Does diversity influence soil nitrate, light availability and productivity in the establishment phase of Australian temperate grassland reconstruction?

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Introduction

There has been a worldwide reduction in the range and diversity of native grassland communities. The recognition of the scale of threat posed to these communities (at all trophic levels) by historic and ongoing human activity has become increasingly focused during the past several decades (Lunt 1998; McDougall & Morgan 2005).

In Australia, there have been limited opportunities for researchers and restorationists to develop or refine methods of grassland restoration using a diverse range of native species. Previous studies investigating the use of indigenous grassland species in restoration (e.g. Lodge 1981; Huxtable & Whalley 1999; Windsor & Clements 2001; Gibson-Roy 2004; Cole et al. 2005; McDougall & Morgan 2005), generally reported less than optimal field results, particularly with forb species.

Interestingly, recent findings from ecological research provide insights that might assist restorationists to re-assemble communities that more effectively use available resources and thereby increase the likelihood they are both functional and persistent (e.g. Naeem et al. 1999; Spohn et al. 2000; Tilman et al. 2006). These studies have shown that both species and functional richness are intimately linked to the efficient use of community resources in plant and animal communities.

It is thought that the processes involved in the maintenance of ecosystem function are the sum of the activities of their constituent organisms and the effects that these biotic activities have on the physical and chemical

Summary

The successful conservation and restoration of the temperate native grasslands of south-eastern Australia is critical to reversing the decline in range and diversity of these threatened plant communities. Yet the goals of high native species diversity and weed management are difficult to achieve in grassland restoration projects. To increase our understanding of whether synergies exist between these goals (i.e. whether early introduction of a larger number of species might improve both outcomes in the reassembly of native grassland), we examined the relationships between plant species number, functional group number and resource use during the establishment phase of direct-sown grassland. We did this by sowing a representative suite of species (at varying levels of species number and functional group number) into experimental plots and then measuring and analysing the extent to which the newly established assemblages captured available resources, i.e. used soil nitrate, absorbed light and produced biomass (vegetative cover).

Statistically significant correlations were common between the predictor variables (species number, functional group number, percentage vegetative cover, plant number, presence of idiosyncratic (dominating) species and responses (soil nitrate concentration, light reduction or ‘extinction’)). Higher diversity was associated with lower soil nitrate, while percentage vegetative cover and the presence of idiosyncratic species best predicted light extinction. The relationship between diversity, and plant biomass (measured as vegetative cover) and plant number was positive in the first year of the study. The diversity/biomass relationship became negative in the second year due to the higher numbers and cover of ‘idiosyncratic’ species. The diversity/plant number relationship also became negative in the autumn of the second year and was reduced to a trend by the winter.

We found that lower nitrate and increasing plant numbers and vegetative cover were most strongly linked to increasing species number in the early stages of this study. This suggests that introducing and maintaining high diversity early in a native grassland reassembly or enhancement project will improve the resistance (e.g. to weed) of these communities. At later stages of grassland development, this function may be provided by the more dominating idiosyncratic species. The maintenance of diversity, an important goal in its own right, will therefore necessitate managed disturbances to periodically reduce the vegetative dominance of idiosyncratic species, releasing resources for the diverse range of other species whose early introduction will have allowed them to persist in the soil seed bank or as suppressed rootstocks.

Key words: functional diversity, grassland restoration, light extinction, soil nitrate, species diversity, resilience, resistance.
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Table 1. Experimental treatments and method of allocation to treatments

| Treatment no. | Treatment description                                      | Allocation method                                                                 |
|---------------|-----------------------------------------------------------|-----------------------------------------------------------------------------------|
| 1             | 1 species                                                 | Randomly drawn from total species pool (n = 64)                                    |
| 2             | 9 species                                                 | Randomly drawn from total species pool (n = 64)                                    |
| 3             | 21 species                                                | Randomly drawn from total species pool (n = 64)                                    |
| 4             | 1 functional group – 1 species                            | Functional group randomly drawn from pool of seven; species randomly drawn from representatives of this functional group |
| 5             | 3 functional groups – 1 species per functional group       | Functional groups randomly drawn from pool of seven; species randomly drawn from representatives of these functional groups |
| 6             | 7 functional groups – 1 species per functional group       | Species randomly drawn from representatives of each functional group              |
| 7             | 1 functional group – 3 species per functional group        | Functional group randomly drawn from pool of five (excludes C4 grass n = 1, Therophytes n = 1); species randomly drawn from representatives of these functional groups |
| 8             | 3 functional groups – 3 species per functional group       | Functional groups randomly drawn from pool of five (excludes C4 grass n = 1, Therophytes n = 1); species randomly drawn from representatives of these functional groups |
| 9             | 5 functional groups – 3 species per functional group       | Species randomly drawn from representatives of five functional groups (excludes C4 grass n = 1, Therophytes n = 1); species randomly drawn from representatives of these functional groups |
| 10            | Most abundant in the reference communities – 9 species    | Species randomly drawn from a pool of 20 species identified as the most abundant in field surveys |

