Invasive grass affects seed viability of native perennial shrubs in arid woodlands

Kaisha M. Edwards · Christine Schlesinger · Mark K. J. Ooi ·
Kris French · Ben Gooden

Abstract It is often assumed that declines in native vegetation associated with alien plant invasion are driven by competition between plants for limited resources. However, invasion can also impact native plants through recruitment limitation mechanisms. We examined the effects of *Cenchrus ciliaris* L. (buffel grass, an alien pasture species) on the seed viability and germination of two native perennial shrubs (*Acacia tetragonophylla* and *A. victoriae*) in arid woodlands of central Australia. Dormancy, germination rate and seed viability were assessed using laboratory-based germination assays on seeds collected from the soil and mature shrubs in: (1) woodland invaded by buffel grass and (2) areas in which buffel grass had been removed and reinvasion prevented for at least 7 years. There was a twofold increase in viability of *A. victoriae* seeds in buffel grass-removed compared with invaded sites, and a faster germination rate (T_{50}) for *A. tetragonophylla* in buffel grass-removed sites. *Acacia victoriae* seed mass was reduced by approximately 25% in invaded areas, associated with decomposed or absent embryos. Invasion may limit native recruitment by reducing the viability and germination rate of native seeds prior to dispersal from parent plants. Reduced seed viability would reduce seed bank accumulation and total available seed for *A. victoriae*, while slower germination rates would minimise the efficiency by which *A. tetragonophylla* responds to sporadic rainfall events. Both mechanisms could lead to long term declines in native plant populations. Reduced seed viability would compound interference of buffel grass on recruiting plants.

K. M. Edwards · M. K. J. Ooi · K. French · B. Gooden
Centre for Sustainable Ecosystem Solutions, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

C. Schlesinger
Research Institute for Environment and Livelihoods, College of Engineering, IT and Environment, Charles Darwin University, Alice Springs, NT 0870, Australia

M. K. J. Ooi
Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

B. Gooden (✉)
CSIRO Health and Biosecurity, GPO Box 1700, Canberra, ACT 2601, Australia
e-mail: Ben.Gooden@csiro.au

Introduction

Alien plant invasion significantly reduces biodiversity and disrupts the composition and function of native vegetation at a global scale, yet the drivers of impact remain poorly understood (Vilà et al. 2011; Pyšek et al. 2012). Plant invasion can lead to homogenisation of recipient native vegetation, where many native species become replaced by a few (or sometimes single) dominant invasive plants (Olden et al. 2004). Competition for limiting resources, such as light and soil nutrients (e.g. Seabloom et al. 2003; Vilà and Weiner 2004) can increase environmental stress for native species, which has classically been considered as the principal mechanism by which invasive species limit native plant populations (Gioria and Osborne 2014). More recently, research has shown that invasion can alter vegetation communities indirectly by disrupting natural disturbance regimes (e.g. increased fire frequency and severity, D'Antonio and Vitousek 1992), ecosystem processes (e.g. nutrient cycling, Ehrenfeld 2003) and plant-animal interactions that underpin reproduction and population persistence (e.g. pollination and dispersal, Traveset and Richardson 2006).

Alien plant invasion can also disrupt reproductive and recruitment processes. Invasion has been shown to reduce rates of pollinator visitation, pollen transfer and seed set in neighbouring native plants (Morales and Traveset 2009), leading to significant reductions in native seed density and richness in soil stored seed banks (e.g. Gioria et al. 2014; Gooden and French 2014). Seed banks are important reservoirs of genetic and species diversity, and a key life-history stage that enables reestablishment of the community following disturbance of the standing vegetation (see review by Hopfensperger 2007). Invasion-induced seed bank degradation can therefore reduce the long term resilience of native vegetation to ecosystem change (Gioria et al. 2014).

Although patterns of seed bank decline associated with invasion have been well-documented for a variety of ecosystems (see review by Gioria et al. 2014), the mechanisms underpinning such impacts are poorly understood. Most studies determine seed bank composition using passive germination assays, whereby soil samples from invaded and native habitats are placed within glasshouses under optimal germination conditions and seedling emergence is used as a surrogate for seed presence (Gioria et al. 2014). Because seeds are rarely extracted directly from the soil in such studies, it remains unknown whether invasion impacts the seed bank by reducing the supply of seeds to the soil through limiting seed production or dispersal (Gooden et al. 2014), or by inhibiting the post-settlement germination of seeds into the standing vegetation (i.e. recruitment limitation, Ens and French 2008) via changes to dormancy or viability.

In crop systems, environmental stress is known to affect seed yield and viability (e.g. Farooq et al. 2009; Hampton et al. 2013). Greater levels of competition can decrease yield, as well as produce changes in seed morphology and size, germinability and dormancy (e.g. Jordan et al. 1982; Nurse and DiTommaso 2005). It is likely, therefore, that alien plants could produce changes in the seeds of co-occurring native plants that impact the seed bank in natural systems. Stress on native plant species is likely to be a driver of changes to reproduction, in turn potentially leading to recruitment limitation. This is supported by evidence from studies that show stress induced by drought and temperature change can cause variation in seed dormancy, germination rate and viability (Steadman et al. 2004; Hoyle et al. 2008; Segura et al. 2015).

