MR. DAVID P. JOHNSON (Orcid ID : 0000-0001-8728-8382)

Article type : Research article

# Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland

Authors: David P. Johnson, Jane A. Catford, Don A. Driscoll, Philip Gibbons

Johnson, D. P. (Corresponding author, david.johnson@anu.edu.au)[1]
Catford, J. A. (j.a.catford@soton.ac.uk)[1,2,3]
Driscoll, D. A. (d.driscoll@deakin.edu.au)[4]
Gibbons, P. (philip.gibbons@anu.edu.au)[1]

[1]Fenner School of Environment and Society, The Australian National University, Building 141, Canberra, ACT 6201, Australia
[2]Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK
[3]School of BioSciences, The University of Melbourne, Vic 3010, Australia
[4]School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Burwood Campus, Vic 3125, Australia

Printed journal page estimate: 6780 words (8.5 pages), display items 1.7 pages, total 10.2 pages.

Keywords: Temperate grassland; Grassland restoration; Grassland structure; Leaf litter; Resource availability; Seed addition; Seedling emergence; Native forb; Exotic plant invasion; Recruitment limited; Seed limited.

## Abstract

Questions

Long-term restoration of native forb diversity can only be achieved if native forb species can recruit (colonise and establish) and reproduce. We asked whether native forbs in a temperate grassland were seed limited, and how the recruitment of native and exotic forbs is affected by grassland structure and resource availability.

Location

Australian Capital Territory, south-eastern Australia.

Methods

We conducted a field experiment in a temperate grassland dominated by a native tussock grass to assess effects of: 1) addition of native forb seed, 2) thinning of native grass tussocks, 3) leaf litter removal, and 4) exotic plant removal on the recruitment of native and exotic forbs. These four actions can alter grassland structure and the availability of soil nutrients, soil moisture, and light. We used generalised linear mixed models to determine the importance of seed addition, grassland structure and resource availability on the richness and abundance of sown native forbs, and the abundance of exotic forb seedlings and unsown native forbs.

Results

Adding seed increased the species richness and abundance of native forbs. Tussock thinning and litter removal increased species richness and abundance of sown native forbs, and the abundance of exotic forb seedlings. Exotic plant removal also increased the abundance of sown native forbs. Abundance of unsown native forb species was unaffected by the experimental treatments. Species richness and abundance of native forbs and abundance of exotic forbs declined with increasing tussock grass cover. Leaf litter restricted the abundance of native forb species more than exotic forb species.

Conclusion

Native forb recruitment predominantly relied upon seed addition, suggesting that seed limitation is a major barrier to the recovery of degraded grasslands. Reducing the cover of living grass tussocks facilitated recruitment of native and exotic forbs, and removing litter disproportionally increased recruitment of native forbs compared with exotics. Combining seed addition with the reduction of both living and dead grass biomass should help restore native grassland forbs.

# Introduction

Forb diversity has declined in many parts of the world following the conversion of grasslands for agriculture, and often remains low even when agriculture is subsequently abandoned (Wheeler et al. 2015; Fensham et al. 2016). Forbs represent a large proportion of plant species richness in natural grasslands (Tremont & Mcintyre 1994; Klimek et al. 2007; Jacquemyn et al. 2011; Mitchell & Bakker 2016) and their decline reduces the functional diversity of grassland ecosystems (Hooper et al. 2005). The decline of grassland diversity has flow-on impacts on pollinator diversity (Wilkerson et al. 2014), resistance to invasion (Hulvey & Zavaleta 2012), and the availability of habitat resources for other grassland dependent taxa (Barrett et al. 2008). Restoring and maintaining forb diversity is thus a key conservation goal for grasslands around the world (Hobbs et al. 2013). The Australian Government lists the ecosystem under consideration in this study as Critically Endangered, largely on the basis of lost forb diversity (https://www.environment.gov.au/system/files/resources/be2ff840-7e59-48b0-9eb5-4ad003d01481/files/box-gum.pdf; accessed 12-10-2017).

Low rates of seedling emergence and survival may explain why few forb species recover once agriculture has ceased (Fayolle et al. 2009; Donath & Eckstein 2010). Poor seedling emergence and survival can result from limited availability of both seeds and other resources that are needed for early survival and growth (Zobel et al. 2000; Brandt & Seabloom 2012; Dybzinski & Tilman 2012). Seed supply may be limited because of an absence of source populations, poor dispersal from source populations, and the lack of a persistent soil seedbank (Brandt & Seabloom 2012). If forb species are seed-limited, increasing seed availability (e.g. through direct seeding) will be required (Jacquemyn et al. 2011; Morgan & Williams 2015). Even where adequate seed is present, recruitment requires resources essential for plant growth (light, nutrients and moisture), which may be influenced by grassland structure and competition (Morgan 1998b; Hellström et al. 2009; Frances et al. 2010).

Understanding the ways in which different management actions affect native and exotic forb recruitment will help optimise restoration (Fig. 1). For example, the actions needed to restore native forb diversity will differ if they are physically restricted by accumulated leaf litter, or if they are resource-limited due to competition from established vegetation (Moles & Westoby 2004). If restricted by litter or competition from native grasses, litter removal and control burns would be effective management actions (Fynn et al. 2005), whereas weed control would be more effective in the case of restriction by exotic species (Prober & Thiele 2005). Understanding how exotic forbs respond to management is also important because management actions designed to increase native forb recruitment may also inadvertently facilitate exotic species recruitment (Faithfull et al. 2012; Driscoll 2017).

