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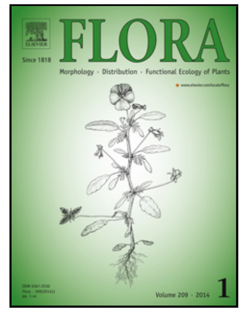

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Early recovery signs of an Australian grassland following the management of *Parthenium hysterophorus* L.

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Highlights

- We quantified the impact of parthenium weed upon species diversity in grassland.
- We assessed the resulting shifts in plant community composition following management.
- High infestations of parthenium weed significantly affected species diversity and native species abundance.
- Herbicide applications were beneficial to the recovery of the grassland with grazing exclusion.
- Productivity of these grasslands increased, being a key step in determining an environmentally stable management strategy.

ABSTRACT Parthenium weed (*Parthenium hysterophorus* L.) is believed to reduce the above- and below-ground plant species diversity and the above-ground productivity in several ecosystems. We quantified the impact of this invasive weed upon species diversity in an Australian grassland and assessed the resulting shifts in plant community composition following management using two traditional approaches. A baseline plant community survey, prior to management, showed that the above-ground community was dominated by *P. hysterophorus,* stoloniferous grasses, with a further high frequency of species from Malvaceae, Chenopodiaceae and Amaranthaceae. In heavily invaded areas, *P. hysterophorus* abundance and biomass was found to negatively correlate with species diversity and native species abundance. *Digitaria didactyla* Willd. was present in high abundance when *P. hysterophorus* was not, with these two species, contributing most to the dissimilarity seen between areas. The application of selective broad leaf weed herbicides significantly reduced *P. hysterophorus* biomass under ungrazed conditions, but this management did not yet result in an increase in species diversity. In the above-ground community, *P. hysterophorus* was partly replaced by the introduced grass species *Cynodon dactylon* L. (Pers.) 1 year after management began, increasing the above-ground forage biomass production, while *D. didactyla* replaced *P. hysterophorus* in the below-ground community. This improvement in forage availability continued to strengthen over the time of the study resulting in a total increase of 80% after 2 years in the ungrazed treatment, demonstrating the stress that grazing was imposing upon this grassland-based agro-ecosystem and showing that it is necessary to remove grazing to obtain the best results from the chemical management approach.

Key words: chemical control, grazing, invasive species, plant community composition, species diversity.

Introduction

Native to the tropical and subtropical Americas (Navie et al., 1996), *Parthenium hysterophorus* L. (parthenium weed) is now a major weed in many regions around the world, including Africa, Asia, the Pacific and Australia (Adkins and Navie, 2006). The species has also been shown to significantly reduce both the diversity and productivity of grasslands (Navie et al., 2004; Timsina et al., 2011; Javaid and Riaz, 2012). In such circumstances the weed dominates, forming stands that reduce the presence of all other species (Chippendale and Panetta, 1994; Navie et al., 1996; Riaz and Javaid, 2011). Previous studies have therefore reported the weed to cause major habit alterations including the rapid replacement of native grasses and other herbaceous species, and causing a reduction in community species diversity (Evans, 1997; Navie et al*.,* 2004; Yaduraju et al*.,* 2005). This loss of diversity usually leads to a reduction in fodder quantity and quality as the remaining herbaceous ruderal plants are poorly palatable (Archer and Smeins, 1991; Milton et al., 1994). This, in turn, leads to a lowering of the carrying capacity of the grassland making profitable beef cattle production very difficult (Nigatu et al., 2010). Currently in Queensland, Australia, *P. hysterophorus* is mostly a weed of grasslands, affecting beef cattle production by as much as *ca.* AU$95 million annually (Adamson, 1996) in up to 60 million ha of land (Department of Primary Industries 2011).

Parthenium hysterophorus is susceptible to a number of selective herbicides and therefore chemical management is usually effective over short periods of time (Navie et al., 1996). However, chemical management can also trigger secondary successional trends within grassland communities (Connell and Slatyer, 1977) as they can open up the vegetation matrix, which in turn allows for the recruitment of fugitive species capable of rapid establishment (Lavorel et al., 1999). Therefore, selective herbicides use to manage weeds are thought to shift, quantitatively and qualitatively, the community composition, but this has never been studied in detail in a *P. hysterophorus*-invaded grassland.

The most common design of studies assessing the response of a plant community to a certain disturbance such as a weed management strategy, is by eliminating the weed (either manually or chemically) from an area and monitoring the subsequent vegetation recovery in comparison to that of a matched weedy area (e.g. Fowler, 1981; Hejda and Pysek, 2006). This technique is one that involves time-sequence studies (Adair and Groves 1998). Due to the patchiness of species distribution and the kinds of temporal change that go on in grassland communities, data exhibit great variation in density and species composition over very small areas as well as over time (Bigwood and Inouye, 1988; Bullock, 2006). To avoid possible problems due to great data variation, focus on a local ecological problem on a small scale controlled field experiment contains very useful information.

Despite *P. hysterophorus* has been studied intensively over the past 30 years in Australia, no previous studies have examined how the plant community composition and diversity can be shifted after specific management interventions have been applied for this weed. Following the traditional succession sequence (Connell and Slatyer, 1977) we would expect a significant turnover of species in this type of grasslands after 2 or 3 year of the chemical control of *P. hysterophorus* with a gain in perennial species. The validation of successional theory to guide restoration of an invasive plant dominated grassland (Sheley et al., 2006) has previously shown successful, but this has never been studied for a *P. hysterophorus*-invaded grassland.

In this study, we evaluate the impact of long term *P. hysterophorus* invasion and then management upon the above- and below-ground community composition during a 2-year period. An initial community survey formed a baseline onto which all subsequent changes resulting from management could be compared. We asked the following questions: (1) what is the current species composition of this grassland-based agro-ecosystem that has been grazed and invaded by *P. hysterophorus* for more than 25 years?, (2) has *P. hysterophorus* altered the above- or below-ground species composition, plant diversity or productivity of this grassland, and (3) does chemical and grazing management lead to an improvement in species composition, diversity and productivity in a 2 year-period?

Materials and methods

Study area

This investigation was conducted in the Kilcoy district (26.94^{o} S, 152.49^{o} E) of south-east Queensland, Australia. The site (*ca.* 231 m above sea level) was gently sloping with good drainage and consisted of a typical soil for the region (i.e. a brown-grey dermosol, pH 6). The vegetation of the region was native grassland that has been under continuous grazing for at least 100 years and infested with *P. hysterophorus* for at least 25 years (D. Youles, personal communication, 2011). This continuous grazing practice has led to the replacement of certain desirable native species (such as *Heteropogon contortus* L.), with other less desirable species (Loi and Malcom, 1998). The majority of the species present within these grasslands are perennial grasses with also a high presence of forbs and graminoids (Nguyen, 2011). A two-strata vegetation system has developed, as is commonly found in long-term grazed, sub-humid grasslands, and consists of a low dense stratum, no more than 5 cm tall and a taller stratum of bunched grasses and small woody plants (Altesor et al., 2005).