properties of their environment (Naeem et al. 1999). The particular form and degree of contribution varies significantly from species to species and from individual to individual. Biotic contributions are, in turn, influenced by stochastic abiotic factors such as fluctuations in temperature, rainfall or soil fertility, which impact on individuals and communities at differing local and regional scales (Fridley 2001). The summed contribution of individuals and species has led many ecologists to hypothesize that the maintenance of community function is intimately linked to the level of diversity within a community (Tilman et al. 1996; Fridley 2001). However, debate continues as to the cause and form of the relationship (Cardinale et al. 2000).

Our study investigated relationships between initial diversity (number of species, number of functional groups) and plant-specific effects ('idiosyncratic' species, plant number and vegetative cover) and key community resources (soil nitrate, light). The dual purposes of the study were to identify whether (i) the ultimate goal of high diversity was achievable in the early stages of the re-assembly of communities from Australian temperate grassland species, and (ii) whether the multiple mechanisms for capturing resources (nutrients, light and space) provided by a higher number of native species, may confer improved function to these communities at an early stage in their development.

**Methods**

**Study design**

The study was initiated in autumn (May) 2002 at the Burnley Campus field station, of the University of Melbourne, Richmond, Victoria, Australia (coordinates 37°49'55.3"S, 144°01'24.9"E). Before sowing, the site was excavated to a depth of 100 mm to remove the soil seed and bud bank. The area was then covered to 250 mm depth with imported basaltic subsoil sourced from a residential development situated approximately 2 km east of three reference grassland communities. Sub-soil was used because it contained fewer seeds and vegetative propagules than the topsoil layer. Details of site preparation and sowing procedures were reported in Gibson-Roy et al. (2007b).

We investigated the influence of species number, functional group number and individual species characteristics on the use of root zone nitrate and photosynthetically active radiation (PAR) by newly sown grassland communities (Gibson-Roy 2004). Ten treatments (Table 1) were applied to 1-m² plots within eight blocks. For treatments investigating functional group effects (treatments 4 to 9), each of the 64 species collected for the study was allocated to one of seven functional groups based on morphological or physiological characteristics (Gibson-Roy et al. 2007b) (Table 2).

To avoid confounding the effects of species number with individual species traits (species identity), species were allocated randomly into each of the treatment replicates (e.g. Spehn et al. 2000). The 20 most abundant species within the reference sites, included in treatment 10 (Table 3), were identified in a survey of the four source remnant communities during spring-summer growing season of 2001/2002 (Gibson-Roy 2004).

The species pool for this study was sourced from a series of remnant grassland communities that occur approximately 20 km to the north of metropolitan Melbourne. All herbaceous native species located at the reference sites were collected, subject to the conditions that the population contained at least 10 plants and that seed was available in sufficient quantities. For most species, seed was collected from 50 or more plants. The seeds of 64 species were harvested in the spring/summer (September to February) of 2001/2002 and dry stored until the field sowing in May 2002.
Table 2. Functional group categories (Raunkiaer 1934; Tremont & McIntyre 1994) to which each of the 64 species was allocated

| Life form            | Functional group | Description                                                                 | Species pool size |
|----------------------|------------------|-----------------------------------------------------------------------------|-------------------|
| Grasses              | C3 Grasses       | Possess a C3 photosynthetic pathway                                          | 11                |
|                      | C4 Grasses       | Possess a C4 photosynthetic pathway                                          | 1                 |
| Non-leguminous forbs | Therophytes      | Annual plants that survive the unfavourable season as seed                  | 1                 |
|                      | Chamaephytes     | Plants with buds or shoot-apes situated on stems that lie on or near to     | 30                |
|                      |                  | the soil surface                                                            |                   |
|                      | Hemicryptophytes | Plants with buds or shoot-apes situated at the lowest part of the stem,     | 10                |
|                      |                  | protected by soil and withered leaves in the soil surface                   |                   |
|                      | Geophytic Cryptophytes | Plants with bud or shoot apices borne on subterranean stems               | 7                 |
| Leguminous forbs     | Legumes          | Plants that utilize symbiotic bacterial root associations to acquire        | 4                 |
|                      |                  | inorganic soil nitrogen                                                    |                   |