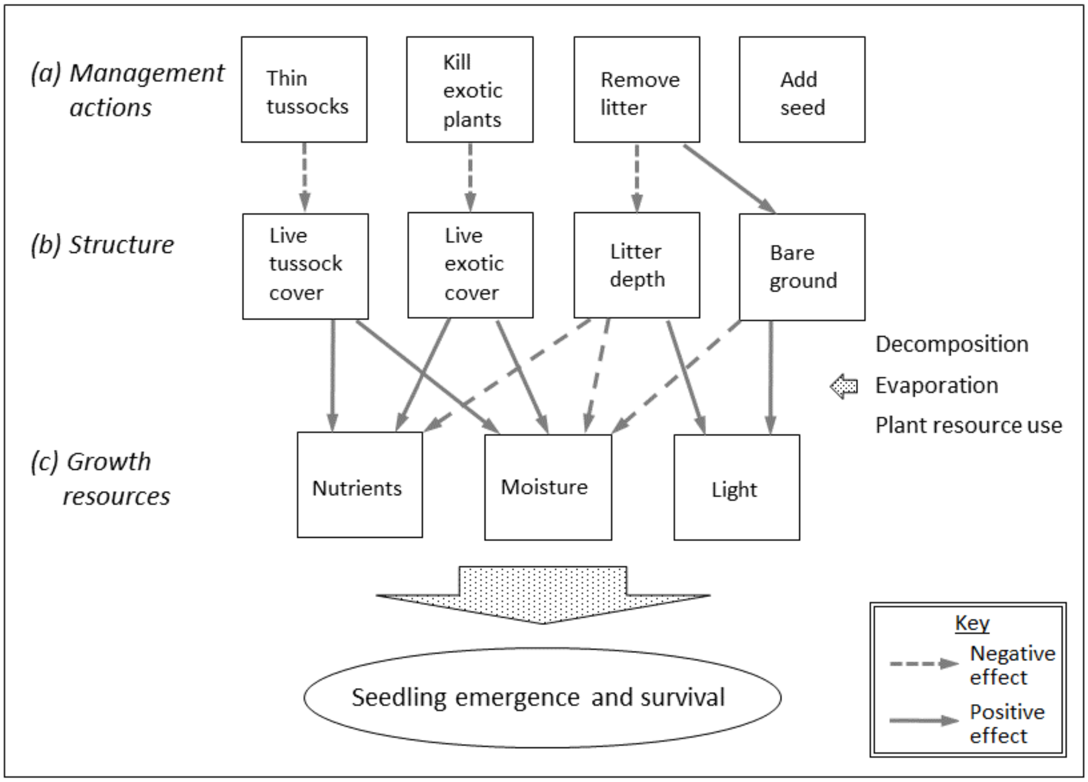

**Fig. 1.** Hypothesised impacts of management actions on grassland structure, resource availability, and native forb recruitment. Management actions (a) change grassland structure (b), and structural changes influence resource availability (c) by initiating changes in the rates of decomposition, evaporation and plant resource use. The changes flowing from each of these management actions are hypothesised to have a positive effect on seedlings.

Temperate grasslands in south-eastern Australia exemplify the challenges faced when attempting to restore forb diversity in grasslands. Agriculture practices over the last 200 years have drastically simplified Australian grasslands, with fertiliser application, herbicide use, sowing of exotic pasture grasses, livestock grazing, and suppression of fires all being common practice. As a result, less than 0.5% of the original extent of diverse grassland ecosystems remains (Prober et al. 2013), and remnant grassland is now isolated in small fragments (Tremont & Mcintyre 1994), limiting opportunities for seed dispersal among patches.

In this experimental study, we implemented four treatments (thin tussocks, kill exotic species, remove litter, and add seed) that represent key management actions for restoring grassland forbs. Our aims were to: i) assess the necessity of seed addition for the re-establishment of native forbs, ii) determine which treatments, structural components and resources are associated with native forb recruitment (Fig. 1), and iii) compare how the abundance of native forbs and exotic forbs respond to these treatments.

# Methods

## Study site

We conducted the experiment in a temperate grassland in the Australian Capital Territory (ACT) in south-eastern Australia (35.270562° S, 149.026425° E, 574 m above sea level). The site was located in a nature reserve, and has a median annual rainfall of 650 mm. The 1.5 $km^2$ reserve was previously a pastoral lease starting with sheep grazing from the 1920s and more recently (1985-2005) low intensity cattle grazing. It was declared a nature reserve in 2010. In 2011 and 2012, a non-residual selective broadleaf herbicide (fluroxypyr) was applied (via boom-spraying) in parts of the reserve to control (successfully in the short-term) an invasive exotic forb, *Hypericum perforatum*.

We established the experiment on a gentle (5 degrees), south-facing slope that was dominated by *Themeda triandra* (Kangaroo grass) before the experiment began. *Themeda triandra* is a summer growing native perennial tussock grass, which can become dominant when present. In productive conditions, *T. triandra* accumulates a thick mat of dead leaf litter—from leaves that die over winter—if not removed (e.g. by fire). The litter was up to 3 cm deep in parts of the study site, with an average dry mass of 310 $g.m^{-2}$. Before the start of the experiment, 43 native forb species (up to 10 species per 0.5 $m^2$) were observed across the whole reserve (Johnson 2013, Unpubl.), but only 10 forb species (up to 4 species per 0.5 $m^2$) were observed in the experimental site, probably due to previous herbicide use and lack of disturbance. Exotic grasses and forbs are abundant in the surrounding area (>2 m from experimental site), but made only a minor contribution to vegetation in the experimental site.

## Experimental design

The experiment was a fully crossed factorial design with all 16 ($2^4$) treatment combinations randomly arranged within each of six blocks, making a total of 96 plots (Table 4 in Appendix S1). The plots, measuring 0.75 m x 0.75 m and separated by at least 75 cm, were located within a single 1000 $m^2$ area of homogenous grassland to minimise variation due to topography, soils, weather, and vegetation type. The area was fenced to minimise grazing by vertebrate herbivores (kangaroos, rabbits).

The experimental plots within each block were randomly treated with every combination of: (a) *T. triandra* tussocks thinned by ~50% by spraying with glyphosate (7.2 g.L), (b) all leaf litter on the ground removed by hand, (c) all exotic grasses and forbs killed by painting individuals with glyphosate (7.2 g.L), and (d) addition of seed for 14 native forb species that occur naturally in the region (Tables 3 and 4 in Appendix S1). Three of the sown species already occurred in the site. Plants killed with glyphosate were not removed. All treatments were applied in the austral spring (5-6 Oct

2014), and exotic plant removal was done three more times: late-spring (Nov 2014), summer (Jan 2015) and early autumn (Mar 2015). The plots were watered with a known amount applied evenly using a spray nozzle and a timer as required to encourage germination in the first month, and after that at a rate equivalent to the 75th percentile of historic rainfall to simulate a good growing season.