Despite the well-known effects of competition on seeds and plant reproduction in crop species (Craine and Dybzinski 2013), the impacts of alien plant invasion on seeds of co-occurring native species are surprisingly understudied. Such an effect could represent a novel mechanism by which native vegetation community structure and diversity are disrupted by invasion over the long term. A pre-dispersal reduction in the viability of native seeds due to alien plant invasion would reduce seed banks and population size over time, with detrimental effects on long-term vegetation persistence and ecosystem function.

Buffel grass (*Cenchrus ciliaris* L.) is a C4 tussock grass native to Africa and southern Asia, which was introduced to Australia as a pasture species and is now highly invasive in arid regions (see review by Marshall et al. 2012). Buffel grass is a significant invader of arid woodlands worldwide, with evident negative effects on native vegetation richness (Franklin and Molina-Freaner 2010), seedling recruitment and population structure (Morales-Romero and Molina-Freaner 2008). It has a deep, strong root system, is drought tolerant and grows rapidly in response to rain, thereby enhancing its competitive ability in arid and semi-arid

areas. Buffel grass can also modify native vegetation structure by promoting fire (e.g. McDonald and McPherson 2011), although there is little understanding of the other mechanisms by which invasion may alter the composition of native shrubs and trees and ecosystem function.

Using a long term weed-removal experiment and additional areas where buffel grass had been managed for more than a decade, our aim was to examine the effects of buffel grass invasion on seed dormancy, viability and germination of two native perennial shrubs: *Acacia victoriae* Benth. (Victoria wattle) and *A. tetragonophylla* (dead finish) F.Muell. These species are widely distributed in arid woodlands throughout semi-arid Australia and are typical woody perennials of arid ecosystems, with long-term persistence of populations dependent on the development of a bank of dormant seeds which gradually lose dormancy over time, so that recruitment can occur in response to sporadic rainfall events or disturbance (Grice and Westoby 1987; Auld 1995). Dormancy can prevent germination during unfavourable ecological conditions, when the likelihood of seedlings successfully reaching adulthood is low (Baskin and Baskin, 2014), and maintaining a dormant fraction in the seed bank is, therefore, a favourable adaptation for spreading risk (Ooi et al. 2009). Increasing competition can change seed morphology including the structure of the seed coat (Jordan et al. 1982). In plants with physically dormant seeds (i.e. those that have dormancy imposed by a hard impermeable seed coat typical of the Fabaceae and Malvaceae), changes to the seed coat could affect the level of dormancy and reduce the risk-spreading capacity. In crop systems, both competition and drought have been reported to change dormancy levels, with stressful conditions generally increasing the dormant fraction of seeds (Nurse and DiTommaso 2005; Hudson et al. 2015). We predict, therefore, that buffel grass invasion will be associated with reduced seed viability for the two native shrub species, and disrupt dormancy. Specifically, we asked:

1. Does the fraction of non-dormant seeds or the proportion of viable seeds of *A. victoriae* and *A. tetragonophylla* differ between buffel grass-invaded and buffel grass removed plots?
2. Is there a difference in seed mass or germination rate between the two treatments indicating the potential for changes to provisioning?

3. What are the potential mechanisms by which buffel grass invasion affects seed viability of resident native plants?

Materials and methods

Study area and habitat

This study was situated in arid woodlands of the West MacDonnell Ranges, near Alice Springs, Northern Territory, Australia. The climate is classified as hot desert (Köppen climate classification, BWh; Vallam and Qin 2017), with mean minimum/maximum daily temperatures of approximately 22/36 °C in January (austral summer) and 4/20 °C in July (austral winter), respectively, and mean annual rainfall of 280 mm (BOM 2018). Seed sampling for the two perennial shrubs *A. victoriae* and *A. tetragonophylla* was undertaken at separate locations (Simpsons Gap and Desert Park, respectively) with different levels of replication (as described below).

Sampling of seeds for *Acacia victoriae*

Three 50 m × 70 m buffel grass-removed plots were established in 2008 at Simpsons Gap, Tjoritja National Park (~ 12 km west of Alice Springs; 23°43′S, 133°43′E; described by Schlesinger et al. 2013). The plots were positioned on an alluvial, sandy floodplain dominated by *A. victoriae* and scattered *Hakea divaricata*, interspersed with *A. murrayana* and *Eremophila longifolia* shrubs, with a mixed ground-layer of ephemeral grasses and forbs. Buffel grass removal commenced in February 2008 during a relatively dry period, when buffel grass was the dominant ground cover with a mean cover of 40% (C. Schlesinger, unpublished data), with all other ground-layer plants making up less than 1% cover. Buffel grass cover was initially reduced by mechanical means with a slasher attached to a tractor. Coarse woody debris, native tree and shrub seedlings and adult plants were avoided if possible. Secondary control of buffel grass commenced in December 2008 and continued opportunistically until 2016 by spot-spraying regrowth and seedlings with 10% glyphosate (Roundup Biactive® containing isopropylamine salt of glyphosate) as per Schlesinger et al. (2013). The sites were maintained in

a state where only small amounts (< 10% foliage cover and usually much less) of buffel grass was present and native grasses and forbs were dominant, whereas buffel grass continued to dominate the surrounding vegetation (> 40% cover).