Table 3. Twenty species identified as occurring most frequently in the reference grasslands communities for this study. These species were included in a pool of frequency dominants used for treatment 10; functional group, functional group designation (C3 = C3 grass, C4 = C4 grass, Ch, chamaephyte; G, geophyte; H, hemicryptophyte; L, legume; T, therophyte); Foliage orientation = general characteristics of foliage orientation and structure

| Frequency rank | Species                   | Functional group |
|----------------|---------------------------|------------------|
| 1              | Themeda triandra          | C4               |
| 2              | Asperula conferta         | Ch               |
| 3              | Veronica gracilis         | Ch               |
| 4              | Poa labillarderi          | C3               |
| 5              | Solenogyne domini         | H                |
| 6              | Convolvulus remotus       | H                |
| 7              | Eryngium ovirum           | H                |
| 8              | Calocephalus lacteus      | H                |
| 9              | Austrodanthonia caespitosa | C3             |
| 10             | Acaena echinata           | H                |
| 11             | Wahlenbergia communis     | Ch               |
| 12             | Tricoryne elatior         | G                |
| 13             | Lomandra filiformis       | H                |
| 14             | Austrodanthonia setacea   | C3               |
| 15             | Arthropodium strictum     | G                |
| 16             | Geranium retrorsum        | H                |
| 17             | Linum marginale           | H                |
| 18             | Leptorrhynchus tenulifolius | H              |
| 19             | Senecio quadridentatus    | Ch               |
| 20             | Brachycome basaltica      | H                |

Gibson-Roy et al. (2007a) reported details of the viability and germination capacity of each species, and Gibson-Roy et al. (2007b) reported detailed emergence and recruitment characteristics from the sowing reported in this paper.

On 5 May 2002, each 1-m² plot was sown with various combinations of species containing 1, 3, 7, 9 or 21 species, representing various levels of species and functional group diversity (Gibson-Roy et al. 2007b). Each plot was sown with 300 seeds, divided equally between the species allocated to that replicate. Therefore, the numbers of seeds sown varied between species, depending on the number of species in a treatment and the number of times a given species was randomly allocated to treatments. A detailed description of sowing and maintenance procedures was reported by Gibson-Roy et al. (2007b).

Measured variables

Soil nitrate

Soil nitrate was measured on four occasions (spring and summer, 2002/2003, and autumn and winter, 2003) as an indicator of available soil nitrogen. Nitrate levels were determined (depth 100 mm) for each of the 80 experimental plots. Soil core samples were removed using a 19-mm diameter stainless steel corer. For each sample, four randomly located cores were harvested per plot. Once removed, each soil core was placed into a plastic bag, labelled and stored in a freezer until analysis. All holes created by the soil corer were re-filled with stored sub-soil of the same origin.

Samples were oven dried at 90°C for 48 h and then reduced to a fine powder. From each, a 10-g sub-sample was added to 100 mL of distilled water and shaken mechanically for 1 h. Solutes were then filtered (Whatman no. 1, 90 mm filter paper). Nitrate measurements were taken from 50 mL sub-samples of these filtrates using a nitrate electrode (direct-ION ISE 3021). Readings were taken in millivolts and converted to parts per million (mg/kg) NO₃⁻.

Light extinction

Light extinction through the plot canopies was measured seasonally (spring and summer, 2002/2003 and autumn and winter, 2003) using a ceptometer probe (Decagon Devices Inc., Pullman, Washington, USA). Analysis of measurements compared the mean amount of ambient photosynthetically active radiation (PAR) occurring at 100 mm above plot canopies with that occurring at the soil surface beneath canopies. Measurements were taken from four randomly generated points in each plot within 1 h of noon, under cloudless conditions. Readings were expressed in micromoles of photons per square metre per second (μmol/m²/s). Light extinction through the canopy was expressed as a percentage of ambient light levels that did not reach the ground surface within plots.
The total cover of vegetation within each plot and the cover per species per plot were recorded as the percentage of ground area covered by live plants. Vegetation cover was recorded monthly from May 2002 until February 2003 and thereafter in May 2003 (autumn) and August 2003 (winter).

Plant number

The number of plants within each plot and the number of plants per species per plot were recorded by counting individuals from species included in the initial sowings. Plant number was recorded monthly from May 2002 until February 2003 and thereafter in May 2003 (autumn) and August 2003 (winter). All non-sown plant species were removed by hand from the experimental plots as soon as they were identified as not belonging to the species pool. The identity of non-sown species emerging from the imported soil during the first year (2002) was recorded from eight randomly located 250 × 250-mm sample plots that were cut into the weed matting that surrounded each plot (Gibson-Roy et al. 2007b).

Idiosyncratic identity

Individual species were identified as idiosyncratic when their total vegetative cover was equal to or greater than 70% of a plot surface. Idiosyncratic identity was recorded monthly from May 2002 until February 2003 and thereafter in May 2003 (autumn) and August 2003 (winter).

