed dispersal. These ants are seed predators, but may occasionally abandon seeds before they are delivered to the nest, or discard viable seeds outside their nests.

Although a total of 22,500 seeds were applied in the seed treatment, only 204 plants had established on the experimental plots at the time of biomass harvest (0.9 % of the individuals applied). Some of the same factors that may have resulted in movement of seed to unseeded plots may have been responsible for disappearance of seed from the treated areas. Ants are the most likely cause of major losses. None of the bird species frequently observed foraging on the ground in the Iramoo grassland are specialised granivores, although all are known to consume grass seeds (Barker and Vestjens 1989 1990).

Natural resistance to invasion

It has been recognised for many years that natural temperate grasslands in good condition are strongly resistant to invasion by exotic plants. Patton (1935 p. 175) noted in respect of the grasslands of the Victorian basalt plains that “So long as the natural vegetation covering, open though it be, is maintained, entrance to new-comers is denied.” The dominant tussock grasses not only command most of the space and light but may also starve the intertussock species of moisture and nutrients (Keith 2004). Prober and Lunt (2009) argued convincingly that *T. triandra* provides much of this biotic resistance in these grassy ecosystems: it is a keystone species that regulates N cycling and so controls invasion by exotic species. The experiment at Iramoo confirms this established understanding: removal of *T. triandra* enabled *N. neesiana* invasion.

The claim of Muyt (2001 p. 73) that fire “generates bare ground and reduces immediate competition, conditions that are ideal for seed germination” of *N. neesiana* has been proven false. There was little evidence in the experiment that more than minor *N. neesiana* establishment occurred in untreated plots, despite the very recent fire, open vegetation structure and low cover by native grasses. Bare ground is probably occupied beneath the soil surface, mainly by roots of the dominant grasses, which presumably would in part at least survive the fire or recover rapidly post-fire and pre-empt resources for potential invaders; the native grasses may even be stimulated to take up additional nutrients, as the dead material in their crowns is removed, stimulating faster rates of growth. Pot studies have demonstrated that below-ground competition for water and nutrients in grasslands is generally of much greater importance than aboveground competition (Schmidt *et al.* 2008). The native flora is adapted for frequent fire and is not significantly impacted when fire intervals are <5 years

(Wong and Morgan 2007). However very long fire intervals may allow the development of very high standing plant biomass, enabling hotter, longer-lasting, more destructive fires, that may kill the dominant native grasses, or reduce their capacity to take up and hold nutrients and water, and thus allow *N. neesiana* invasions. Fires at intervals of >5 years in *T. triandra* grasslands are too infrequent to maintain native plant diversity (Wong and Morgan 2007).

Sharp (1997) created 1 m² areas of bare ground experimentally using glyphosate herbicide in Dry *T. triandra* and *Austrodanthonia* grasslands in the ACT and studied colonisation of the gaps for 18 months. As found in the Iramoo experiment after 16 months, native grass cover did not recover to pre-treatment levels after 18 months. She found that exotic grass cover and richness initially increased, but after 18 months decreased to levels similar to those prior to treatment. Native and exotic forb richness and cover was increased. Evidently the continuity of occupation by invading species may not be assured. However there was no indication in the Iramoo experiment that the gains made by *N. neesiana* might later be lost.

Burke and Grime (1996) demonstrated that the creation of bare ground in limestone grassland by removal of indigenous plant cover, particularly the dominant grass, directly controlled the subsequent cover of sown introduced species, and that fertilisation (with NPK) greatly magnified the establishment success of the invading species. Resistance to invasion was hypothesised to result from “the combined effects of biomass and litter production by the residents” (Priour-Richard and Lavorel 2000 p. 3). In contrast, Tilman (1997) demonstrated that addition of seeds of new native or exotic species to 1 m² plots of intact native grassland resulted in increased plant diversity, indicating that the assembled community was not saturated and could readily accommodate additional species with little effect on the existing components. However this was in a sod-grass rather than a tussock grass grassland, so different mechanisms might be operating.

Nassella neesiana was able to occupy the disturbed areas at Iramoo because there was little competition from native species. Native seed banks in Australian temperate grasslands are generally small and ephemeral (Lunt 1990b 1995a 1996, Morgan 1995a 1998c, Lunt and Morgan 2002). None of the native perennial inter-tussock species in existing native temperate grasslands are obligate seed regenerators, almost all being obligate resprouters, or resprouting and with limited seedling production, and mostly able to set, and actually setting seed within 12 months of regeneration (Lunt 1990c, Morgan 1996 1998c, Lunt and Morgan 2002). Thus disturbance that kills the existing native plant population effectively leaves clear ground for invasion by an invader such as *N. neesiana* that produces high propagule pressure. Restoration of these grasslands, as in native grasslands elsewhere in the world (Callaway and Maron 2006), is arguably constrained by lack of native seeds, rather than the presence of a dominant exotic competitor.