Four reproductively mature plants were randomly sampled in 2015 from each of the three buffel grass-removed plots and adjacent buffel grass-invaded areas ($n = 12$ plants per invasion treatment and 24 in total). Two cohorts of seeds were collected: (1) post-dispersal seeds from the soil that represented reproductive events from 2014 and earlier and (2) pre-dispersal seeds from the canopies of parent plants that were produced in 2015. At least 80 mature seeds were collected randomly from the leaf litter and soil surface (to a depth no greater than 2 cm) beneath the canopies of each replicate *A. victoriae* plant in October 2015. Litter and soil were sieved through a 3 mm mesh on-site to assist with separating the seeds from soil. More than 100 seeds were collected from plant canopies in early December 2015. Plants were selected randomly on the basis that they were producing ripe seeds borne on fully-opened seed pods. Seeds were harvested at random across the plant canopy when hard and dark, and when pods had split open. Seeds were contained in sealed paper bags for transport and stored at room temperature prior to viability assays in March to May 2016. Previous studies (Liyanage and Ooi 2017) found that viability and the non-dormant fraction of *Acacia* (and other Fabaceae) seeds did not change after 6 to 18 months in laboratory storage.

The maximum height (m) and width (m) were measured for each plant to control for size effects on fecundity and seed viability. A spherical volumetric surrogate for plant biomass was derived from average canopy diameter ($\pi \times$ mean diameter/6). Plant volume was similar between invasion treatments for each of the two seed cohorts (canopy seeds: $t = 0.19$, $P = 0.85$; soil seeds: $t = 0.17$, $P = 0.87$).

Sampling of seeds for *Acacia tetragonophylla*

The vegetation community at Alice Springs Desert Park (ASDP, 23°42′S, 133°49′E) consisted of an open sclerophyll woodland, dominated by *A. kempeana* and *A. aneura* interspersed with scattered *A. tetragonophylla* shrubs and *Corymbia opaca*, *C. aparrerinja* and *A. estrophiolata* trees. During 2000 and 2001, buffel grass infestations adjacent to the entrance road to

ASDP were controlled using a mix of mechanical and chemical controls (10% glyphosate herbicide as described above). Subsequent to this initial control, secondary buffel grass invasion was suppressed mechanically by chipping individual plants.

During October 2015, at least 100 ripe seeds were sampled from 18 reproductively-mature plants ($n = 9$ plants per invasion treatment). Plants were selected opportunistically on the basis that they were producing ripe seeds borne on fully-opened seed pods, and so that plants in invaded and buffel-removed areas were interspersed across the study area. Ripe seeds had well-developed, black, hard seed coats with an attached aril. Seeds were not sampled from the soil for *A. tetragonophylla*, since preliminary soil cores extracted to a depth of ∼ 5 cm beneath mature canopies contained extremely low densities of seeds (i.e. < 1 per 4000 cm^3; B. Gooden pers. obs). Plant volume, measured as per *A. victoriae*, did not differ between invasion treatments ($t = 0.56$, $P = 0.58$). Seeds were stored in the lab as per *A. victoriae*.

Assessment of seed viability, dormancy and germinability

Laboratory-based germination experiments were used to determine the effects of buffel grass invasion and removal on the viability and dormancy of *A. victoriae* and *A. tetragonophylla* seeds. Up to 80 seeds per plant for each of the three seed cohorts were randomly selected from available seeds, resulting in a total of between 700 and 955 seeds tested per invasion treatment for each species (see data provided in Electronic supplementary material). Seeds per plant were evenly distributed amongst four 9 cm-diameter petri dishes on filter paper (i.e. resulting in up to four replicate dishes of seeds per plant sample). Seeds were dampened with distilled water until the filter paper was saturated. Petri dishes were wrapped in cling film to retain moisture. Petri dishes were placed in an incubator (day/night cycle of 25/18 °C), and checked every 1 to 3 days for germination. Germination was recorded upon emergence of the radicle, and seeds were counted as unviable if they were soft, discoloured and/or rotten. Germinating and dead seeds were removed upon detection.