Before this study, the area had been used for cattle grazing at a stocking rate of *ca.* 0.5 cows ha^{-1} during the drier winter months (June to August) and 0.8 cows ha^{-1} during the wetter summer months (December to February; D. Youles, personal communication, 2011). For the last 6 years, the study area had also been subjected to an annual aerial herbicide application of a mixture of metsulfuron-methyl and 2,4-D (8 g a.i. and 200 mL a.e., respectively in every 100 L of water) for the chemical management of *P. hysterophorus*, except during the study. The climate at the site is sub-tropical, with a long-term average annual precipitation of 950 mm, occurring mainly in the summer months of January and February. The minimum and maximum temperatures at the site ranged between 7 ^{o}C for night-time lows in winter to 29 ^{o}C for day-time highs during summer (Australian Bureau of Meteorology, 2012).

This study was undertaken over two contiguous sites (each *ca.* 300 m^2), one being grazed and the other ungrazed, both with similar infestation levels of *P. hysterophorus* (*ca.* 7 plants m^{-2}), within a 105 ha paddock. Within each site there were two treatments, so over the two sites four treatments were compared: 1) an ungrazed treatment with a herbicide application (UWH); 2) an ungrazed treatment with no herbicide application (UNH); 3) a grazed treatment with herbicide application (GWH) and 4) a grazed treatment with no herbicide application (GNH). The herbicide was a mixture of metsulfuron-methyl and 2,4-D (8 g a.i. and 200 mL a.e., respectively in every 100 L of water). It was applied twice in the autumn of the first year (on 17 March 2010 and on 18 April 2010).

Assessment of the above-ground plant community

Vegetation sampling was done between the summer of 2010 and the summer of 2012. Prior to the first application of herbicide and change in grazing management (i.e. February 2010), the species composition of the community (above- and below-ground) was assessed within each of the four treatments (for this first survey so-called plots as herbicide application has yet not occurred) and this composition used to form a baseline against which all future community comparisons could be made. The remaining species composition surveys were undertaken in August (winter) of 2010 and 2011 and February (summer) of 2011 and 2012. Each survey was undertaken by sampling 40 random quadrats (each 1 m^2 and 10 $treatment^{-1}$, considered replicates). Within each quadrat, species identity and abundance were determined. Exceptions to this protocol were for two stoloniferous grass species, blue couch (*Digitaria didactyla* Willd.) and green couch (*Cynodon dactylon* L. Pers.), whose abundances were estimated by a visual estimate of the proportion of the quadrat occupied by these two species (Smart et al., 2006). As such, a conversion to individual plants was necessary for data analysis purpose, being assumed that 2% cover corresponded to an individual (i.e. 50% of coverage was equal to 25 individuals; S. Navie, personal communication, 2010).

Twenty-five percent of the vegetation from each quadrat was then separated into two groups, either *P. hysterophorus* or all other species, and dry biomass samples (90 ± 5 °C for *ca.* 72 hours) taken.

Assessment of the below-ground plant community

Two soil cores (7.2 cm diameter, 10 cm deep) were collected at random from within each quadrat and mixed together. Hence, a total of 10 samples were collected from each of the four treatments and on each survey occasion. The soil samples were returned to the glasshouse at the University of Queensland, Brisbane and spread thinly (*ca.* 5 mm) over a sterilized soil mixture (University of California mixture; forming a 3 cm thick basal layer) that was contained within shallow plastic germination trays (20 cm × 25 cm × 6 cm, w/l/h; one quadrat sample per tray). These trays, plus two control trays of the sterilized soil mixture, were then distributed randomly onto benches in the glasshouse, with the temperature being maintained similar to that of the ambient temperature outside. Soil moisture content was maintained close to field capacity by daily watering. Trays were assessed weekly for any newly emerging seedlings. Once emerged, seedlings were identified and counted, removed and discarded. In the case where immediate identification was not possible, representative individuals were transplanted into small pots and grown to maturity, to allow for later taxonomic identification. When no further emergence was recorded for a period of 2 weeks (*ca.* 26 weeks after the start of the study), the soil was allowed to dry for 1 week, stirred, then rewetted to trigger further germination. Each seed bank assessment, from each of the five survey occasions, was run for a total of 6 months to allow for all of the species in the seed bank to be identified, including those with long-term seed dormancy.

Data analysis

Frequency and abundance of each species, as baseline descriptive parameters were calculated using the following formulae:

$$\text{Frequency of species A (\%)} = \frac{\text{Number of quadrats species A occurs}}{\text{Total number of quadrats sampled}} \times 100$$

$$\textit{Parthenium}\text{ weed frequency} = \frac{\text{Number of } \textit{Parthenium} \text{ weed individuals}}{\text{Total number of individuals in the quadrat sampled}}$$

$$\text{Abundance of species A (plants m}^{-2}\text{)} = \frac{\text{Total number of individuals of species A}}{\text{Total number of quadrats where species A occurs}}$$

For all survey occasions, species diversity within the above-ground vegetation and the soil seed bank was assessed using the Shannon-Wiener index. This was undertaken for the whole plant community (*H'* total) and for the species that were considered to be forage species consumed by domestic livestock (*H'* forages):

$$H' = -\sum_{i=1}^{S} p_i \log_e p_i$$

where S is the number of species, p_i is the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: $p_i = n_i / N$, where n_i is the number of individuals in species $_i$ (i.e. the abundance of species $_i$ and N is the total number of all individuals; Krebs 1989).

The evenness within the above-ground community and the soil seed bank was assessed using Pielous' evenness index:

$$J' = H' / H'_{max}$$

where H'_{max} is the theoretical maximum value for *H'* if all species in the sample were equally abundant, and calculated as:

$$H'_{max} = -S(1/S)(\log 1/S)$$

where S is the number of species or species richness (Pielou, 1966). In order to obtain a set of species that have the same role in the ecosystem, species were grouped into four main plant functional types: summer grasses (SG), shrubs (Sh), forbs (Fo) or sedges (Cyperaceae, C) and rushes (Juncaceae, J). In a separate grouping, species were also classified according to their life cycle (i.e. annual, perennial, annual/biennial or annual/perennial), their growth habit (i.e. erect, prostrate or prostrate/ascending) and their origin to Australia (i.e. native or introduced). As species richness is strongly influenced by abundance (Peet, 1974 cited in Scott and Morgan, 2012), species richness for all survey occasions and for the plant functional types, life cycle, growth habit and origin groups, was assessed using *Margalef's richness index*, calculated as:

$$d = S - 1/\ln N$$

where *S* is the number of species, and *N* is the total number of all individuals (Margalef, 1951).