Data analysis

All data were log transformed to satisfy the assumption of homogeneity of variance. The analysis was conducted using the statistical package MINITAB 15 (Minitab Inc.). It was anticipated that seedling recruitment across the experimental site in the second season would alter imposed compositions within plots and confound direct treatment comparisons. However, germination and establishment on the site was initially highly variable and even in the first year few treatment gradients (number of species, number of functional groups and target number of individuals per plot) were achieved. Consequently, regression analysis was used for statistical modelling, with plots grouped according to the actual characteristics of the vegetation community at the time, rather than the targeted characteristics. Measures were taken from each experimental plot (n = 80), and used to model the relationship between the predictor variables (species number, functional group number, percentage vegetative cover, plant number) and response variables (soil nitrate, PAR) at four separate, seasonal intervals.

The predictor variables, species number and functional group number, were highly correlated and were not included in the same analysis. Similarly, the variable idiosyncratic species was highly correlated with percentage vegetative cover and so was considered separately. Analysis of variance was used to compare differences in the mean numbers of plants occurring between treatments at the end of the first season’s growth.

In presenting the results of the multiple regression models, the slope of each predictor variable and its associated P value (for testing the hypothesis of zero slope) and $R^2$ are presented for the models that include predictors that are significant at the 0.05 level.

Results

Establishment of the vegetation

Following the sowing of a total of 24 000 seeds on the 5th May 2002, emergence of the sown communities began in July 2002. By December 2002, there was an average of 13 plants per 1-m² plot. This represented approximately 4% of sown seed and approximately 8% of sown seed when adjusted to take account of TZ assessments of seed-lot viability. There was no significant difference in the number of established seedlings per plot between treatments ($P = 0.24$) at the end of the first growing season. This indicated that those species identified in treatment 10 as frequency dominants in reference communities did not establish more successfully than species chosen at random from the larger species pool. Nine species were identified as idiosyncratic because of their vegetative cover (70% of plot or greater) (Table 4). Mean vegetative cover within plots rose from 37% in spring to a peak of 54% in autumn and winter, and mean vegetative cover in plots containing an idiosyncratic species was, in all cases, greater than 75% (Table 5).

Although there was no significant difference in the number of individuals occurring on plots at the end of the first growing season, often the species and functional group targets were not achieved. For example, treatments that examined species number effects imposed a target gradient of between 1 and 21 species. The actual species gradient across the site in year one was between one and nine species. Treatments examining functional group effects imposed a gradient of between one and seven functional groups, but five functional groups was the largest number recorded in any plot in year one. The vegetation complexity on plots increased over time with...
later emergence from the original sown seed and second season recruitment from plants established in the first season of growth (Gibson-Roy et al. 2007b).

**Emergence of non-sown species from the sub-soil**

No indigenous species emerged from the weed survey plots in the first season of the experiment. In the second year, one native tree species (River Red Gum, *Eucalyptus camaldulensis*), which occurs at the experimental site, was recorded. All other non-sown species recorded were exotics. All non-sown species were removed.

**Nitrate levels**

Average soil nitrate concentrations at 100 mm declined from 18.7 mg/kg in the first spring (2002) to 6.6 mg/kg in the summer, and to 3.5 mg/kg in autumn 2003, after which it increased to 8.3 mg/kg by August (winter) 2003. There were significant correlations ($P < 0.05$) in at least one season between each of the four predictor variables and soil nitrate levels (Table 6). The contribution made by the predictor variables altered substantially over time. Species number was the variable that contributed most consistently to a reduction in soil nitrate during the study period. The presence of an idiosyncratic species was not a significant predictor of soil nitrate levels (Table 7).

**Light extinction**

Vegetative cover was the main predictor of light extinction through the canopy in each season (Table 8). As the vegetation established, the strength of this relationship increased at each of the four seasonal measurements. The proportion of variation explained by vegetative cover increased from around 42% in spring 2002 to around 83% in winter 2003. In winter 2003 functional group number also made a significant positive contribution to the relationship.

The presence of idiosyncratic species also increased light extinction through canopies (Table 7). This relationship continued throughout the study.

**Vegetation cover and plant number**

There was a significant relationship between percentage vegetative cover and species number although the direction and the strength altered over time (Table 9). The relationship was positive soon after emergence (spring and summer) and negative in autumn and winter. During the first two seasons (spring and summer), there was a significant positive relationship between the predictive variable species number and number of plants per plot (Table 9). This remained significant but reversed in autumn and reduced to a trend in winter when a few species produced large numbers of seedlings.

Plant number and the presence of an idiosyncratic species were the primary predictors of increased vegetation cover (Table 10). These relationships continued in each season, though the effect of plant number was substantially reduced in autumn and winter.