Only a small proportion of *N. neesiana* seeds applied in this experiment resulted in established plants. The number of established plants within plots after 16 months was accounted for by only 0.9% of the seeds applied. In individual plots, the maximum recruitment was in plot 30 where 63 plants resulted from 500 viable seeds (12.6%), 69 weeks after seed application. Prior testing indicated >80% viability of the *N. neesiana* seeds that were applied, and inspections indicated that there was very minor amount of seed lodgement outside plot edges. High levels of seed or seedling mortality or removal are therefore indicated.

Harvester ants (*Pheidole* sp.) are known to harvest large numbers and high proportions of broadcast grass seeds in some circumstances in Australia (Campbell 1966), and observations during this experiment indicated that they probably destroyed or removed a large proportion of the seed applied. Indeed, *Pheidole* sp. devoted extraordinary efforts to excavating and removing some of the *N. neesiana* seeds applied to plots that were firmly embedded upright in the soil. Campbell (1966) found that pasture grass seeds become unattractive to *Pheidole* spp. when they have swelled prior to germination. He demonstrated that the proportion of seed harvested depends directly on the foraging time available between seed application and the occurrence of rainfall sufficient to stimulate germination. In the Iramoo experiment c. 16 weeks elapsed from the time of *N. neesiana* seed application to the time of major germination, during the winter and early spring period when seed production by the existing vegetation would have been at a minimum. Reduced availability of other food sources might therefore have resulted in more thorough ant harvesting of *N. neesiana* seeds than under other circumstances. Nevertheless it is clear that biotic resistance to invasion arises not just from the flora.

Competitive effects of *N. neesiana*

The *N. neesiana* plants that established had a marked suppressive effect on the growth of native grasses. This is probably best characterised in part as a priority effect due to the establishment of plants in the absence of other competition. Space and resources pre-empted by *N. neesiana* were unavailable for use by other species. Simultaneous application of native seed would be required to properly examine competitive effects.

Primary juvenile period and first seed production

The demonstration that the primary juvenile period, leading to viable seed production, can be less than one year reinforces the need to control new outbreaks as an urgent priority to minimise propagule pressure at the expanding edges of infestations.

Contrasting ecology of cleistogenes

The experiment described above examined establishment from panicle seeds, which have a very different biology to that of stem and basal cleistogenes (Gardener 1998). Dyksterhuis

(1945) found that cleistogenes of the closely related *N. leucotricha* commonly failed to germinate within a year of their production and germinated especially within old, closely grazed, dead tussocks, while panicle seeds, in contrast, germinated in areas cleared of litter, outside of tussocks. Observations reported by Slay (2001) indicate that *N. neesiana* cleistogenes, particularly the basal cleistogenes, behave similarly to those of *N. leucotricha*. Panicle seeds of *N. neesiana* have previously been reported to germinate only in bare areas (Gardener *et al.* 1996a) or when gaps are created in pastures (Gardener *et al.* 1999). The limited evidence suggests *N. neesiana* stem and basal cleistogenes probably have higher rates of germination in closed swards or in unkilld areas of vegetation than panicle seeds, and juvenile plants of cleistogene origin might have higher survival rates than panicle seeds in areas with existing plant cover.

Conclusions

Nassella neesiana was able to establish or colonise at the experimental site only where there was propagule pressure accompanied by disturbance involving the death of the dominant grasses. Establishment in the absence of competition from established dominant grasses is arguably a trivial result that conforms with general understanding of the conditions required for recruitment of perennial grasses. Removal of competition by other plants generally results in increased seedling survivorship (Fenner 1987). That disturbance of ground previously unoccupied by *N. neesiana* enables *N. neesiana* invasion is also unsurprising, although the concept appears notably absent from the agriculturally-oriented Australian *N. neesiana* literature (e.g. Snell *et al.* 2007). Probably of more significance is the incapacity of *N. neesiana* to establish in areas occupied by native grass tussocks, even though there was little competition above-ground for light. Also significant is that some of this resistance to invasion was maintained even in ‘half kill’ of native tussocks – so that there was significantly less than a directly proportional outcome for *N. neesiana* establishment on the ‘half kill’ plots – that is, removal of half the native tussocks resulted in much less than half the replacement by *N. neesiana* plants than occurred in ‘full kill’ plots. The results do however greatly clarify the issue of whether *N. neesiana* is an ‘active’ invader, i.e. that it possesses superior competitive abilities, or instead is a ‘passive’ invader that follows disturbance. The low levels of *N. neesiana* establishment in half kill plots also suggests that native grasslands are significantly resistant to invasion, and that large ecological gaps are required before *N. neesiana* can establish in any numbers.