A correlation analysis was used to examine associations between *P. hysterophorus* abundance and biomass, species groupings and the remaining species biomass and all other calculated indexes (i.e. *H'* total, *H'* forages and *J'*) in the first survey occasion only, before treatments were applied. All data sets were analysed using Minitab, version 16 (Minitab Inc., USA).

Multivariate tests were performed to examine patterns of plant species composition and temporal changes in response to the managements in the area. As such, a non-metric multi-dimensional scaling (NMDS) approach in two dimensions, on both species abundance and presence/absence data, based on a Bray-Curtis dissimilarity matrix (Clarke and Warwick, 2006) was carried out. The extent of clustering according to chemical and grazing management was assessed using a maximum of 5,000 permutations in an analysis of similarity (ANOSIM), an analogue of the ANOVA specifically developed for ecological data. This tests for differences between and within groups of samples (i.e. multivariate) from different times and managements, and does not require normality and homoscedasticity assumptions, generating the statistic R. Values of R range from -1 to +1, with values approaching R = +1 indicating a strong dissimilarity among samples (Clarke and Warwick, 2006). When differences between treatments at $\alpha < 0.05$ or lower were detected a 'similarity percentage' routine (SIMPER) was used in order to identify which species were primarily contributing to the similarities/dissimilarities among managements. Species abundance was transformed as ($\sqrt{\text{number of plants m}^{-2}}$) to enhance their fit to the model. Both abundance and presence or absence data sets gave similar results and so only those from square root transformed abundance data are presented. All multivariate analyses were performed using the software Primer version 6.0 (Clarke and Gorley, 2006). Principal component analysis (PCA) was used, decomposing a correlation matrix, to summarize patterns, reduce dimensionality of the plant community, and to detect which species were causing response patterns to chemical and grazing management. The results presented from this analysis are a reduced data set, which includes only those species present at higher densities (i.e. achieved higher Eignvalues).

Results

Impact of P. hysterophorus on the baseline community

In total, 48 species were recorded in the above-ground baseline plant community prior to the application of the chemical and grazing management (Table 1). Of these, 12 species belonged to the Poaceae with *D. didactyla and Paspalidium distans* Trin. being more frequent (78% and 68%, respectively; Table 1), three to the Asteraceae and the remaining 37 species came from 16 other families. The frequency and abundance of *P. hysterophorus* prior to the application of management, were moderate to high (62.5% and 6.33 plants m^{-2}; Table 1) but significantly lower in one plot (R = 0.10, $P < 0.05$, and R = 0.11 $P < 0.05$, respectively), and significantly higher in another (Table 2). Within the highest *P. hysterophorus* infested plot (13 plants m^{-2}), there was a significant negative association between weed abundance and species diversity (*H*' total) and evenness (*J*'), prostrate and native species abundance and annual species richness (Table 3). *Parthenium hysterophorus* biomass was also negatively correlated with the abundance of facultative annual and erect species and the richness of perennial and erect species (Table 3).

The below-ground abundance of *P. hysterophorus* (11,912 seeds m^{-2}) was greatest in one plot (R = 0.19, $P < 0.001$) but this did not result in a significant reduction in species richness or abundance of any of the plant groups, neither with *H*' total, and *J*' (not shown).

Above-ground plant community shifts after management

By the summer of 2011, the above-ground biomass of *P. hysterophorus* was greatest in the UNH treatment (R = 0.16, $P < 0.05$). However, *P. hysterophorus* abundance and frequency did not follow the same trend being similar in all treatments (Table 2). Two years after the start of the study (summer of 2012), *P. hysterophorus* abundance and frequency was greater in the GNH treatment (R = 0.10, $P < 0.05$; R = 0.20, $P < 0.01$, respectively), while its above-ground biomass was similar in all treatments (Table 2).

The *H*' total and *J*' was greatest for the UNH treatment ($P < 0.01$ and $P < 0.05$, respectively; Table 2), however; *H*' forage species was not significantly different between treatments.

The NMDS showed a significant effect of the management upon the species composition (R = 0.28, $P < 0.01$) one year after the application of the management (i.e. summer 2011). The greatest dissimilarity in species abundance was seen between the ungrazed treatments (UWH and UNH), with *D. didactyla* and *C. dactylon* being the species that contributed the most to this difference (dissimilarity 69.46%; supplementary materials).

The two grazed treatments (GNH and GWH) were the most similar in species composition, with still some overlap between treatments (Fig. 1b).

In the summer 2012 survey, 2 years after the application of the initial managements, the NMDS still showed a significant effect of the managements and dissimilarities in most of the pair-wise comparisons between treatments (R = 0.46, $P < 0.001$), continuing to show some overlap on species composition between treatments (Figure 1b). The greatest dissimilarity in species abundance was now seen between the two most contrasting treatments; UWH and GNH, with *D. didactyla* and *C. dactylon* being the species that contributed the most to this difference (dissimilarity 81.25%). The treatments GNH and GWH were still the two that were the most similar in species composition.

Principal components analysis indicated that *D. didactyla* and *P. hysterophorus* presented unusual values above-ground for all four plots in the summer 2010 survey (Fig. 2a). When examining the effects of the managements on species composition during summer 2011, three of the treatments (GNH, GWH and UNH) were shown to be highly correlated and the first principal component removed 92.6% of the variation from the data set, which subsequently removed the average effect of the factors. After 1 year without grazing but with chemical management (UWH) *P. hysterophorus* was seen to be replaced by *C. dactylon*. Two years after the application of the management, PCA indicated that three of the treatments (GNH, GWH and UNH) were shown to be still highly correlated and the first principal component removed 83.9% of the variation from the data set, which removed the average effect of the factors. After 2 years without grazing and with chemical management (UWH), *P. hysterophorus* was still being replaced by *C. dactylon* (Figure 2b).

The herbicide application decreased the number of other broadleaved species and thus the number of botanical families in the herbicide applied areas also decreased, with greater reduction under the ungrazed situation (data not shown).

The above-ground biomass of all the remaining species was still similar between treatments 1 year after the start of the management. Two years after the application of the treatments, however, the UWH treatment showed greater biomass (R =0.48, $P < 0.001$), with an improvement in forage availability of 80% increase (Table 2).

Below-ground plant community shifts after management

In the summer of 2011, *P. hysterophorus* below-ground abundance was greatest in the UNH treatment (2,492 seeds m^{-2}; R = 0.21, $P < 0.001$). One year later (i.e. summer of 2012), *P. hysterophorus* abundance was still greater in the UNH treatment (1,584 seeds m^{-2}; R = 0.25, $P < 0.001$). The *H*' forage species did not show significant differences between treatments (R = 0.01, $P = 0.3$; R = 0.063, $P = 0.056$). However, *J*' was greater for the GWH treatment (R = 0.17, $P < 0.01$). By the summer 2012, *H*' total and *J*' was similar for all four treatments.