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**Table 5.** Differences between average percentage vegetative cover over four seasonal measurements in: all plots; plots containing no idiosyncratic species; plots containing one (or more) idiosyncratic species

|               | All plots | Plots containing no idiosyncratic species | Plots containing idiosyncratic species |
|---------------|-----------|------------------------------------------|---------------------------------------|
|               | Mean | SD | Mean | SD | Mean | SD |
| Spring        | 37 ($n = 80$) | 28 | 22 ($n = 73$) | 17.0 | 75 ($n = 7$) | 7.0 |
| Summer        | 47 ($n = 80$) | 31 | 28 ($n = 60$) | 23.4 | 81 ($n = 20$) | 16.9 |
| Autumn        | 54 ($n = 80$) | 32 | 40 ($n = 60$) | 28.2 | 85 ($n = 20$) | 12.9 |
| Winter        | 54 ($n = 80$) | 33 | 43 ($n = 58$) | 31.2 | 83 ($n = 22$) | 13.7 |

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**Table 6.** Summary of multiple regressions examining the effect of the predictor variables species diversity, percentage vegetative cover, plant number (A) and functional group number, percentage vegetative cover, plant number (B) on nitrate (100 mm soil depth) at seasonal points (spring, summer, autumn and winter)

|          | A Species no. | Percentage cover | Plant no. | B Functional group no. | Percentage cover | Plant no. | $R^2$
|----------|---------------|------------------|-----------|-----------------------|------------------|-----------|------
|          | Slope | $P$ value | Slope | $P$ value | Slope | $P$ value | Slope | $P$ value |
| Spring   | $-1.609$ | 0.027 | $-2.157$ | 0.008 | $0.338$ | 0.042 | 15.5% |
| Summer   | $-1.231$ | 0.001 |                   |          |                   |          | 14.5% |
| Autumn   | $-0.242$ | 0.050 |                   |          |                   | $-0.001$ | 0.010 | 9.9% |
| Winter   | $-0.556$ | 0.070 |                   |          |                   |          | 3.9% |

|          | Slope | $P$ value | Slope | $P$ value | Slope | $P$ value |
|----------|-------|-----------|-------|-----------|-------|-----------|
| Spring   | $-1.769$ | 0.003 | $-1.481$ | 0.021 |       |           |
| Summer   | $-0.001$ | 0.034 |       |          |       |           |
| Autumn   |       |           |       |          |       |           |
| Winter   |       |           |       |          |       |           |

$+$ or $-$ indicate the direction of the slope. $N = 80$. $R^2$ refers to the model with significant predictors only.
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Table 7. Summary of ANOVA comparisons of means of measured resources (soil nitrate and photosynthetically active radiation (PAR)) between plots containing one or more idiosyncratic species (+idio, species exhibits percentage vegetative cover = / >70%) and plots that do not contain an idiosyncratic species (=idio, species exhibit percentage vegetative cover 0–100%) over seasonal measurements (+ ± SD)

| Nitrate (100 mm) | PAR |
|-----------------|-----|
| Treatment       | Treatment |
| +Idio           | -Idio | P value |
| +Idio           | -Idio | P value |
| Spring          | 14.0 ± 3.4 | 19.2 ± 13.7 | 0.321 |
| Summer          | 4.3 ± 3.4  | 6.8 ± 7.3  | 0.150 |
| Autumn          | 3.8 ± 0.5  | 3.5 ± 2.1  | 0.635 |
| Winter          | 9.0 ± 6.6  | 8.3 ± 5.8  | 0.640 |
| 46.7 ± 30.3     | 11.5 ± 12.1 | 0.001 |
| 64.6 ± 24.6     | 28.1 ± 25.6 | 0.001 |
| 85.3 ± 8.5      | 48.7 ± 31.2 | 0.001 |
| 80.2 ± 10.6     | 48.4 ± 30.3 | 0.001 |

Discussion

**Grasslands as functional models**

Species from herbaceous or grassland communities are often used to assemble communities, usually from sown seed, to investigate diversity/function relationships (e.g. Tilman 1996; Spehn et al. 2000; Dukes 2001). Factors such as reference community composition, species identity and sampling effects have been debated in the literature because of concerns that they confound treatment variables and limit the interpretation of results (Tilman 1996; Fridley 2001). These concerns were addressed in this study by using all available co-habiting species from a reference community, classifying each to a functional group and then randomly allocating those species to treatment replicates.

There are uncertainties with achieving experimental treatment targets when biotic and abiotic factors such as disease, herbivory or moisture availability can affect the germination and establishment of plants (Polley et al. 2003). Few authors have reported emergence and establishment issues impacting on experimental targets; largely because they use devices such as sowing experimental plots at very high rates, (e.g. Spehn et al. 2000; Tallowin & Smith 2001). Yet, our study encountered such issues because we sowed at lower rates to avoid the possibility that individuals or dominant species may saturate plots. Only 43 of the 64 sown species emerged and established in the plots, thereby altering treatment targets (Gibson-Roy et al. 2007b). This meant that multiple regression analysis examined actual, rather than targeted characteristics of the plot communities, although the large number of observations (n = 80) improved precision.