After approximately 19 to 23 days, when seeds were no longer imbibing moisture and swelling, the seeds that did not germinate were scarified with a

scalpel to break the impermeable seed coat. Seeds that germinated before scarification were considered to be viable and non-dormant, whilst seeds that germinated only once the seed coat had been broken were considered to be viable and dormant. Scarified seeds were moistened with water, placed back into the incubator and monitored every 1 to 3 days. Between 38 and 41 days after scarification, the remaining seeds that did not germinate were dissected in order to examine the embryo. White, firm embryos were deemed to be viable, whilst soft, mushy and discoloured embryos, or seeds lacking an embryo, were deemed to dead (Ooi et al. 2004).

Seed masses of up to 20 additional seeds from each plant for each of the three cohorts were weighed (AB204-S model balance, Mettler Toledo Ltd.) after arils were removed from *A. tetragonphylla*.

Data analysis

For each of the three seed cohorts we derived the total proportion of viable seeds by dividing the sum of the number of seeds that germinated plus seeds that did not germinate but contained a living embryo, by the total number of seeds collected per plant. The non-dormant viable seed fraction was calculated by dividing the number of seeds that germinated *before* scarification by the total number of viable seeds per plant. To compare speed of germination for fresh seeds, we calculated T_{50} (defined as time elapsed until 50% of seeds germinated relative to the maximum germination percentage, Ritz et al. 2013) for canopy collected seeds using the R package *drc*. T_{50} rates were compared between treatments using the *compParm* function in R. Any plants with less than 10 viable seeds were excluded from the T_{50} analysis. The unit of replication for these two response variables was the individual plant and not a single seed or petri dish, given the unequal seed sample sizes between some plants.

The effects of buffel grass invasion on seed germination for all three seed cohorts were assessed to compare the dormant fraction and total viable seeds between treatments. Analyses were conducted using R (R Core Team 2017). For *A. victoriae*, a Generalised Linear Mixed Model (GLMM) with logit link function and binomial distribution was used to account for the random effects of plot on seed germination, in addition to the main fixed effect of invasion treatment, using

the *lme4* package. Data were checked to ensure the fulfilment of normality and homoscedascity assumptions, and for evidence of overdispersion. *P* values were obtained using Likelihood Ratio Tests by comparing the full model against a null model, using the *anova* function. For *A. tetragonophylla*, plants were not clustered around discrete plots within each of the two invasion treatments, so we used a Generalised Linear Model (GLM) only containing the main effect of invasion treatment. In this case, data were overdispersed, so a quasi-binomial distribution was used. *P* values were obtained as described above.

The effect of invasion treatment on seed mass for the three seed cohorts was assessed with nested analyses of variance. Replication was at the level of the individual seed. The model included the nested effect of plant (with up to 20 replicate seeds per plant) in addition to the main effect of invasion treatment. For *A. victoriae*, plot was initially included in the model as a random effect but was found to have no significant influence on seed mass (canopy seeds: $P = 0.14$, soil seeds: $P = 0.06$). Data were thus pooled across the three plots in order to increase the power to detect the main effect of invasion category. Data were square root transformed as necessary to improve normality of residuals.

Results

Acacia victoriae

For seeds collected from the soil and canopies, the non-dormant fraction of viable seeds was generally low (ranging from 2 to 10% on average, Fig. 1). This did not differ significantly between buffel grass invaded and removed treatments. Following scarification, the percentage of seeds germinating for both seed cohorts increased significantly, reaching a maximum of 20–55% after approximately 13 days. Total seed viability was significantly lower in buffel grass invaded compared to removed habitats (Table 1). Germination of seeds collected from the canopy and the soil were approximately 40 and 65% lower, respectively, in habitats invaded by buffel grass (Fig. 1). Very few (and in most cases none) of the seeds that failed to germinate contained living embryos. This indicated that the 65% (from canopies) to 80% (from soil) of seeds from buffel-invaded areas

that did not germinate were unviable and not simply unresponsive to our experimental protocols.