The NMDS on the below-ground species abundance showed a significant effect of the management on the species composition (R = 0.33, $P < 0.01$) and dissimilarities in most of the pair-wise comparisons between treatments (supplementary materials). The greater dissimilarity in species abundance was now, similarly to the above-ground vegetation, between the ungrazed treatments (UWH and UNH), with *Lepidium didymum* L. *Digitaria didactyla* and *P. hysterophorus* being the species that contributed the most to this difference (dissimilarity 66.68%). The grazed treatments (GNH and GWH) were similar in species composition. However, the NMDS on species abundance still showed some overlap on species composition between treatments

In the summer 2012, the NMDS on below-ground species abundance still showed a significant effect of the management on the species composition (R = 0.31, $P < 0.001$) and dissimilarities in most of the pair-wise comparisons between treatments. The greater dissimilarity in species abundance was now between the UWH and the GNH treatments, with *C. ciliaris*, *D. didactyla* and *C. dactylon* being the species that contributed the most to this difference (dissimilarity 57.60%).

When examining the effect of the managements on species composition through PCA analysis, three of the treatments (GNH, GWH and UNH) were shown to be highly correlated and the first principal component removed 51.7% of the variation from the data set, which removed the average effect of the factors. Principal components analysis indicated that *C. gracilis*, *P. oleracea*, *O. exilis* and *P. hysterophorus* presented unusual values for all four treatments in the summer 2010 survey (supplementary materials). After 1 year of grazing and chemical management *P. hysterophorus* was replaced by *D. didactyla*. Two years after the application of the managements, the first principal component removed only 49.3% of the variation from the data set, which removed the average effect of the factors. After 2 years of removal of grazing and herbicide control (UWH) *P. hysterophorus* was still being replaced by *D. didactyla*.

Discussion

The results of this study showed that the current species composition of this grassland-based agro-ecosystem that has been grazed and invaded by *P. hysterophorus* for more than 25 years, was dominated by weed species with only two forage grasses being present in high frequencies (*i.e. D. didactyla* and *P. distans*).

Previous observations of the region's native grasslands have shown that this kind of plant community is indicative of a history of heavy grazing (Loi and Malcom, 1998). This grazing history has probably resulted in the current plant community having a relatively low resistance to the invasion by weeds (Tracy et al., 2004; Tozer et al., 2008). Even though aerial herbicide applications were in place, the rapid adaptation, colonization and strong allelopathic and competitive ability of *P. hysterophorus* in highly disturbed areas, may explain its high abundance, high coverage and frequency at the site (Adkins and Navie, 2006).

The overall dissimilarities in species abundance measured on the baseline community were similar for the above- and below-ground plant communities. However, the general composition and the contribution of each species to these different communities differed, as the seed bank was dominated by species that produce large number of seeds, such as *C. gracilis*. In other studies low similarities between the above and below-ground community composition have been frequently observed, and are not related to the infestation level of *P. hysterophorus* (Osem et al., 2006; Nigatu et al., 2010; Tozer et al., 2010). The assessment methodology used for the below-ground community allows for the germination of species that might otherwise remain dormant under field conditions and with these species not being present at the time of the above-ground survey. The above-ground abundance of *P. hysterophorus*, did not reflect its large seed bank size. This result agrees with previous studies that show the germinable soil seed banks of this weed to be very large and persistent, while the above ground communities are represented by only *ca.* 2 to 10 plants m^{-2} (Navie et al., 2004; Nguyen, 2011).

Our investigation demonstrated that greater *P. hysterophorus* abundance affected negatively not only the species diversity and richness but also the species evenness across the community. Previous research has suggested that the species diversity of grassland plant communities is significantly reduced by the presence of *P. hysterophorus* (Nguyen et al., 2010; Nigatu et al., 2010; Timsina et al., 2011). Earlier field studies, however, have not explored the influence of *P. hysterophorus* abundance upon community evenness. This negative association is, nevertheless, expected as when *P. hysterophorus* dominates the plant community it forms pure stands of plants reducing the presence of the remaining species (Chippendale and Panetta, 1994; Navie et al., 1996). Indeed, this present study has shown that greater abundance of *P. hysterophorus* can negatively affect native species abundance (Table 3).

As the PCA analysis confirmed the dominance of *P. hysterophorus* within the study site during the first summer (Figure 2a), we expected the management approaches to have a considerable impact upon this grassland. Our results revealed a shift in species composition both above- and below-ground when the invader was better managed. The PCA analysis clearly showed that *P. hysterophorus* was being replaced by *C. dactylon*

1 year after the application of the herbicide and the exclusion of livestock grazing. This was to be expected, considering the growth habit of the stoloniferous grass species *C. dactylon* that can rapidly colonize bare soil niches created by the herbicide application. These results are consistent with previous studies where a vigorous colonization by *C. dactylon* occurred in the gaps created after removing weeds (Fowler 1981). In our study, the colonization by *C. dactylon* and an exclusion of grazing plan did improve the fodder biomass production of the grassland, and therefore its carrying capacity. Nevertheless, the increased *C. dactylon* abundance did not cause an increase in the total species diversity of the community. As its forage quality and digestibility for domesticated livestock is known to be poor (Arthington and Brown, 2005), it would be desirable that other, native and more valuable forage species (*H. contortus*, *Bothriochloa decipiens* (Hack) C.E. Hubb) colonizes the niches created by the weed management program. Chemical management used for the control of broadleaf species have been previously noticed to favour exotic grass species abundance and biomass rather than native grasses (Sheley et al., 2006).

Despite, high rainfall during all summer sampling periods (1,619 mm for 2010; 1,168 mm for 2011 and 980 mm for 2012; Australian Bureau of Meteorology, 2012), the chemical management was more effective in reducing *P. hysterophorus* abundance under the ungrazed situation (UWH) demonstrating the stress that grazing was imposing upon the grassland-based agro-ecosystem and showing that it is necessary to remove grazing to obtain the best results from the chemical management approach. The reductions of *P. hysterophorus* abundance and biomass caused by the chemical management did not relate to an increase in either the total species diversity or the forage species diversity of the community. The initial species diversity at this site was relatively low.

Despite the chemical management, the grazed treatments (GNH and GWH) still had a similar species composition 2 years after the chemical management was applied, while the exclusion of grazing showed the chemical to create a succession towards a completely different species composition (UNH and UWH; Figure 1b). The removal of grazing also allowed for an increase in the total species diversity and evenness when no chemical management was applied (UNH; Table 3). These results confirm grazing to be one of the most important disturbance factors driving down species community composition, and reducing the beneficial effect of chemical management explaining why the previous aerial herbicide applications effectiveness was being compromised.

Importantly, this study has shown that in this region, a shift in community composition, in terms of replacement of the invasive *P. hysterophorus* by forage species, can be seen in as little as 1 year after putting in place a management plan for *P. hysterophorus* and that this improvement continues to strengthen over the second year. We have demonstrated that after a severe disturbance over an area, such as that caused by a herbicide application, recruitment comes from vegetative growth from neighbour species with 'early succession' characteristics (Connell and Slatyer, 1977). These results support previous suggestions that the reduction of domesticated livestock grazing rates might improve the species composition and the productivity of a long-disturbed grassland community (McGovern et al., 2011), adding that both strategies (chemical management and the removal of grazing) need to be carried out together to effectively control the invader.