It should also be noted that studies such as ours, which are conducted over relatively short periods (1–3 years), document patterns in the early stages of primary colonization (initial establishment/restoration), when dominance is relatively low and resources are ‘freely’ available. Therefore, the observed patterns and interactions may not reflect those in a mature temperate Australian grassland where dominant grasses, characteristically Kangaroo Grass (*Themeda triandra*), commonly account for >95% of biomass.

**Sowing mix composition**

In the early stages of this restoration study (to 12 months following sowing), we observed that including a larger number of species in a sowing mix led to more individuals and a faster establishment of plant cover than on plots sown with fewer species but with the same total number of seeds. Increased plant numbers led to an increased loss of light at the soil surface. A higher number of species per plot were linked to lower available nitrate levels, although this variable explained less than 20% of the observed reduction. By the following autumn, the relationship between species number and vegetation cover had reversed. More species initially led to greater cover but subsequently, high levels of cover led to fewer species as competition progressed. Although

Table 8. Summary of multiple regressions examining the effect of the predictive variable species number, percentage vegetative cover, plant number (A) and functional group number, percentage vegetative cover, plant number (B) on photosynthetically active radiation (PAR) at seasonal points (spring, summer, autumn and winter). + or − indicate the direction of the slope. N = 80. $R^2$ refers to the model with significant predictors only

| Estimated regression parameters for PAR as a response variable |
|---------------------------------------------------------------|
| A | Species no. | Percentage cover | Plant no. | $R^2$ |
|---|-------------|------------------|-----------|------|
| Response variable | Slope P value | Slope P value | Slope P value | |
| Spring | 5.008 0.001 | 0.001 | 42.2% |
| Summer | 7.118 0.001 | 57.9% |
| Autumn | 7.917 0.001 | 63.3% |
| Winter | 8.361 0.001 | 82.7% |

| B | Functional group no. | Percentage cover | Plant no. | $R^2$ |
|---|---------------------|------------------|-----------|------|
| Response variable | Slope P value | Slope P value | Slope P value | |
| Spring | 5.008 0.001 | 42.2% |
| Summer | 7.118 0.001 | 57.9% |
| Autumn | 7.917 0.001 | 63.3% |
| Winter | 3.757 0.05 | 84.6% |
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A suite of early dominant (idiomsyncratic) species were identified in these establishing communities, the characteristic community dominant, Kangaroo Grass, provided only moderate cover by the end of the study. Therefore, these findings shed light on community interactions and trajectories during the initial establishment phase rather than those in later successional phases of grassland development.

The reported relationships are broadly consistent with those of a number of similar studies and they provide further evidence of a complex set of interrelationships (with diversity playing a small but significant part) that influence resource availability and successional trajectories in grassland communities (Tilman 1996; Hector et al. 1999; Fargione et al. 2007).

Soil nitrate

Nitrogen is commonly a limiting resource in native grassland but in excess, it can increase vulnerability to weeds (Prober et al. 2002). In this study, although mean soil nitrate levels fluctuated seasonally, available nitrate reduced as the complexity of species assemblages on plots increased. Species number explained more of the reduction in soil nitrate levels than functional group number, percentage vegetative cover, plant number or idiosyncratic traits. However, although statistically significant, the strength of the relationships was relatively weak. Nonetheless, although our study did not investigate the direct causal relationship between species diversity and soil nitrate levels, the significant correlation suggests that diversity impacts on nutrient levels.

During the early stages of our study (spring 2002), both vegetation cover and plant numbers were also linked to the variation in soil nitrate. Thereafter, functional group number was significant in summer and autumn and plant number was significant again during autumn, demonstrating that a variety of factors had an impact on soil nitrate levels.

The observation that diversity was the variable most consistently linked to soil nitrate levels is particularly interesting because plant biomass has been reported as a major driver of nutrient levels (Jackson et al. 1998). The finding that idiosyncratic species traits (i.e. vegetative dominance) were not a useful predictor of nitrate levels in our plots adds weight to the observation that diversity, rather than plant biomass, most affected soil nitrate levels in these developing plant communities. It should be noted that only above ground biomass (plant cover) were assessed in this study. It is possible that below ground root biomass may have a stronger association with nitrate levels.