The mass of *A. victoriae* seeds from the canopy of adult plants varied significantly amongst individual plants as well as between the two invasion categories
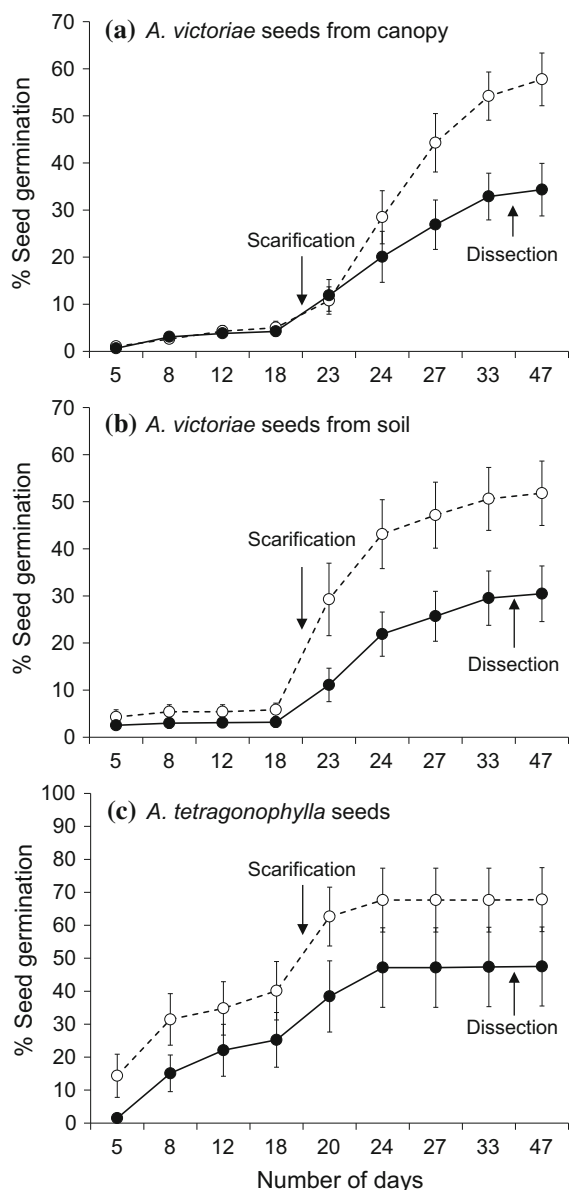
Fig. 1 Variation in percentage of germinant seeds through time in buffel grass invaded (dark circles) and removed (white circles) habitats. Values are means ± standard errors; $n = 12$ for *A. victoriae* and $n = 9$ for *A. tetragonophylla*. Note different *y*-axis ranges. Scarification and dissection of seeds occurred between days 19–20 and 38–41, respectively, as denoted by arrows on each graph

(Table 1). Mean canopy seed mass in buffel grass-removed plots was approximately 20% greater than seeds collected from invaded plots (Table 1). In contrast, there was no significant effect of buffel grass on the mass of seeds from the soil beneath each plant, although there were significant differences among plants independent of invasion category (Table 2). Similarly, there was no significant effect of treatment on T_{50} ($t = 0.19$, $P = 0.85$), with buffel grass-invaded and buffel grass-removed areas producing seeds that germinated at very similar rates (23.53 ± 0.62 SE and 23.66 ± 0.27 SE days respectively).

Acacia tetragonophylla

There was no significant effect of invasion on the percentage of seeds germinating (Table 1), however T_{50} differed significantly ($t = -2.60$, $P = 0.009$), with buffel grass-invaded areas producing seeds with a slower rate of germination (14.05 ± 0.69 SE days) than buffel grass-removed areas (11.59 ± 0.65 SE days). Additionally, initial percentages of non-dormant seed germination were much higher for *A. tetragonophylla* (mean range across treatments of 53.44% to 60.40%) than *A. victoriae* (8.60% to 11.47%) prior to scarification.

There was no significant difference in the mass of seeds from *A. tetragonophylla* canopies between invaded and removed treatments (Table 2). However, mean seed mass did vary significantly amongst individual plants, ranging on average from approximately 0.004 to 0.022 g.

Discussion

Invader impacts on native seed viability: implications for seed bank and recruitment dynamics

By comparing the viability of seeds of mature plants in invaded areas to those where the invasive species had been managed for at least 7 years, we showed that reduced viability and germination performance of native plant seeds is a potential mechanism by which alien plant invasion limits native plant recruitment. Sustained invasive grass removal resulted in a greater than 50% increase in seed viability for *A. victoriae* (as a function of improved embryo development; see

Table 1 Results of models for the three seed lots collected

Species response variable	Error distribution (model used)	DF	Deviance	P
Acacia victoriae (soil collected)				
Non-dormant fraction	Binomial (GLMM)	1	0.30 (χ^2)	0.583
Total viable	Binomial (GLMM)	1	100.23 (χ^2)	**< 0.001**
Acacia victoriae (canopy collected)				
Non-dormant fraction	Binomial (GLMM)	1	0.70 (χ^2)	0.402
Total viable	Binomial (GLMM)	1	107.12 (χ^2)	**< 0.001**
Acacia tetragonophylla				
Non-dormant fraction	Quasibinomial (GLM)	1	0.16 (F)	0.691
Total viable	Quasibinomial (GLM)	1	1.94 (F)	0.183

These are Chi squared analysis of deviance tests for binomial Generalised Linear Mixed Models (GLMMs) and an F test for a quasibinomial Generalised Linear Model. Bold P values (< 0.05) denote significant effects

Table 2 Results of nested ANOVA for response of seed mass to invasion treatment and parent plant