It is likely that a longer timeframe would detect the traditional succession sequence, with a gain in perennial species (Connell and Slatyer, 1977) and the recovery on the species diversity of the community expected by the chemical control of *P. hysterophorus* and the exclusion of grazing. A significant turnover of species in this type of grasslands might be expected after 2 or 3 years of removing grazing (Rodriguez *et. al* 2003). Previous studies have shown that the increase in the abundance of native species after the invader is removed and/or grazing is excluded is slow (Altesor et al., 2005; Flory and Clay, 2009; Cuevas and Zalba, 2010).

The greater number of species found in the below-ground community, and the replacement of *P. hysterophorus* by other species in the early stages of secondary succession, instead of new recruitment of the weed seedlings, indicates that it might be possible to improve the above-ground species composition of this grassland community over time with *P. hysterophorus* management practices in place.

Despite the focus of this present study on a small scale controlled field experiment, studies such as these are the vital foundation for subsequent understanding of how ecological communities work. Thus, these results support the success of using successional theory to guide restoration of an invasive plant dominated grassland (Sheley et al., 2006) and its validation for an *P. hysterophorus* invaded grassland. As such, our results indicate that using grazing management and herbicide applications as restoration techniques of grassland long dominated by *P. hysterophorus* might direct the plant community towards more desired species, but the improvement in species richness and diversity and complete achievement of the restoration will probably be seen in a longer term.

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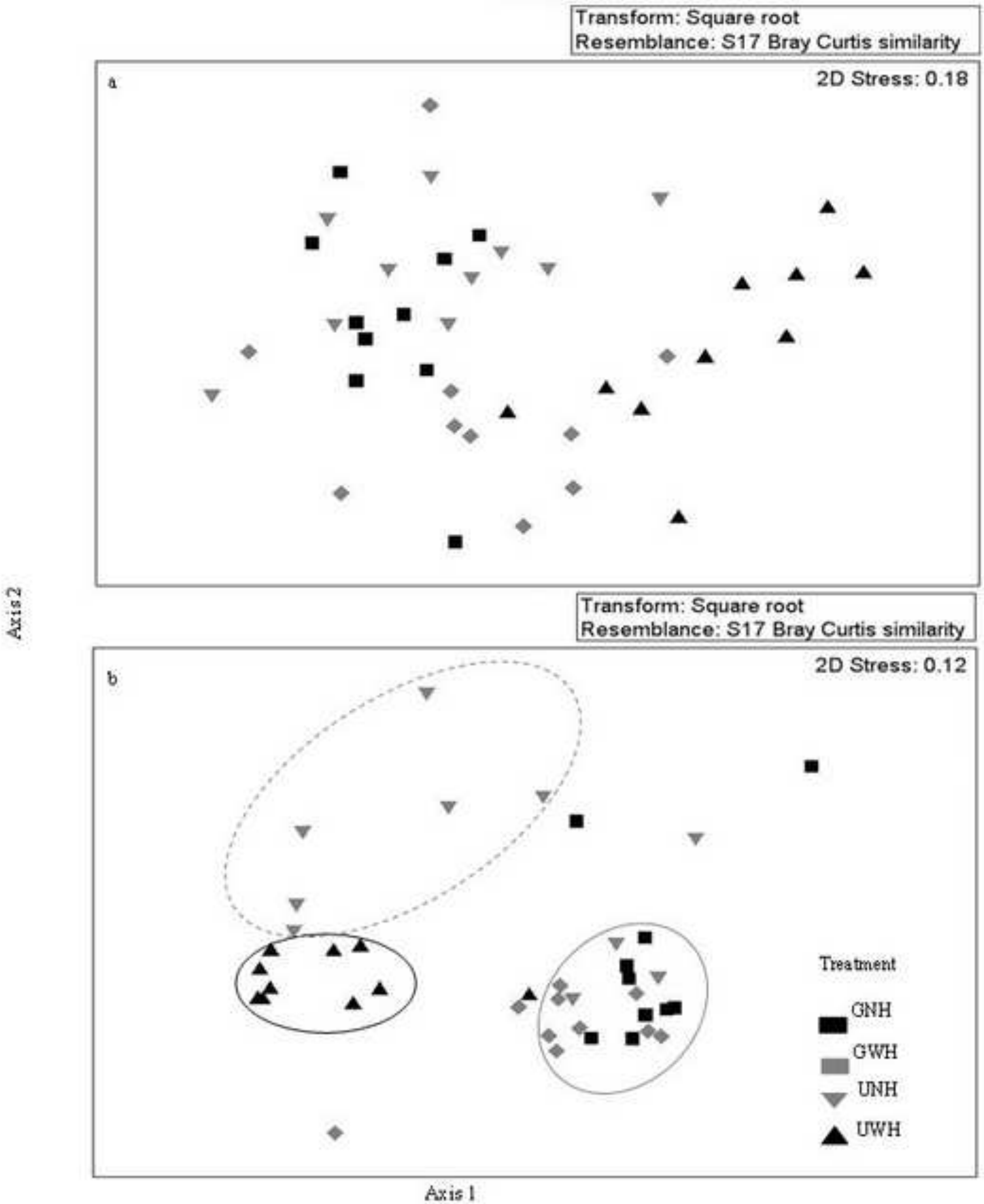
Fig. 1. Results of the two-dimensional non-metric multi-dimensional analysis based on a Bray-Curtis dissimilarity matrix on the above-ground plant community for the four treatment treatments: ungrazed treatment with herbicide application (UWH); ungrazed treatment with no herbicide application (UNH); grazed treatment with herbicide application (GWH) and grazed treatment with no herbicide application (GNH); (a) in the summer of 2010 and (b) in the summer of 2012. Symbols enclosed within different line colours denote dissimilarities between treatments.

Fig. 2. Results of the PCA analysis on the above-ground species composition in (a) summer 2010 and (b) in summer 2012. The first axis explained 59.2% and 83.9% of the total variation of the data set in summer 2010 and summer 2012, respectively. Full names of species are given in Table 1.

Table 1

The frequency and abundance of the species recorded in the above- (AG) and below-ground (BG) plant communities at the studied plots during the summer of 2009/2010 prior to the application of the management treatments.