Soil nitrate fluctuations occur naturally, and peaks during summer and autumn, are thought to favour the growth of annual exotics (Prober et al. 2005). These authors

Table 9. Summary of linear regressions (n = 80) between predictor variable species number, and percentage vegetative cover response variables, vegetative cover and plant number over seasonal measurements

|                | Vegetative cover | Plant no. |
|----------------|------------------|-----------|
|                | Slope  | P value  | R²   | Slope  | P value  | R²   |
| Spring         | 0.222  | 0.026    | 6.2% | 1.557  | 0.010    | 8.1% |
| Summer         | 0.280  | 0.001    | 18.7%| 1.326  | 0.026    | 6.2% |
| Autumn         | –0.215 | 0.002    | 11.8%| –69.56 | 0.011    | 7.9% |
| Winter         | –0.232 | 0.002    | 33.9%| –9.590 | 0.060    | 4.4% |

Table 10. Summary of multiple regressions examining the effect of the predictor variables species number, plant number, idiosyncratic species (yes/no) (A) and functional group number, plant number, idiosyncratic species (yes/no) (B) on percentage vegetative cover at seasonal points (spring, summer, autumn and winter)

|                | A     | B     |
|----------------|-------|-------|
|                | Species no. | Plant no. | Idiosyncratic effects | R² |
|                | Slope  | P value | Slope  | P value | Slope  | P value | R²   |
| Spring         | 0.238  | 0.007   | 0.064  | 0.001   | 4.394  | 0.001   | 61.8%|     |
| Summer         | 0.401  | 0.001   | 0.072  | 0.001   | 4.244  | 0.001   | 67.0%|     |
| Autumn         | 0.002  | 0.001   | 0.423  | 0.001   | 4.812  | 0.001   | 51.6%|     |
| Winter         | 0.013  | 0.001   | 0.423  | 0.001   | 4.423  | 0.001   | 47.1%|     |
|                | Functional group no. | Plant no. | Idiosyncratic effects | R² |
|                | Slope  | P value | Slope  | P value | Slope  | P value | R²   |
| Spring         | 0.328  | 0.009   | 0.069  | 0.000   | 4.142  | 0.001   | 61.6%|     |
| Summer         | 0.620  | 0.001   | 0.066  | 0.003   | 4.328  | 0.001   | 66.0%|     |
| Autumn         | 0.002  | 0.001   | 0.427  | 0.001   | 4.827  | 0.001   | 51.6%|     |
| Winter         | 0.013  | 0.001   | 0.423  | 0.001   | 4.423  | 0.001   | 47.5%|     |

+ or – indicate the direction of the slope. N = 80. R² refers to the model with significant predictors only.
found that nitrate levels could be reduced to <2.5 mg/kg by the addition of sugar (0.5 kg/m² at 3 monthly intervals) with a resulting reduction in weed vigour. In general, these low nitrate levels are indicative of those in undisturbed or slightly disturbed grassy woodland (<5 mg/kg), whereas >50 mg kg is typical of weed dominated 'stock camp' sites. Nitrate levels where highest in our study in spring (18.7 mg/kg) and progressively declined to 3.5 mg/kg in autumn, not dissimilar to those found in Prober et al. (2005) after the additions of sugar. The levels began to rise again in late winter when such increases would be expected. In spring and summer, when natural peaks seem to occur and when weed vulnerability in an establishing grassland is at its highest, the strength of the relationship between species number and lowered nitrate levels was at its greatest. Therefore, knowing that direct-sown grasslands are particularly vulnerable to competition from weeds (both in-situ and invading propagules), this suggests that restoration strategies might consider the early introduction of high diversity to increase the competitiveness (resource use and biomass accumulation) of the developing communities.

**Light relations**

The effective and complementary interception of light in diverse plant communities is an important example of niche differentiation enhancing the opportunity for efficient resource use by co-habiting species. Complementary structural, phenological and physiological characteristics of plants in diverse communities can enhance the use of light in both space and time (Anten & Hirose 1999; Aan et al. 2006). Light levels beneath plant canopies are linked most strongly to plant productivity and asymmetry, rather than species diversity (Foster & Gross 1998; Rajaniemi 2003; Harpole & Tilman 2007). Therefore, a key question in this study was: is diversity a driver of light extinction in these assembled communities, or is it better predicted by levels of vegetation cover or species-specific traits?

In our heterogeneously structured communities, plant cover most reduced the level of light reaching plot surfaces. The number of species on plots did not significantly influence the relationship at any time during the study. However, we found that functional group number contributed to this relationship in the final winter (2003). The functional groups used in this study were an expansion of those commonly used in grassland studies (i.e. grass, forb, legumes) (e.g. Spehn et al. 2000; Polley et al. 2003). In newly sown communities, it is probable that it takes some time for these differences to significantly influence light extinction. Indeed, it is questionable how long lasting these particular functional group effects are likely to be and the extent to which they will diminish as a single functional group (tussock grasses) establishes dominance.