Response variable source	DF	Sum of Squares	F	P	R^2
A. victoriae seed mass from canopy					
Model	23	0.0203	7.0660	**< 0.001**	0.27
Invasion	1	0.0050	7.1429	**0.0139**	
Plant (invasion)	22	0.0154	5.5800	**< 0.001**	
Error	456	0.0570			
Total	479	0.0773			
			Mean ($\pm$ SE) seed mass (per 100 seeds): buffel-invaded, 3.23 ($\pm$ 0.09), n = 240; buffel-removed, 3.85 ($\pm$ 0.20), n = 240		
A. victoriae seed mass from soil					
Model	23	0.0133	6.8818	**< 0.001**	0.26
Invasion	1	< 0.001	0.1431	0.7088	
Plant (invasion)	22	0.0132	7.1483	**< 0.001**	
Error	456	0.0384			
Total	479	0.0517			
			Mean ($\pm$ SE) seed mass (per 100 seeds): buffel-invaded, 3.55 ($\pm$ 0.15), n = 240; buffel-removed, 3.50 ($\pm$ 0.07), n = 240		
A. tetragonophylla seed mass from canopy					
Model	17	0.0088	19.8038	**< 0.001**	0.50
Invasion	1	< 0.001	0.0869	0.7721	
Plant (invasion)	16	0.0087	20.9280	**< 0.001**	
Error	342	0.0089			
Total	359				
			Mean ($\pm$ SE) seed mass (per 100 seeds): buffel-invaded, 1.17 ($\pm$ 0.06), n = 180; buffel-removed, 1.10 ($\pm$ 0.05), n = 180		

Means ($\pm$ SE) and replicate levels are provided for shrubs in buffel-invaded and buffel-removed treatments to accompany each analysis. Bold P values (< 0.05) denote significant effects

details below), which in turn led to increased seed mass for some seed cohorts. Overall seed performance was improved by alien grass removal for both native species, with germination rate (represented by T_{50}) increasing significantly for *A. tetragonophylla*.

Previous investigations have focused on pollination limitation (e.g. McKinney and Goodell 2010), reduced reproductive effort (i.e. flowering) and output (i.e. fruit and seed production, Gooden et al. 2014), and propagule storage in seed banks (Gioria et al. 2014; Gioria and Pyšek 2016) as mechanisms by which invasive plants limit the recruitment of co-occurring native plants. The reduction in the *quality* (i.e. viability—embryo health and germinability) of native seeds before they are released from the parent plant to the invaded soil seed bank indicated by our results represents an additional novel mechanism of recruitment limitation. We did not examine whether seed output, seed rain (i.e. dispersal of the seeds from the parent plant to the soil surface) or density within the soil seed bank varied between buffel grass-invaded and removed plots; our results suggest that irrespective of these factors it is likely that reduced seed viability will lead to a degraded seed bank and disrupted native plant recruitment in the long term. In turn, any disruption to recruitment potential in invaded woodlands will likely limit how native plant populations respond to disturbance events (e.g. severe fires) that damage the standing vegetation.

Our results highlight that the impacts of alien plant invasion may be underestimated if native propagule quality is not considered when assessing native plant recruitment dynamics and seed bank composition in invaded habitats. For example, Morales-Romero and Molina-Freaner (2008) compared plant density and reproductive dynamics of the cactus, *Pachycereus pecten-aboriginum,* between buffel grass-invaded and native habitats in Mexico, and found no negative relationship between invasion and cactus flower density or fruit set; it was suggested, rather, that invasion reduces cactus population density by inhibiting post settlement seedling emergence and survival. However, it is possible that the recruitment of cactus seedlings in invaded areas was also limited through a reduction in the viability of seeds before they reached the soil seed bank. Indeed, our study shows that population models based solely on seed inputs may overestimate fecundity if a large fraction of those seeds do not contain viable embryos. Studies that

estimate impacts of invasion on seed banks by quantifying seed rain or seed density in the soil (see review by Gioria et al. 2014) without examining the quality of those seeds may either not detect invader impacts or underestimate those impacts.

Mechanisms by which alien grass invasion impacted native seed viability

We hypothesise that reduced viability of *A. victoriae* seeds in buffel grass invaded woodlands resulted from embryo abortion post fertilisation. Seed abortion is a common response by plants under stress, and the timing and duration of the stressor can determine at which stage abortion occurs (Sun et al. 2004; Aragón et al. 2008). For species within the family Fabaceae, the seed coat and endosperm develop first, prior to the embryo (Weber et al. 2005; Smýkal et al. 2014). In our study, coats of collected seeds were intact and well developed, and unviable seeds were represented primarily by being empty (or near empty), suggesting that seed abortion occurred post-fertilization. The close proximity of our buffel grass-invaded and removed plots means that pollinators very likely had similar access to maternal plants, meaning that plants were unlikely pollen-limited in invaded areas. However, in many cases we were unable to discern whether non-viable seeds in buffel grass-invaded plots had either never contained an embryo in the first place or the embryo had grown but subsequently decomposed after being aborted.