Transform: Square root
Resemblance: S17 Bray Curtis similarity
a
2D Stress: 0.18
Transform: Square root
Resemblance: S17 Bray Curtis similarity
b
2D Stress: 0.12
Treatment
GNH
GWH
UNH
UWH
Axis 2
Axis 1

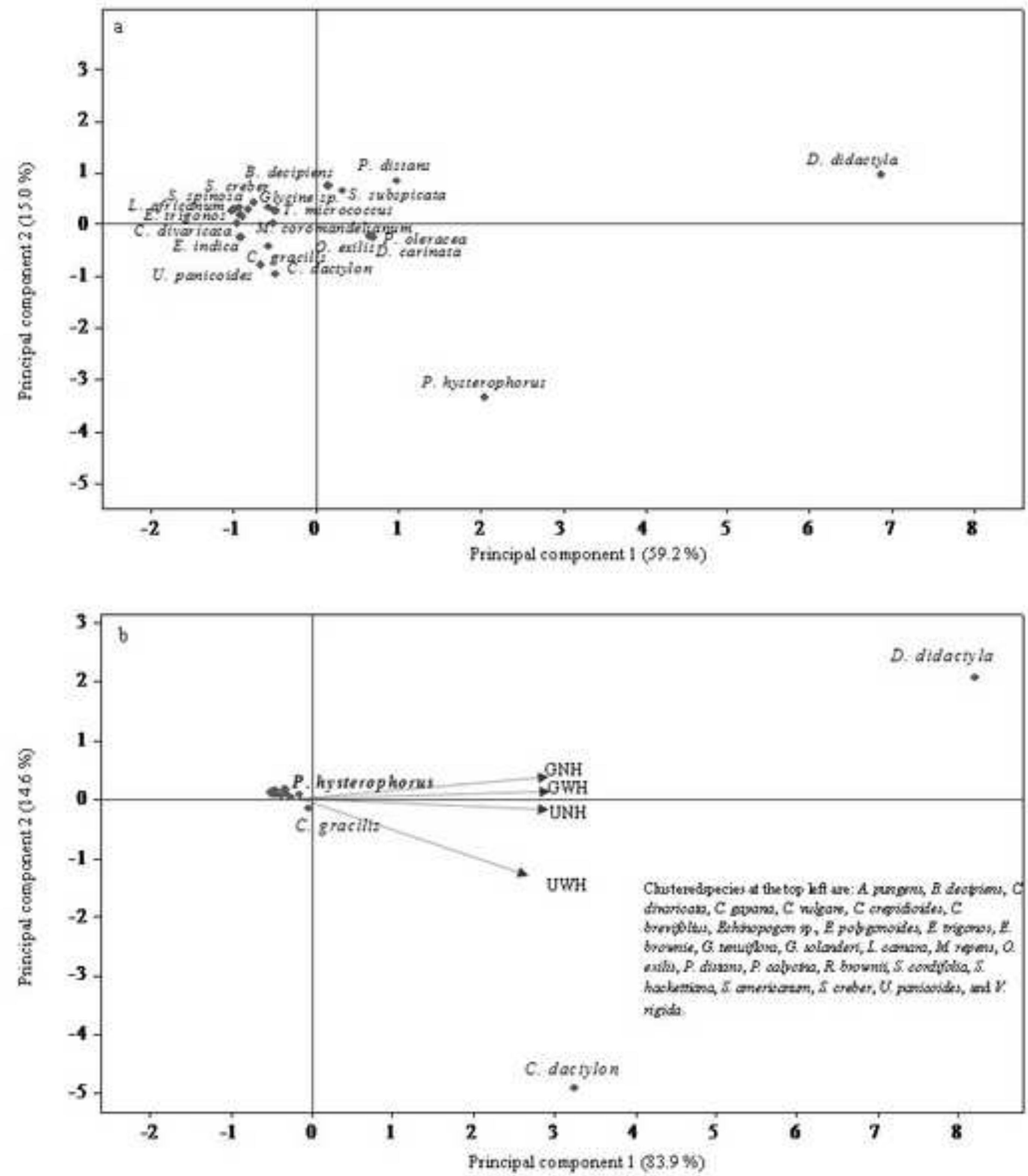

a
D. didactyla
P. dissans
B. decipiens
S. creber
S. spinosa
Glycine sp.
S. subspicata
L. africanum
E. trigonos
T. micrococcus
C. divaricata
M. coromandelianum
P. oleracea
E. indica
O. exilis
D. carinata
C. gracilis
C. dactylon
U. panicoides
P. hysterophorus
Principal component 1 (59.2 %)
Principal component 2 (15.0 %)
b
D. didactyla
P. hysterophorus
GNH
GWH
UNH
UWH
C. gracilis
Clusteredspecies at the top left are: A. pungens, B. decipiens, C. divaricata, C. gayana, C. vulgare, C. crepidioides, C. brevifolius, Echinopogon sp., E. polygonoides, E. trigonos, E. brownie, G. tenuiflora, G. solanderi, L. camara, M. repens, O. exilis, P. distans, P. calycina, R. brownii, S. cordifolia, S. hackettiana, S. americanum, S. creber, U. panicoides, and V. rigida.
C. dactylon
Principal component 1 (83.9 %)
Principal component 2 (14.6 %)

Table 1
The frequency and abundance of the species recorded in the above- (AG) and below-ground (BG) plant communities at the studied plots during the summer of 2009/2010 prior to the application of the management treatments.