### Response variables

The four response variables were: i) the species richness of sown native forbs (age < 8 months); ii) the abundance (count of individuals) of sown native forbs; iii) the abundance (count) of unsown exotic forbs; and iv) the abundance (count) of unsown native forbs (Table 5a in Appendix S1). Response variables were measured in the central 0.5 m x 0.5 m of each 0.75 m x 0.75 m plot to avoid edge effects. For sown native forbs, we used the maximum counts from two surveys conducted in early summer and early winter, 11 weeks and eight months after the treatments, respectively, as the best indication of total recruitment over that period. Count averages were not possible because of difficulty determining the season in which individuals had emerged. We did not measure the species richness of exotic forbs as individuals were too small to identify to species-level (Table 8c in Appendix S2).

### Explanatory variables

We measured six potential explanatory variables to characterise physical structure and resource availability within each plot.

Physical structure within the central 0.5 m x 0.5 m of each plot was represented by i) the percentage area covered by living grass tussocks, not including attached tussock biomass that had senesced due to the thinning treatment or natural die-back over winter; ii) ground litter depth averaged from three measurements; and iii) the visually estimated percentage area of bare ground (Table 5b in Appendix S1). These data were recorded separately for each quarter of a 0.5 m x 0.5 m quadrat, and the results subsequently averaged. We did not include the cover of exotic species as a structural explanatory variable because exotic species made up little cover (mean 2.2% ± SD 7.1%) prior to the final months of the experiment.

Resource availability was represented by: i) the percentage of light penetrating the canopy to above the litter layer, measured with a LI-COR LI-191 line quantum sensor positioned along both diagonals of the 0.75 m x 0.75 m square plots; ii) available soil phosphorus measured within a $NaHCO_3$ extract of the soil using a Lachat QuikChem 8500 flow injection analyser; and iii) the percentage of soil moisture, by volume, measured in the outer 12 cm on opposing sides of each plot with a Delta-T Theta Probe ML3 moisture probe four days after rain (Table 5b in Appendix S1). Soil sampling and moisture measurement was done in the outer 12 cm to avoid disturbance to the central 0.25 $m^{-2}$ where the plant responses and structural variables were measured.

We also measured the oven-dry weight (80° C) of litter lying on the ground—consisting almost entirely of dead tussock leaves—gathered from twelve untreated 0.25 $m^{-2}$ patches, two beside each block, to estimate the overall litter biomass.

### Statistical analysis

We used generalised linear mixed models (GLMM) within the "glmmTMB" package in R statistical software (R Core Team 2016) to model relationships between plant responses to: (a) the experimental treatments, and (b) to explanatory covariates representing physical structure and the availability of resources within each plot that are potentially affected by the treatments. Responses to treatments and covariates were modelled separately as there were strong correlations between the tussock thinning treatment and the percent cover of living tussock ($r$ = -0.65), and between the litter removal treatment and litter depth ($r$ = -0.86). All combinations and subsets of the structural variables (percent cover of living tussock, litter depth, and bare ground) and resource variables (light penetrating the canopy, available soil phosphorus, and soil moisture) were evaluated as potential model terms. Total tussock cover was excluded due to correlation with cover of living tussock ($r$ = 0.82). Light at ground level was excluded due to correlation with light penetrating the canopy ($r$ = 0.72) and bare ground ($r$ = 0.71). The remaining potential explanatory variables were not highly correlated ($r < 0.7$).

We used a Poisson distribution with log-link function to model sown native forb species richness, and negative binomial distributions for plant abundance data because they were more widely dispersed than Poisson distributions due to high numbers of zeros and several high scores. Of the 96 plots in the experiment, 48 were sown with native forbs. Only data from the 48 sown plots were used to model sown native forb responses, because no sown native forbs were found in the unsown plots. We modelled exotic forb responses using data from the 48 plots where the exotic removal treatment was applied to ensure that counts were of seedlings only. Data from all 96 plots were used to model the response of unsown native forb species.

We fitted block number, representing the individual blocks in which treatments were grouped, as a random effect term to account for variation between blocks. We used Bayesian Information Criterion (BIC) information criteria to determine the fixed terms of the 'best fit' models, out of all possible non-correlated combinations. We calculated *Delta BIC*–the difference in BIC between the 'best fit' and the 'best fit minus one term' models–as a basis for comparing the relative influence of individual terms (Burnham & Anderson 2003).

## Results

We recorded a total of 4465 forb individuals: 1264 seedlings for nine of the 14 sown native forb species (Table 8a in Appendix S2), 2001 individuals of exotic species (including 900 seedlings in plots where exotic species had been removed), and 1200 unsown native forb individuals of any age (Table 8b in Appendix S2).

Prior to establishing the treatments, native species richness (mean 1.1 ± SD 1.1) and percent cover of native species (6.2% ± 10.7%) across all plots (not including *T. triandra*) was higher than for exotic species (0.3 ± 0.6, and 2.2% ± 7.1%).

### Responses to treatments

There were statistically significant associations between three of the four response variables (sown native forb richness, sown native forb abundance and exotic forb abundance) and one or more of the treatments (Table 1, Fig. 2). Seedlings of the sown native forb species only emerged where seed had been added, and with no other treatments mean total native richness and abundance increased by 122% and 61% respectively. Where seed was added, mean total richness and abundance of native forbs increased with tussock thinning (+214%, +78%), litter removal (+160%, +64%), or both (+575%, +488%) (Table 4 in Appendix S2).

Sown native species richness and abundance, and exotic abundance were positively associated with tussock thinning and litter removal. Sown native abundance was also positively associated with exotic species removal (Table 1a-c, Fig. 2a-c). Litter removal had a greater influence than tussock thinning on the richness (*Delta BIC* = 49 vs 8) and abundance (*Delta BIC* = 41 vs 25) of sown native forbs (Table 1a-b). However, litter removal and tussock thinning had similar influence on the abundance of exotic forbs (*Delta BIC* = 9 vs 8) (Table 1c). There were no additional effects from interactions between treatments.