A small sub-set of species can exert a disproportionate influence on the way resources are used in some communities, overriding the influence of diversity (Lawton 1994; Berlow et al. 1999). Wardle (1999) state that idiosyncratic species have an important but unpredictable effect on community function regardless of changes to species number. We found idiosyncratic species contributed significantly to light extinction throughout the study. However, the influence of some of the idiosyncratic forb species identified in this study may decline in subsequent years. Their effect was related to faster growth rate rather than longer-term competitiveness.

Nine species were idiosyncratic in their contribution to vegetation cover (three tussock grass species (two C3, one C4), six non-leguminous forbs (three chamaephytes, three hemicryptophytes) and one legume). Their vegetation dominance over and above the species pool was through tall, vertically orientated tussocks, upright and spreading canopies, or low, lateral growth (Table 4). Lunt (1991) and Morgan (2001) identified the capacity of Kangaroo Grass to dominate light relations in many Australian temperate grassland communities through biomass dominance. Factors such as fire, grazing and resource limitations reduce the dominance of such idiosyncratrics from time to time, temporally releasing sub-dominant species from competition. Also, light transmittance to the ground surface is thought to be critical in these communities to enhance opportunities for seedling survival (Wills & Groves 1991).

**Vegetation cover**

Increases in plant cover and biomass in grassland communities are commonly associated with decreases in diversity (Ghilarov 2000), with the relationship attributed to competitive exclusion (Grime 1973; Grace 1999). In the early stages of this study, higher diversity was associated with increased plant cover. However, this relationship later reversed and, 18 months after sowing, a negative trend was evident with idiosyncratic species and plant number the strongest predictors of vegetation cover. This pattern is common in mature south-eastern temperate grasslands of Australia. In the absence of disturbance, the accumulation of biomass from dominant grass species leads to canopy closure and eventual reductions in species diversity (Lunt 1991; Morgan 2001).

**Colonization potential**

By the end of the second season, we had identified 12 species (from the pool of 64 sown) that exhibited high colonizing potential (Gibson-Roy et al. 2007b). This was based on a greater than 100% increase, between December 2002 and August 2003, in the number of plots on which each species was recorded. This indicates that a number of these species are capable of rapid increases in numbers and range under favourable conditions. This observation is important to note when restoration programmes seek continued expansion post-introduction.

One treatment included 20 species identified as frequency dominants from the reference remnant communities (treatment 10). Interestingly, only five of the frequency dominants were noted as colonizing species. At the end of the first season, species in this treatment were not represented in plots in larger numbers than the general species pool. This suggests their higher frequency in reference communities is linked more to competitiveness or persistence than to recruitment from seed or pioneering capacity.

**Implications for grassland restoration**

Species and functional diversity in plant communities support greater diversity at other trophic levels, further enhancing ...
ecosystem services (e.g. Ives et al. 2000; Bever et al. 2002). Consequently, using a diverse range of species and functional groups in restoration programmes is desirable when the aim is to reassemble functional grassland communities. Because declining species diversity is a major threatening process in Australian temperate lowland grasslands, such a policy would have the added advantage of expanding the range and complexity of these threatened communities.

In the early phase following sowing, increasing diversity was associated with higher levels of vegetative cover, plant numbers and lowered soil nitrate levels. These observations suggest that, through a combination of rapid niche utilization and increased resource use efficiency, diverse communities may be quicker to consolidate and therefore less vulnerable to invasion by non-target species. As the sown communities began to mature, we found that diversity declined with increasing vegetative cover. This does not reduce resistance to invasion by non-target species while the dominant species responsible for vegetative cover persist. It does, however, indicate that managers must find ways to maintain diversity over time. In particular, our research confirms the findings of others (e.g. Lunt & Morgan 1999) that restoration practitioners should consider the ongoing management of native species that demonstrate a potential to dominate vegetatively. This is especially relevant in mature grasslands where, under favourable rainfall conditions, grass dominants can quickly accumulate biomass to levels that swamp sub-dominant species. Management strategies would include the application of appropriate disturbance regimes such as burning, periodic grazing or mowing, which release resources to the diverse mix of initially sown species. We can reliably predict that these species are capable of re-sprouting and/or germinating in response to the disturbance.

In summary, the study demonstrated that high levels of temperate grassland species diversity can be established in the early phase of a restoration project and that higher diversity led to higher capture of resources early in the establishment phase. In a restoration context, this is likely to confer improved resistance to weed and other non-target species, and improved resilience to the cyclic disturbances that are needed to maintain high diversity in grasslands. A recognition by restorationists, land managers and perhaps, most importantly, by policy-makers, of the importance of species and functional diversity to community processes, tempered by an understanding that other factors also influence the development of plant communities, will enhance capacity to more effectively preserve and restore these highly threatened communities.

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