Botanical family	Species	LC[1]	PFT[2]	GH[3]	Frequency (%)		Abundance (plants m^{-2})	
					AG	BG	AG	BG
Amaranthaceae	*Alternanthera nana* R.Br.	A	Fo	P	5.0	5.0	0.44	3.11
	**Alternanthera pungens* Kunth	A/P	Fo	P	45.0	10.0	1.10	12.28
	**Amaranthus spinosus* L.	A	Fo	E	2.5	2.5	0.03	21.50
	**Amaranthus viridis* L.	A	Fo	E	0.0	2.5	0.00	3.07
	**Gomphrena celosioides* Mart.	A/P	Fo	P	12.5	5.0	0.18	6.14
Apiaceae	**Cyclospermum leptophyllum* (Pers.) Sprague	A	Fo	E	0.0	20.0	0.00	36.83
	Hydrocotyle acutiloba (F.Muell.) Wakef.	P	Fo	P	0.0	27.5	0.00	55.30
	**Soliva* sp.	A	Fo	P	0.0	17.5	0.00	1,096.00
Asteraceae	**Conyza bonariensis* (L.) Cronq.	A/B	Fo	E	0.0	37.5	0.00	187.30
	**Conyza sumatrensis* (Retz.) E.H. Walker	A/B	Fo	E	7.5	17.5	0.15	52.20
	**Gamochaeta pensylvanica* (Willd.) Cabrera	A	Fo	E	0.0	32.5	0.00	125.90
	***Parthenium hysterophorus* L.**	**A**	**Fo**	**E**	**62.5**	**67.5**	**6.33**	**3,856.00**
	Pterocaulon redolens (Willd.) Fern.-Vill.	A/P	Fo	E	0.0	5.0	0.00	6.15
	**Sonchus oleraceus* L.	A	Fo	E	0.0	5.0	0.00	6.14
	Vittadinia sulcata N.T.Burb.	A	Fo	E	0.0	2.5	0.00	3.37
	**Xanthium spinosum* L.	A	Fo	E	42.5	0.0	0.08	0.00
Boraginaceae	**Heliotropium amplexicaule* Vahl	P	Fo	E	0.0	5.0	0.00	18.42
Brassicaceae	**Lepidium africanum* (Burm.f.) DC.	A/B	Fo	E	15.0	37.5	0.23	239.50
	**Lepidium bonariense* L.	A/B	Fo	E	0.0	45.0	0.00	101.30
	**Lepidium didymum* L.	A	Fo	P	0.0	57.5	0.00	512.70
Campanulaceae	*Wahlenbergia gracilis* (G.Forst) A.DC.	P	Fo	E	0.0	67.5	0.00	331.60
Chenopodiaceae	*Dysphania carinata* (R.Br.) Mosyakin & Clemants	A	Fo	P	70.0	50.0	3.20	319.00
	Dysphania pumilio (R.Br.) Mosyakin & Clemants	A	Fo	P	0.0	70.0	0.00	596.00
	Einadia polygonoides (Murr.) Paul G. Wilson	P	Fo	P	20.0	72.5	0.20	362.30
	Einadia trigonos (Schult.) Paul G. Wilson	P	Fo	P	20.0	45.0	0.40	138.20
Convolvulaceae	*Ipomoea* sp.	P	Fo	P/A	2.5	0.0	0.03	0.00
Crassulaceae	*Crassula sieberiana* (Schult. & Schult.f.) Druce	A/P	Fo	P/A	0.0	7.5	0.00	9.21
Cyperaceae	**Cyperus brevifolius* (Rottb.) Hassk.	P	C	E	0.0	30.0	0.00	86.00
	Cyperus gracilis R.Br.	P	C	E	55.0	97.5	1.23	4,501.00
	Cyperus iria L.	A	C	E	0.0	10.0	0.00	15.35
Fabaceae	*Galactia tenuiflora* (Willd.) Wight & Arn.	P	Fo	P	17.5	0.0	0.25	0.00
	Glycine sp.	P	Fo	P	22.5	0.0	0.33	0.00
	**Macroptilium atropurpureum* (DC.) Urb.	A/P	Fo	E	2.5	0.0	0.30	0.00
Gentianaceae	**Schenkia spicata* (L.) Mansion	A/B	Fo	E	0.0	87.5	0.00	1,145.00
Iridaceae	**Sisyrinchium* sp. Peregian	A	Fo	E	0.0	2.5	0.00	6.14
Juncaceae	*Juncus usitatus* L.A.S.Johnson	P	J	E	0.0	5.0	0.00	6.14
Lamiaceae	**Stachys arvensis* (L.) L.	A	Fo	E	0.0	2.5	0.00	3.07
Malvaceae	**Malva parviflora* L.	A/P	Fo	E	5.0	5.0	0.08	6.14

Botanical family	Species	LC[1]	PTF[2]	GH[3]	Frequency (%)		Abundance (plants m^{-2})	
					AG	BG	AG	BG
Malvaceae	**Malvastrum americanum* (L.) Torr.	A/P	Sh	E	2.5	10.0	0.03	15.36
	**Malvastrum coromandelianum* (L.) Garcke	A/P	Sh	E	25.0	2.5	0.40	3.08
	**Sida cordifolia* L.	A/P	Sh	E	5.0	2.5	0.05	6.14
	**Sida rhombifolia* L.	A/P	Sh	E	32.5	7.5	0.50	9.22
	**Sida spinosa* L.	A/P	Sh	E	37.5	17.5	0.53	36.8
	Sida hackettiana W. Fitzg.	A/P	Sh	E	50.0	0.0	2.58	0.00
Myrsinaceae	**Lysimachia arvensis* (L.) U.Manns & Anderb.	A/P	Fo	P	0.0	2.5	0.00	3.07
Nyctaginaceae	*Boerhavia dominii* Meikle & Hewson	A/P	Fo	P	2.5	0.0	0.03	0.00
Ophioglossaceae	*Ophioglossum reticulatum* L.	P	Fo	E	5.0	0.0	0.00	0.00
Oxalidaceae	*Oxalis exilis* A. Cunn.	A/P	Fo	E	50.0	95.0	0.95	2,042.00
	Oxalis debilis var. corymbosa.	A/P	Fo	P	7.5	57.5	0.13	414.50
Plantaginaceae	*Plantago debilis* R.Br.	A/P	Fo	E	7.5	25.0	0.10	52.20
Poaceae	*Aristida* sp.	P	G	E	5.0	0.0	0.08	0.00
	Bothriochloa decipiens (Hack.) C.E.Hubb.	P	SG	E	57.5	7.5	2.23	9.22
	Chloris divaricata R.Br.	P	SG	E	12.5	27.5	0.18	70.60
	Chloris ventricosa R.Br.	P	SG	E	0.0	7.5	0.00	24.60
	**Cynodon dactylon* (L.) Pers.	P	SG	P	17.5	5.0	1.00	12.27
	**Digitaria didactyla* Willd.	P	SG	P	77.5	90.0	14.20	1,857.00
	**Eleusine indica* (L.) Gaertn.	A	SG	E	22.5	22.5	0.32	55.30
	**Eragrostis cilianensis* (All.) Janch.	A	SG	P	2.5	32.5	0.03	49.14
	Paspalidium distans (Trin.) Hughes	P	SG	E	67.5	60.0	3.83	267.10
	Sporobolus creber De Nardi	P	SG	E	2.5	32.5	0.65	187.30
	Sporobolus elongatus R.Br.	P	SG	E	2.5	27.5	0.03	95.20
	**Urochloa panicoides* Beauv.	A/B	SG	E	2.5	2.5	1.30	6.14
Polygonaceae	**Polygonum aviculare* L.	A/P	Fo	E	0.0	2.5	0.00	3.07
	Rumex brownii Campd.	P	Fo	E	15.0	12.5	0.15	36.80
Portulacaceae	**Portulaca oleracea* L.	A	Fo	P	85.0	95.0	3.43	2,708.00
	**Portulaca pilosa* L.	A	Fo	P	5.0	37.5	0.05	98.30
Solanaceae	**Datura ferox* L.	A	Fo	P/A	2.5	0.0	0.05	0.00
	**Solanum nodiflorum* Jacq.	A/P	Fo	E	30.0	5.0	0.03	6.15
Urticaceae	**Urtica incisa* Poir.	P	Fo	E	0.0	2.5	0.03	3.07
Verbenaceae	**Verbena litoralis* Kunth	P	Fo	E	2.5	7.5	0.03	15.40
	**Verbena rigida* (Hayek) Moldenke	P	Fo	E	2.5	20.0	0.08	33.78
Zygophyllaceae	*Tribulus micrococcus* Domin	A/B	Fo	P	45.0	0.0	1.10	0.00
-------	Unknown 1(shrub)	------	Sh	-------	0.0	10.0	0.00	0.00
	Unknown 2			-------	0.0	2.5	0.00	12.29
	Unknown 3			-------	0.0	2.5	0.00	3.07

[1]LC = Life cycle consists of longevity (A = annual, P = perennial A/B = annual/biennial, A/P = annual/perennial);[2]PFT = Plant Functional Types (Fo = forb, SG = summer grass, C = Cyperaceae, J =Juncaceae and Sh = shrub); [3]GH = Growth habit (E = erect, P = prostrate, P/A = prostrate/ascending). *Introduced species (exotic) after Stanley and Ross 1983, 1986, 1989, PlantNET – National Herbarium of New South Wales website and Hussey et al., 2007).