The important question of “which disturbances are most likely to lead to invasions” (Hobbs 1991 p. 100) is also clearly answered by the Iramoo experiment: disturbances that kill the dominant existing grasses. The finding that sugar application results in a major reduction in *N. neesiana* establishment further elaborates this understanding. It is presumed that sugar

functioned as a ‘reverse fertilisation’ treatment by stimulating soil microbe populations. Major increases in available soil nutrients would have occurred in the ‘full kill’ plots due to rapid decay of the killed vegetation, and temporary immobilisation of these nutrients using sugar presumably severely reduced *N. neesiana* recruitment. Deliberately added nutrients were presumably immobilised in the same way, or in vegetated plots were preferentially sequestered by the existing vegetation. This again is in general conformity with existing knowledge: disturbance is important for weed establishment when it increases the availability of a limiting resource (Hobbs 1989 1991, Davis *et al.* 2000). Recruitment may be expected to be first limited by whatever is the most limiting resource for seedling establishment and growth. Rainfall driven *N. neesiana* seedling establishment, along with the observed patterns of juvenile plant consolidation away from plot edges, and mortality due to drought, plus the reported results of nutrient addition suggest that the most critical resource for recruitment in this experiment may have been water, not N or P.

Within 18 months of the application of 500 *N. neesiana* seeds m⁻² to cleared ground, a population of *N. neesiana* plants was established that was able to produce >200 panicle seeds m⁻². Under conditions of continuing strong disturbance *N. neesiana* appears precocious and fecund enough to provide the propagule pressure necessary to continue to invade new areas from sites it has only recently occupied.

Maintenance of native plant diversity (mainly inter-tussock forbs) in the natural temperate grasslands of south-eastern Australia requires *inter alia* the reduction of cover of dominant grasses, prevention of senescence dieback of *T. triandra*, the retention of intertussock spaces and the creation of recruitment opportunities for native plants (Reynolds 2006, Wong and Morgan 2007). The conundrum for these grasslands is that such activities at the same time may create more open areas and relief from competition above and below ground that may increase susceptibility to invasion by *N. neesiana* and other exotic weeds. Regular opening of gaps (at least the maintenance of intertussock space) is required for forb seedling recruitment in *T. triandra* grasslands (Morgan 1995b, Sharp 1997) and recruitment of many forb species may require more severe small scale soil disturbances formerly achieved by native vertebrates (Robinson 2003 2005, Reynolds 2006). In the experiment reported in this chapter, it was found that the creation of large gaps (1 m²) by killing the native vegetation (in ‘full kill plots’) had the strongest influence on *N. neesiana* establishment, and that small gaps (10-30 cm) enabled very little survival of juvenile *N. neesiana* to maturity (in ‘half kill’ plots). Gaps are colonised by the species present in the seed bank and by dispersed seeds. If soil seed banks are absent, the timing of gap creation determines which dispersed seeds arrive first and can gain an advantage. The timing of the disturbance in relation to the availability of seed of potential colonising species is of critical importance (Körner *et al.* 2008). Where a soil seed

bank is present, its species composition is likely to have an influential role in determining the vegetation that subsequently develops. Where the soil seed banks of native species is generally low, as commonly occurs in these grasslands (Lunt 1990b, Morgan 1998c) and was evidently the case in the experimental area, gaps are likely to be filled by the exotics in the seed bank and whatever more highly dispersible species exist in the immediate area. If native grasses are dispersed onto the disturbed ground before the arrival of *N. neesiana* and prior to conditions suitable for germination, then native species may well occupy the disturbed ground. Evidence that this happens when *T. triandra* seeds are deliberately applied after strong disturbance in these temperate native grasslands has been provided by Phillips (2000) and Mason (1998 2004).

Apart from the above considerations there is little reason to suspect that the results obtained at this one site cannot be generalised to other similar grasslands. *Nassella neesiana* may also invade in the absence of major disturbance, but the experiment indicates that if it does, invasion would be at a much slower rate and in greatly reduced numbers. Testing of such a proposition is difficult given that anthropogenic alteration of the native grassland environment is nearly universal, and 'natural' disturbance is widespread and appears also to be a requirement for establishment of native grassland species.