**Table 1.** GLMM models fitted to predict the effects of the experimental treatments on: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only) and (c) exotic forb seedling abundance (exotic removal plots only). There were no significant models predicting the response of (d) unsown native forb abundance to the treatments (all plots). Block number was included as a random effect in each model.

| Plant group response | BIC | df | Treatment | Estimate | Std. Err. | F pr. | Delta BIC |
|---|---|---|---|---|---|---|---|
| (a) Sown native forb species richness | 204.2 | 43 | Thin tussocks | 0.498 | 0.146 | <0.001 | 8 |
| | | | Remove litter | 1.105 | 0.163 | <0.001 | 49 |
| (b) Sown native forb abundance | 353.6 | 42 | Thin tussocks | 1.697 | 0.201 | <0.001 | 25 |
| | | | Remove litter | 2.284 | 0.240 | <0.001 | 41 |
| | | | Remove exotics | 0.492 | 0.149 | <0.001 | 6 |
| (c) Exotic forb abundance | 367.9 | 43 | Thin tussocks | 1.152 | 0.311 | <0.001 | 8 |
| | | | Remove litter | 1.212 | 0.317 | <0.001 | 9 |
| (d) Unsown native forb abundance | N/A | | | | | | |

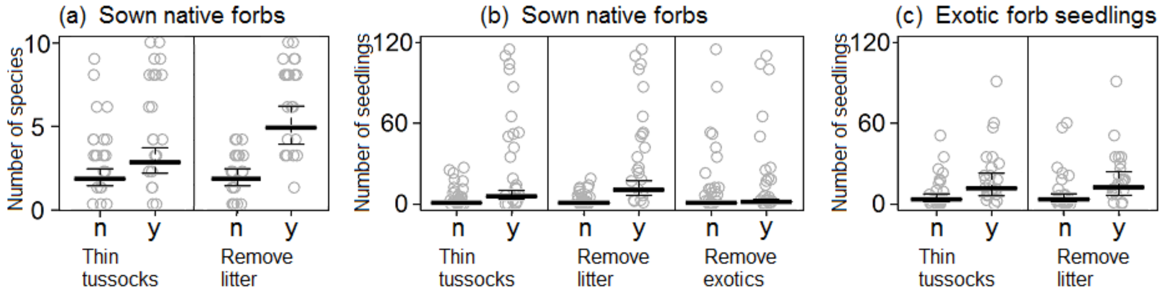

**Fig. 2.** Effects of the three treatments (thin tussocks, remove litter, remove exotics) on (a) sown native forb species richness, (b) sown native forb abundance and (c) exotic forb abundance. Predicted responses (mean ± 95% confidence intervals) to individual treatments (y), assuming all other treatments are controls (n), were obtained from GLMMs in which the block number was fitted as a random effect. Observed values are indicated by open circles [two observed counts not shown – maximum (b)(193) and maximum (c)(188)].

### Responses to structure

Sown native species richness and abundance, and unsown native forb abundance were significantly negatively associated with the explanatory variables representing grassland structure (live tussock cover and litter depth). Litter depth had the greatest relative influence on these models (*Delta BIC* = 26, 27 and 5 respectively). In addition, unsown native forb abundance was negatively associated with the percent area of bare ground. Exotic forb abundance was associated with live tussock cover but not litter depth (Table 2a-d, Fig. 3a-d). Weak correlations between live tussock cover and the availability of the measured resources (light penetrating the canopy, *r* = -0.38; light at ground level, *r* = 0.00; phosphorus, *r* = -0.13; soil moisture, *r* = -0.24) suggest the effects of live tussock cover cannot be explained by its effect on these resources. A negative correlation between litter depth and light at ground level (*r* = -0.85) demonstrates a strong shading effect, but weak correlations between litter and the other measured resources (phosphorus, *r* = 0.00; and soil moisture, *r* = 0.33) suggested the effect of litter was not because of its influence on these resources.

**Table 2.** GLMM models predicting the responses of: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only), (c) exotic forb seedling abundance (exotic removal plots only), and (d) unsown native forb abundance (all plots) to the experimental covariates. Block number was included as a random effect in each model.

| Plant group response | Covariate | BIC | df | Estimate | Std. Err. | F Pr. | Delta BIC |
|---|---|---|---|---|---|---|---|
| (a) Sown native forb species richness | %Live tussock cover | 206.7 | 42 | -0.012 | 0.005 | 0.031 | 1 |
| | Litter depth | | | -0.671 | 0.134 | <0.001 | 26 |
| | %Light penetrating canopy | | | 0.017 | 0.007 | 0.021 | 1 |
| | Phosphorus | | | -0.124 | 0.051 | 0.014 | 2 |
| (b) Sown native forb abundance | %Live tussock cover | 371.5 | 43 | -0.044 | 0.011 | <0.001 | 9 |
| | Litter depth | | | -1.475 | 0.204 | <0.001 | 27 |
| (c) Exotic forb abundance | %Live tussock cover | 352.7 | 43 | -0.022 | 0.010 | 0.027 | 1 |
| | %Light penetrating canopy | | | 0.065 | 0.012 | <0.001 | 17 |
| (d) Unsown native forb abundance | %Live tussock cover | 686.3 | 89 | -0.016 | 0.006 | 0.005 | 3 |
| | Litter depth | | | -0.530 | 0.173 | 0.002 | 5 |
| | Soil moisture | | | 0.058 | 0.025 | 0.019 | 0 |
| | Bare ground | | | -0.022 | 0.008 | 0.007 | 3 |