We speculate that buffel grass invasion led to a reduction in native plant reproductive performance by competing for limited resources, most likely soil moisture. Buffel grass germinates (or resprouts) and grows rapidly in response to rainfall pulses, and is also strongly competitive under water-limited conditions (Ward et al. 2006; Stevens and Fehmi 2009; van Klinken and Friedel 2018). Other experimental studies have demonstrated enhanced native flower production and seed set in response to alien plant removal, likely as a result of alleviated competition for resources such as light, moisture and soil nutrients (e.g. D'Antonio et al. 1998; Gould and Gorchov 2000; Miller and Gorchov 2004; McKinney and Goodell 2010). However, it is also possible that reduced seed viability was caused by chemical interference (i.e. allelopathy) of maternal native plants by buffel grass during seed development. Cheam (1984) experimentally

demonstrated inhibition of *Calotropis procera* (an alien shrub of Northern Australia) seedlings and seed germination by allelochemicals exuded by buffel grass roots. However, Cheam (1984) identified that allelochemicals were restricted to the top 20 cm of soil profile where buffel grass roots are most dense. We know of no evidence that alien plants can interfere with the development of native plant embryos by allelochemical interference of mature shrubs and trees whose roots occupy different soil strata. We believe that competition for water resources—which is key to embryo development in arid plants—is a more plausible mechanism by which buffel grass affected seed viability in our study system.

Aside from stress associated with competition, exposure to fire may have contributed to reduced seed viability for *A. victoriae* shrubs in buffel grass-invaded areas. The promotion of more frequent and intense fires as a result of increased fuel loads could be the most severe long-term ecological impact of alien grass invasion of arid woodlands (Butler and Fairfax 2003; Rossiter et al. 2003; Miller et al. 2010; McDonald and McPherson 2011). During fires at Simpsons Gap in 2011, 83–100% of buffel grass invaded areas were burnt compared with only 12–20% of vegetation in buffel grass-removed plots (Schlesinger et al. 2013). Many of the *Acacia victoriae* shrubs that were directly affected by fire in 2011 had lost their entire canopy but subsequently re-sprouted from basal stems (C. Schlesinger, pers. obs). Although we were not able to categorise shrubs as unburnt or resprouted whilst collecting seeds for the current study, we estimate that most of the shrubs in invaded areas were resprouted individuals and more than half of those in buffel removed areas were unburnt individuals. It is possible therefore that modification of natural fire regimes by buffel grass indirectly limited seed viability of native shrubs in invaded areas. However, we are not aware of any evidence that seeds produced by resprouted plants are less vigorous or retain lower rates of viability than those produced by non-burnt plants. Further research testing viability of seeds produced by shrubs exposed to different levels of fire would help to clarify the mechanisms driving differences in seed viability between *A. victoriae* shrubs in invaded and non-invaded areas.

We also found a significant reduction in seed mass for *A. victoriae* in response to buffel grass invasion. A reduction of seed mass is a common response for

physically-dormant species in crop systems under water stress (Dornbos and Mullen 1991), which points again to resource competition being the main mechanism by which buffel grass invasion reduces viability. Furthermore, studies from arid regions have shown that smaller seeds produce smaller seedlings that are less likely to survive and are slower to grow (e.g. Benard and Toft 2007). Seed vigour in our study, represented by germination rate (T_{50}), was also significantly reduced by buffel grass invasion, which in arid systems can reduce the chances of seeds germinating quickly enough to utilise short duration, isolated rainfall events. Taken together, these findings suggest that subsequent performance of native plant seeds from buffel grass invaded areas is likely to be compromised, potentially limiting seedling success and leading to a loss of population fitness.

Conclusion and future research directions

Our results show that alien plant invasion reduced native seed quality (i.e. embryo condition, viability and germinability) prior to dispersal to the soil stored seed bank. We predict that a reduction in seed viability during the reproductive phase is imposed by stress in the maternal environment (most likely caused by competition between the native and invasive plant for limited soil resources, such as water), which in turn reduces the accumulation of viable seeds in the soil seed bank. Although many studies have clearly shown that invaded seed banks have lower richness and density of native seeds than non-invaded seed banks, there is very poor understanding of the mechanisms by which plant invasion leads to degraded seed banks. There is often an assumption that lower native seed density is a function of reduced standing vegetation density and in turn seed rain, yet our results reveal that reduced seed quality may be equally important. Future research will be required to evaluate the importance of disrupted seed viability relative to other well-studied processes in limiting the richness and density of seeds in the soil seed bank. In any case, a reduction in seed viability across invaded habitats potentially limits both the ability of a population to recruit during rainfall events and the capacity to bet-hedge against stochastic disturbances, such as fire or false-start rainfall pulses (Ooi et al. 2009; Segura et al. 2015). The negative effects of invasion on seed viability will likely compound resource competition and other

interferences already faced by native plants across invaded habitats, thereby contributing to the eventual collapse in native populations.