Table 2

The total species diversity (*H*' total), the forage species diversity (*H*' forages), frequency, abundance and biomass of *Parthenium hysterophorus* L. and biomass of the remaining species in the above-ground plant communities in four treatment treatments: ungrazed with herbicide application (UWH); ungrazed with no herbicide application (UNH); grazed with herbicide application (GWH) and grazed with no herbicide application (GNH). Mean values (± 2 SE).

Variable	Treatment	Summer survey occasion		
		2010	2011	2012
H' Total	UWH	1.76 (± 0.13)	0.96 (± 0.15)†	0.44 (± 0.09)†††
	UNH	1.90 (± 0.15)	1.48 (± 0.14)**	1.06 (± 0.19)††
	GWH	1.73 (± 0.16)	0.65 (± 0.14)†	0.59 (± 0.09)†††
	GNH	1.93 (± 0.12)	0.67 (± 0.17)†	0.48 (± 0.12)†††
H' forages	UWH	0.90 (± 0.13)	0.85 (± 0.13)	0.29 (± 0.08)††
	UNH	1.04 (± 0.12)	0.69 (± 0.09)	0.14 (± 0.07)†††
	GWH	0.79 (± 0.17)	0.59 (± 0.14)	0.42 (± 0.07)
	GNH	1.01 (± 0.10)	0.60 (± 0.14)	0.17 (± 0.06)††
J'	UWH	0.76 (± 0.04)	0.58 (± 0.08)	0.37 (± 0.06)†††
	UNH	0.75 (± 0.05)	0.76 (± 0.05)**	0.58 (± 0.07)
	GWH	0.78 (± 0.04)	0.47 (± 0.07)†	0.44 (± 0.06)††
	GNH	0.74 (± 0.05)	0.46 (± 0.07)†	0.31 (± 0.07)†††
Parthenium hysterophorus L. frequency	UWH	0.33 (± 0.08)***	0.01 (± 0.00)††	0.00 (± 0.00)†††
	UNH	0.15 (± 0.03)***	0.01 (± 0.04)†	0.01 (± 0.01)†
	GWH	0.07 (± 0.05)	0.00 (± 0.00)	0.00 (± 0.00)
	GNH	0.06 (± 0.03)	0.03 (± 0.02)	0.04 (± 0.03)**
Parthenium hysterophorus L. abundance (plants m^{-2})	UWH	13.10 (± 3.31)	0.30 (± 0.30)†	0.00 (± 0.00)†
	UNH	5.80 (± 2.82)	0.00 (± 0.00)	0.40 (± 0.27)†
	GWH	3.60 (± 2.70)	0.00 (± 0.00)	0.00 (± 0.00)
	GNH	2.80 (± 1.65)*	0.00 (± 0.00)	1.40 (± 0.88)*
Parthenium hysterophorus L. biomass (g m^{-2})	UWH	189.60 (± 87.7)	0.48 (± 0.48)††	0.00 (± 0.00)††
	UNH	40.60 (± 33.9)	32.12 (± 24.4)*	20.70 (± 15.70)
	GWH	50.40 (± 31.9)	0.00 (± 0.00)	0.00 (± 0.00)
	GNH	10.60 (± 10.6)*	9.92 (± 9.74)	28.50 (± 23.50)
Remaining species biomass (g m^{-2})	UWH	148.00 (± 29.4)	706.00 (± 102.0)††	758.60 (± 54.80)***††
	UNH	195.40 (± 43.2)	826.80 (± 95.6)††	479.00 (± 61.40)**†
	GWH	182.50 (± 16.1)	385.50 (± 75.2)†	268.70 (± 37.6)†
	GNH	235.50 (± 22.5)	531.00 (± 82.9)††	185.4 (± 21.6)

For each treatment N = 10 per survey occasion. All data sets, unless shown otherwise, exhibited no significant differences; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ indicate significant differences from ANOSIM across treatments (columns), † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$ indicate significant differences across survey occasions (rows, with respect to the first survey of the summer 2010).

Table 3

Pearson correlation coefficients for the in the above-ground plant communities between the abundance and biomass of *P. hysterophorus* (PW) and the total species diversity, forage species diversity, species evenness (*H*’ total, *H*’ forage and *J*’) and species abundance and richness.

	Variable	*H*' Total	*H*' forage	*J*'	Biomass PW	Species richness			Abundance				
						Annuals	Perennials	Erect	PW	Annual/ perennial	Erect	Prostrate	Natives
	H' Total	-------	0.53	0.96	-0.09	0.82**	0.16	0.13	**-0.64***	0.36	-0.43	0.63	0.48
	H' forage	NS	-------	0.31	-0.05	0.4	0.06	0.42	-0.03	0.3	0.01	0.06	-0.01
	J'	NS	NS	-------	0.16	0.77**	-0.12	-0.02	**-0.75***	0.2	-0.69*	0.52	0.38
	Biomass PW	NS	NS	NS	-------	-0.10	**-0.68***	**-0.64***	-0.22	**-0.77***	**-0.66***	-0.21	-0.24
Species richness	Annuals	NS	< 0.01	< 0.01	NS	-------	-0.25	0.38	**-0.86****	0.04	-0.51	0.73*	0.76*
	Perennials	NS	NS	NS	< 0.05	NS	-------	0.43	0.44	-0.25	0.6	0.15	-0.03
	Erect	NS	NS	< 0.05	< 0.05	NS	NS	-------	0.01	0.38	0.47	0.31	0.4
Abundance	PW	< 0.05	NS	< 0.05	NS	< 0.01	NS	NS	-------	0.15	0.75*	**-0.7***	**-0.69***
	Annual/ perennial	NS	NS	NS	< 0.05	NS	NS	NS	NS	-------	0.39	-0.05	-0.13
	Erect	NS	NS	NS	NS	NS	NS	NS	< 0.05	NS	-------	-0.29	0.4
	Prostrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-------	0.91***
	Natives	NS	NS	NS	NS	NS	NS	NS	< 0.05	< 0.05	NS	< 0.001	-------

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ and not significant (NS). Values in bold type are significant negative correlations with management implications.