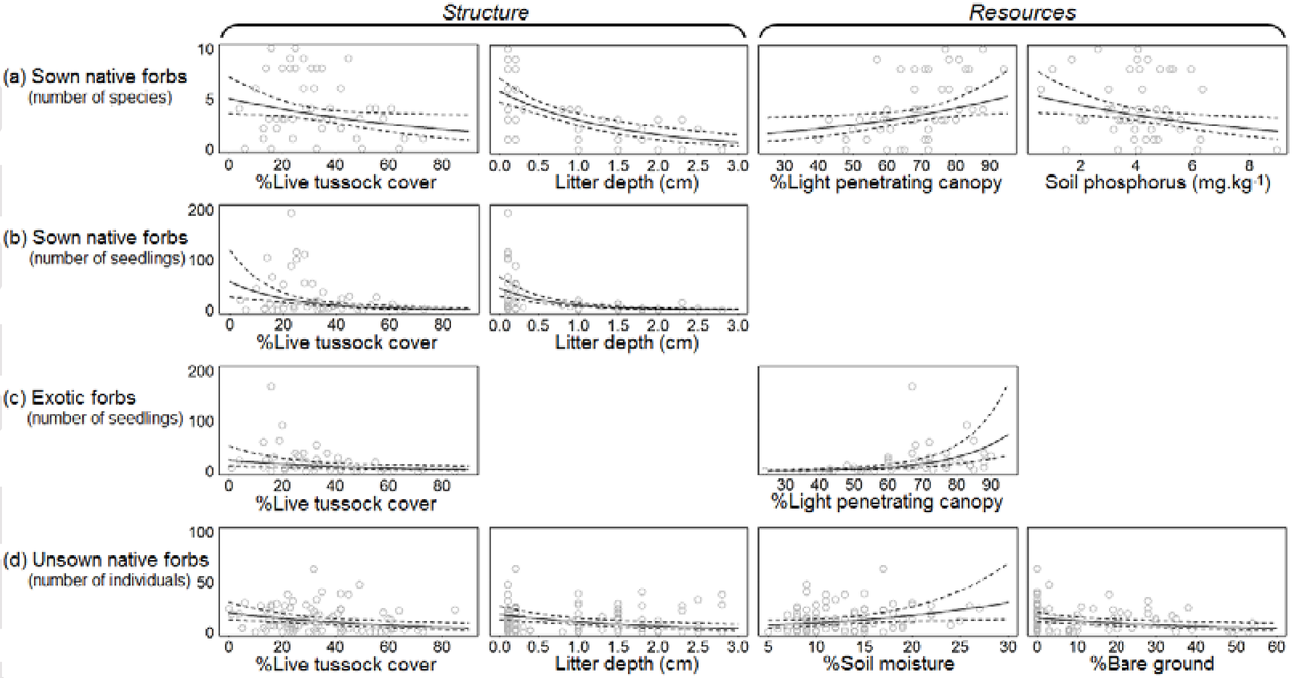

**Fig. 3.** Predicted responses to structural and resource variables (mean ± 95% confidence intervals) from the fitted GLMMs for a) sown native forb species richness, b) sown native forb abundance, c) exotic forb seedling abundance and d) unsown native forb abundance. Predictions were calculated across the range of values observed for each fixed effect in the GLMM with other fixed effects held at their mean. Observed values are indicated by open circles.

### Responses to resource availability

Sown native forb species richness was positively associated with the percent light penetrating the canopy and negatively associated with soil phosphorus. Sown native forb abundance was not associated with any of the measured resources. Unsown native forb abundance was positively associated with soil moisture. Exotic forb seedling abundance was positively associated with percent light penetrating the canopy, which had a greater influence on this response than percent live tussock cover (*Delta BIC* = 17 vs 1), the structural variable in that model. In all other models, resource availability had a relatively minor influence compared with structure (Table 2a-d, Fig. 3a-d).

## Discussion

We investigated whether the addition of seed is needed to restore native forbs in a temperate grassland; and compared responses by native and exotic forbs to tussock thinning, litter removal and weed control. We found that the richness and abundance of native forbs were significantly positively associated with the addition of seed, removal of living biomass (native tussock grasses and exotic species) and removal of litter. Our results agreed with studies conducted in other parts of the world that demonstrate the negative influence that competition from established plants and litter can have on forb seedling recruitment (Dybzinski & Tilman 2012; Scott & Morgan 2012; Loydi et al. 2013). We found that native forb seedlings were more restricted by litter than exotic forb seedlings, and thus, controlling litter build-up is essential for maintaining the richness and abundance of native forbs. However, our results indicated that tussock thinning and litter removal will not result in successful establishment of native forb species without an adequate supply of seed.

## The need for seed

Seed addition will probably be necessary when restoring forb diversity in degraded temperate grasslands, as there is little potential for restoring lost forb diversity though natural dispersal (Hobbs & Yates 2003; Heinken & Weber 2013). While the use of herbicides may have reduced the supply naturally occurring native forb seed at the experiment site, small and fragmented populations of persisting species are universal symptoms of grassland degradation (Hobbs & Yates 2003). And lost species above ground are also likely to be lost from the soil seedbank (Morgan 2001). Clark et al. (2007) argue that the availability of suitable sites is more limiting than seed limitation for recruitment in grasslands, but both were important in our experiment. The richness and abundance of native forbs increased with seed addition and the amount of increase depended on which other treatments were applied (Table 4 in Appendix S2).

Recruitment of the three sown species that were already present at the site relied entirely on seed addition, and nine of the 11 unsown native forb species recorded in our plots (Table 8c in Appendix S2) also failed to recruit. This is probably due to seed limitation as tussock thinning and litter removal had created many sites suitable for germination and establishment (Clark et al. 2007; Dybzinski & Tilman 2012). We cannot be sure of the age of the extant forbs or when the last natural recruitment event occurred, but the unsown native forbs on our site (predominantly perennial) may not have successfully recruited for many years–perhaps since the last major disturbance event (Lauenroth & Adler 2008) 12 years earlier.

Seed limitation is common in plant communities and often occurs in combination with a limitation of resources needed for germination and establishment (Eriksson & Ehrlén 1992; Clark et al. 2007). The arrival of seed from source populations may be affected by interactions between landscape factors (e.g. habitat and population fragmentation and isolation, pollinator availability) and species attributes (e.g. population sizes, dependence on pollinators, genetic self-compatibility/incompatibility) (Hobbs & Yates 2003; Aguilar et al. 2006; Heinken & Weber 2013). Dispersal success is influenced by seed characteristics (e.g. size, dispersal appendages), release height (Thomson et al. 2011), and landscape and site conditions (Soons et al. 2005). Dispersal over time is limited by seedbank longevity, which for Australian native forb species is generally short (Morgan 1998a). Our results suggest that seed for the sown native species and almost all the unsown existing native species were neither present in the seedbank nor dispersing to the site in sufficient quantities—probably a consequence of insufficient numbers of reproductive individuals within dispersal range (Nathan & Muller-Landau 2000; Scott & Morgan 2012).

### Structural influence

Structure influences the recruitment of native forbs directly through physical effects and indirectly by moderating the availability of resources (Davis et al. 2000). Tussocks and litter take up space and create a physical barrier that can restrict seedling emergence (Donath & Eckstein 2010) or prevent seeds from reaching mineral soil (Ruprecht & Szabó 2012). Live tussock cover influences forb recruitment indirectly by competing for available soil resources and light (Dybzinski & Tilman 2012; Loydi et al. 2015). Litter reduces seedling emergence indirectly by reducing the amount of light at ground level (Foster & Gross 1998), although accumulated leaf litter can also be beneficial for seedling emergence in dry conditions through temperature moderation and increased water retention (Loydi et al. 2013).

Exotic forb seedling abundance increased with litter removal, but we found that the abundance of sown native forb seedlings benefited relatively more than exotic species from litter removal than other treatments (based on *Delta-BIC*, Table 1b-c). We were unable to determine the degree to which litter depth restriction on native seedlings was related to physical obstruction or the availability of light, but exotic seedlings were not significantly restricted by litter depth. Our results suggest that exotic forb seedlings can cope with a greater litter depth. Therefore, in productive grasslands where litter accumulates, periodic removal of litter build-up is essential for maintaining the richness and abundance of native forb species as a persistent litter layer will favour the recruitment of exotic species over natives, leading to an increased proportion of exotic forbs. There were no positive forb seedling responses to litter in our study; even the abundance of established unsown native forbs was negatively associated with the litter depth existing before the experiment.

### Response to resource availability

Although the richness and abundance of native forbs generally exhibited the strongest associations with structural attributes of grassland (i.e., litter depth and tussock cover), there were significant associations with some of the measured resources. For example, native forb species richness was negatively associated with soil phosphorus and positively associated with light penetrating the canopy (measured above the litter). Negative associations between elevated soil phosphorus (e.g., from the application of fertilizer or introduction of livestock) and the richness of native forbs has been widely observed (Dorrough & Scroggie 2008; Seabloom et al. 2015; Morgan et al. 2016). Most native species are unable to compete with exotic species in soils with high soil phosphorus levels, as many exotic species evolved in, and are better adapted to, soils high in phosphorus (Daehler 2003). Increased light penetrating the canopy benefits seedling and adult forbs that have grown above the litter, and it may also benefit seedlings that need light to grow through the litter (by increasing the amount of light penetrating into the litter), in this way reducing the severity of litter restriction, as per Hautier et al. (2009). The abundance of exotic forbs was more strongly associated with the amount of light penetrating the canopy than the depth of the litter. Exotic forb species in our study are generally better adapted for rapid growth and therefore have a greater need for resources, including light (Borer et al. 2014; Neuenkamp et al. 2016).

While native forb seedling abundance was not associated with resource availability, most seedlings were counted while very small, and it is likely that resources would become more limiting with increasing competition among growing seedlings.

## Implications for management

Experimental seed addition increased the richness and abundance of native forbs, especially when combined with tussock thinning and litter removal. Living grass tussock cover can be reduced by fire, selective herbicide application, or physically removing a proportion of individual plants. Litter build-up can also be minimised by periodic burning, or physical removal. Grazing can also reduce grass cover and litter build-up, but frequent grazing may be counter-productive as it leads to a reduction in native forbs and an increase in exotic species (Dorrough et al. 2004). Care should be taken that management actions to reduce grass cover and litter build-up do not exceed thresholds required by vulnerable grassland biota. For example, Howland et al (2014) found that the species richness and abundance of ground-dwelling reptiles declined following a change in grassland structure caused by grazing. However, environmental thresholds are likely to be species-specific and may require additional research and choices of which species to favour.

The removal of exotic species was also found to benefit sown native forb abundance to some extent, even though exotic species were initially scarce at our site (Table 1b, Fig. 2b). The removal of exotic species is likely to cost less and be more effective in the long-term if populations are removed while small (Rejmánek & Pitcairn 2001; Simberloff et al. 2013). It would also be preferable to control exotic species before taking actions to reduce tussock cover or litter depth, as reduced biomass may encourage the expansion of existing exotic species.

A key result of our study was that litter restricted the abundance of emerging sown native forb seedlings more than exotic forb seedlings. The most likely reason for this is that Australian grassland species have evolved where the amount of litter was generally less than in Europe—due to greater biomass productivity in European grass species (Groves et al. 2003), and a lower likelihood of fire that removes litter (Bond et al. 2005). Indeed, we demonstrated negative impacts on native forbs where the average ground litter mass was greater than 310 g.m$^{-2}$, considerably less than the 500 g.m$^{-2}$ threshold suggested by Loydi et al (2013) based on research carried out mainly in Europe and USA. *Themeda triandra* grasslands, found mainly in the southern hemisphere (Hodgkinson et al. 1989), were poorly represented in the meta-analysis by Loydi et al (2013). It is reasonable to conclude that litter levels can directly influence the composition of native and exotic forbs in grassland communities, and grasslands with litter levels above the native tolerance threshold are likely to become progressively dominated by exotic forbs. Such a trend may trigger a positive feedback mechanism accelerating the decline of native forbs, due to increased exotic competition for resources and a proportionally reduced native seed supply. Strategic use of litter removal on sites dominated by native tussock grasses combined with the addition of native forbs in spring can benefit native forb richness and abundance in preference to exotic forb abundance; initially by reducing litter restriction on emerging native forbs, and subsequently through greater seed supply and competition for resources from an increased presence of native species.

## Acknowledgements

We acknowledge Ken Hodgkinson's comments on the experimental design, Wade Blanchard's and Jeff Wood's assistance with statistical design and analysis, and John Stein's assistance with spatial data. Dean Ansell, Kat Ng, and Andrew O'Reilly Nugent, helped in the field and Richard Groves provided comments on an earlier draft. Greening Australia provided support with watering. The ACT Government funded this research and provided access to the nature reserve and management information; Michael Mulvaney, Geoff King, Joel Patterson, Andrew Halley, and Richard Milner in particular. JAC acknowledges support from the Australian Research Council (DE120102221) and ARC Centre of Excellence for Environmental Decisions.

## References

Aguilar, R., Ashworth, L., Galetto, L. & Aizen, M.A. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* 9: 968-980.

Barrett, G.W., Freudenberger, D., Drew, A., Stol, J., Nicholls, A.O. & Cawsey, E.M. 2008. Colonisation of native tree and shrub plantings by woodland birds in an agricultural landscape. *Wildlife Research* 35: 19-32.

Bond, W.J., Woodward, F.I. & Midgley, G.F. 2005. The global distribution of ecosystems in a world without fire. *New Phytologist* 165: 525-538.

Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., Adler, P.B., Alberti, J., Anderson, (…) & Yang, L.H. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508: 517-520.

Brandt, A.J. & Seabloom, E.W. 2012. Seed and establishment limitation contribute to long-term native forb declines in California grasslands. *Ecology* 93: 1451-1462.

Burnham, K.P. & Anderson, D.R. 2003. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer New York.

Clark, C.J., Poulsen, J.R., Levey, D.J. & Osenberg, C.W. 2007. Are Plant Populations Seed Limited? A Critique and Meta ‐ Analysis of Seed Addition Experiments. *The American Naturalist* 170: 128-142.

Daehler, C.C. 2003. Performance Comparisons of Co-Occurring Native and Alien Invasive Plants: Implications for Conservation and Restoration. *Annual Review of Ecology, Evolution, and Systematics* 34: 183-211.

Davis, M.A., Grime, J.P. & Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88: 528-534.

Donath, T. & Eckstein, R.L. 2010. Effects of bryophytes and grass litter on seedling emergence vary by vertical seed position and seed size. *Plant Ecology* 207: 257-268.

Dorrough, J., Ash, J. & McIntyre, S. 2004. Plant responses to livestock grazing frequency in an Australian temperate grassland. *Ecography* 27: 798-810.

Dorrough, J. & Scroggie, M.P. 2008. Plant responses to agricultural intensification. *Journal of Applied Ecology* 45: 1274-1283.

Driscoll, D.A. 2017. Disturbance maintains native and exotic plant species richness in invaded grassy woodlands. *Journal of Vegetation Science*: n/a-n/a.

Dybzinski, R. & Tilman, D. 2012. Seed and microsite limitation in a late-successional old field: the effects of water, adults, litter, and small mammals on seeds and seedlings. *Plant Ecology* 213: 1003-1013.

Eriksson, O. & Ehrlén, J. 1992. Seed and microsite limitation of recruitment in plant populations. *Oecologia* 91: 360-364.

Faithfull, I.G., Hocking, C. & McLaren, D.A. 2012. Managing native grasslands to minimise invasion by Chilean needle grass. In: Victoria, W.S.o. (ed.) *Eighteenth Australasian Weeds Conference*, pp. pp. 59-62, Melbourne, Australia.

Fayolle, A., Violle, C. & Navas, M.-L. 2009. Differential impacts of plant interactions on herbaceous species recruitment: disentangling factors controlling emergence, survival and growth of seedlings. *Oecologia* 159: 817-825.

Fensham, R.J., Butler, D.W., Fairfax, R.J., Quintin, A.R. & Dwyer, J.M. 2016. Passive restoration of subtropical grassland after abandonment of cultivation. *Journal of Applied Ecology* 53: 274-283.

Foster, B.L. & Gross, K.L. 1998. Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. *Ecology* 79: 2593-2602.

Frances, A.L., Reinhardt Adams, C. & Norcini, J.G. 2010. Importance of Seed and Microsite Limitation: Native Wildflower Establishment in Non-native Pasture. *Restoration Ecology* 18: 944-953.

Fynn, R.W.S., Morris, C.D. & Edwards, T.J. 2005. Long-term compositional responses of a South African mesic grassland to burning and mowing. *Applied Vegetation Science* 8: 5-12.

Groves, R.H., Austin, M.P. & Kaye, P.E. 2003. Competition between Australian native and introduced grasses along a nutrient gradient. *Austral Ecology* 28: 491–498.

Hautier, Y., Niklaus, P.A. & Hector, A. 2009. Competition for Light Causes Plant Biodiversity Loss After Eutrophication. *Science* 324: 636-638.

Heinken, T. & Weber, E. 2013. Consequences of habitat fragmentation for plant species: Do we know enough? *Perspectives in Plant Ecology, Evolution and Systematics* 15: 205-216.

Hellström, K., Huhta, A.-P., Rautio, P. & Tuomi, J. 2009. Seed introduction and gap creation facilitate restoration of meadow species richness. *Journal for Nature Conservation* 17: 236-244.

Hobbs, R.J., Suding, K.N., International, P.S.E.R., Cale, P. & Allen-Diaz, B.H. 2013. *New Models for Ecosystem Dynamics and Restoration*. Island Press.

Hobbs, R.J. & Yates, C.J. 2003. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany* 51: 471-488.

Hodgkinson, K.C., Ludlow, M.M., Mott, J.J. & Baruch, Z. 1989. Comparative responses of the Savanna grasses Cenchrus ciliaris and Themeda triandra to defoliation. *Oecologia* 79: 45-52.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, (…) & Wardle, D.A. 2005. EFFECTS OF BIODIVERSITY ON ECOSYSTEM FUNCTIONING: A CONSENSUS OF CURRENT KNOWLEDGE. *Ecological Monographs* 75: 3-35.

Howland, B., Stojanovic, D., Gordon, I.J., Manning, A.D., Fletcher, D. & Lindenmayer, D.B. 2014. Eaten Out of House and Home: Impacts of Grazing on Ground-Dwelling Reptiles in Australian Grasslands and Grassy Woodlands. *PLoS ONE* 9: e105966.

Hulvey, K.B. & Zavaleta, E.S. 2012. Abundance declines of a native forb have nonlinear impacts on grassland invasion resistance. *Ecology* 93: 378-388.

Jacquemyn, H., Mechelen, C.V., Brys, R. & Honnay, O. 2011. Management effects on the vegetation and soil seed bank of calcareous grasslands: An 11-year experiment. *Biological Conservation* 144: 416-422.

Klimek, S., Richter gen. Kemmermann, A., Hofmann, M. & Isselstein, J. 2007. Plant species richness and composition in managed grasslands: The relative importance of field management and environmental factors. *Biological Conservation* 134: 559-570.

Lauenroth, W.K. & Adler, P.B. 2008. Demography of perennial grassland plants: survival, life expectancy and life span. *Journal of Ecology* 96: 1023-1032.

Loydi, A., Donath, T.W., Otte, A. & Eckstein, R.L. 2015. Negative and positive interactions among plants: effects of competitors and litter on seedling emergence and growth of forest and grassland species. *Plant Biology* 17: 667-675.

Loydi, A., Eckstein, R.L., Otte, A. & Donath, T.W. 2013. Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. *Journal of Ecology* 101: 454-464.

Mitchell, R.M. & Bakker, J.D. 2016. Grass abundance shapes trait distributions of forbs in an experimental grassland. *Journal of Vegetation Science* 27: 557-567.

Moles, A.T. & Westoby, M. 2004. Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* 92: 372-383.

Morgan, J.W. 1998a. Composition and seasonal flux of the soil seed bank of species-rich *Themeda triandra* grasslands in relation to burning history. *Journal of Vegetation Science* 9: 145-156.

Morgan, J.W. 1998b. Importance of Canopy Gaps for Recruitment of some Forbs in <i>Themeda triandra</i>-dominated Grasslands in South-eastern Australia. *Australian Journal of Botany* 46: 609-627.

Morgan, J.W. 2001. Seedling recruitment patterns over 4 years in an Australian perennial grassland community with different fire histories. *Journal of Ecology* 89: 908–919.

Morgan, J.W., Dwyer, J.M., Price, J.N., Prober, S.M., Power, S.A., Firn, J., Moore, J.L., Wardle, G.M., Seabloom, (…) & Camac, J.S. 2016. Species origin affects the rate of response to inter-annual growing season precipitation and nutrient addition in four Australian native grasslands. *Journal of Vegetation Science*: n/a-n/a.

Morgan, J.W. & Williams, N.S.G. 2015. The ecology and dynamics of temperate native grasslands in south-eastern Australia. In: Williams, N.S.G., Marshall, A.J. & Morgan, J.W. (eds.) *Land of sweping plains: managing and restoring the native grasslands of south-eastern Australia*. CSIRO Publishing.

Nathan, R. & Muller-Landau, H.C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution* 15: 278-285.

Neuenkamp, L., Lewis, R.J., Koorem, K., Zobel, K. & Zobel, M. 2016. Changes in dispersal and light capturing traits explain post-abandonment community change in semi-natural grasslands. *Journal of Vegetation Science*: n/a-n/a.

Prober, S.M. & Thiele, K.R. 2005. Restoring Australia's temperate grasslands and grassy woodlands: integrating function and diversity. *Ecological Management & Restoration* 6: 16-27.
Prober, S.M., Thiele, K.R. & Speijers, J. 2013. Management legacies shape decadal-scale responses of plant diversity to experimental disturbance regimes in fragmented grassy woodlands. *Journal of Applied Ecology* 50: 376-386.
R Core Team 2016. R: A language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria
Rejmánek, M. & Pitcairn, M.J. 2001. When is eradication of exotic pest plants a realistic goal? In: Veitch, C.R. & Clout, M.N. (eds.) Turning the Tide: The eradication of invasive species., pp. 249-253. IUCN, Gland, Switzerland and Cambridge, UK in collaboration with IUCN/SSC Invasive Species Specialist Group, Aukland, New Zealand., University of Aukland.
Ruprecht, E. & Szabó, A. 2012. Grass litter is a natural seed trap in long-term undisturbed grassland. *Journal of Vegetation Science* 23: 495-504.
Scott, A. & Morgan, J. 2012. Dispersal and microsite limitation in Australian old fields. *Oecologia* 170: 221-232.
Seabloom, E.W., Borer, E.T., Buckley, Y.M., Cleland, E.E., Davies, K.F., Firn, J., Harpole, W.S., Hautier, Y., Lind, (…) & Yang, L. 2015. Plant species/' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. *Nat Commun* 6.
Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, (…) & Vilà, M. 2013. Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28: 58-66.
Soons, M.B., Messelink, J.H., Jongejans, E. & Heil, G.W. 2005. Habitat fragmentation reduces grassland connectivity for both short-distance and long-distance wind-dispersed forbs. *Journal of Ecology* 93: 1214-1225.
Thomson, F.J., Moles, A.T., Auld, T.D. & Kingsford, R.T. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology* 99: 1299-1307.
Tremont, R. & Mcintyre, S. 1994. Natural Grassy Vegetation and Native Forbs in Temperate Australia: Structure, Dynamics and Life-Histories. *Australian Journal of Botany* 42: 641-658.
Wheeler, M.M., Neill, C., Loucks, E., Weiler, A., Von Holle, B., Pelikan, M. & Chase, T. 2015. Vegetation removal and seed addition contribute to coastal sandplain grassland establishment on former agricultural fields. *Restoration Ecology* 23: 539-547.
Wilkerson, M.L., Ward, K.L., Williams, N.M., Ullmann, K.S. & Young, T.P. 2014. Diminishing Returns from Higher Density Restoration Seedings Suggest Trade-offs in Pollinator Seed Mixes. *Restoration Ecology* 22: 782-789.
Zobel, M., Otsus, M., Liira, J., Moora, M. & Mols, T. 2000. Is Small-Scale Species Richness Limited by Seed Availability or Microsite Availability? *Ecology* 81: 3274.

Supporting Information
Appendix S1. Tables related to experiment design and results.
Appendix S2. List of species.