References

Aragón CF, Escudero A, Valladares F (2008) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. J Ecol 96:222–229. https://doi.org/10.1111/j.1365-2745.2007.01320.x

Auld TD (1995) Soil seedbank patterns of four trees and shrubs from arid Australia. J Arid Environ 29:33–45. https://doi.org/10.1016/S0140-1963(95)80062-X

Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography, and evolution of dormancy and germination, 2nd edn. Academic Press, San Diego

Benard RB, Toft CS (2007) Effect of seed size on seedling performance in a long-lived desert perennial shrub (*Ericameria nauseosa*: Asteraceae). Int J Plant Sci 168:1027–1033. https://doi.org/10.1086/518942

Bureau of Meteorology (2018) Climate statistics for Australian locations. Commonwealth of Australia. http://www.bom.gov.au/climate/averages/tables/cw_015590.shtml. Accessed 6 Dec 2018

Butler BDW, Fairfax RJ (2003) Buffel grass and fire in a gidgee and brigalow woodland: a case study from central Queensland. Ecol Manag Restor 4:120–125. https://doi.org/10.1046/j.1442-8903.2003.00146.x

Cheam AH (1984) A 'natural herbicide' against calotrope? J Dep Agric 25:42–43

Craine JM, Dybzinski R (2013) Mechanisms of plant competition for nutrients, water and light. Funct Ecol 27:833–840. https://doi.org/10.1111/1365-2435.12081

D'Antonio CM, Vitousek PM (1992) Biological Invasions by exotic grasses, the grass/fire cycle, and global change. Annu Rev Ecol Syst 23:63–87

D'Antonio CM, Hughes RF, Mack M, Hitchcock D, Vitousek PM (1998) The response of native species to removal of invasive exotic grasses in a seasonally dry Hawaiian

woodland. J Veg Sci 9:699–712. https://doi.org/10.2307/3237288

Dornbos DL, Mullen RE (1991) Influence of stress during soybean seed fill on seed weight, germination, and seedling growth rate. Can J Plant Sci 71:373–383. https://doi.org/10.4141/cjps91-052

Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503–523. https://doi.org/10.1007/s10021-002-0151-3

Ens EJ, French K (2008) Exotic woody invader limits the recruitment of three indigenous plant species. Biol Conserv 141:590–595. https://doi.org/10.1016/j.biocon.2007.12.012

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Veronique S, Alberola C (eds) Sustainable agriculture. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2666-8

Franklin KA, Molina-Freaner F (2010) Consequences of buffelgrass pasture development for productivity and species richness of plants in the drylands of Sonora, Mexico. Conserv Biol 24:1664–1673. https://doi.org/10.1111/j.1523-1739.2010.01540.x

Gioria M, Osborne BA (2014) Resource competition in plant invasions: emerging patterns and research needs. Front Plant Sci 5:501. https://doi.org/10.3389/fpls.2014.00501

Gioria M, Pyšek P (2016) The Legacy of plant invasions: changes in the Soil Seed Bank of invaded plant communities. Bioscience 66:40–53. https://doi.org/10.1093/biosci/biv165

Gioria M, Jarošík V, Pyšek P (2014) Impact of invasions by alien plants on soil seed bank communities: emerging patterns. Perspect Plant Ecol Evol Syst 16:132–142. https://doi.org/10.1016/j.ppees.2014.03.003

Gooden B, French K (2014) Impacts of alien grass invasion in coastal seed banks vary amongst native growth forms and dispersal strategies. Biol Conserv 171:114–126. https://doi.org/10.1016/j.biocon.2014.01.005

Gooden B, French K, Robinson SA (2014) Alien grass disrupts reproduction and post-settlement recruitment of co-occurring native vegetation: a mechanism for diversity decline in invaded forest? Plant Ecol 215:567–580

Gould AM, Gorchov DL (2000) Effects of the exotic invasive shrub *Lonicera maackii* on the survival and fecundity of three species of native annuals. Am Midl Nat 144:36–50

Grice AC, Westoby M (1987) Aspects of the dynamics of the seed-banks and seedling populations of *Acacia victoriae* and *Cassia* spp. in arid western New South Wales. Aust J Ecol 12:209–215. https://doi.org/10.1111/j.1442-9993.1987.tb00944.x

Hampton JG, Boelt B, Rolston MP, Chastain TG (2013) Effects of elevated CO_2 and temperature on seed quality. J Agric Sci 151:154–162. https://doi.org/10.1017/S0021859612000263

Hopfensperger KN (2007) A review of similarity between seed bank and standing vegetation across ecosystems. Oikos 116:1438–1448. https://doi.org/10.1111/j.0030-1299.2007.15818.x

Hoyle GL, Steadman KJ, Daws MI, Adkins SW (2008) Pre- and post-harvest influences on seed dormancy status of an

Australian Goodeniaceae species, *Goodenia fasicularis*. Ann Bot 102:93–101. https://doi.org/10.1093/aob/mcn062

Hudson AR, Ayre DJ, Ooi MKJ (2015) Physical dormancy in a changing climate. Seed Sci Res 25:66–81. https://doi.org/10.1017/S0960258514000403

Jordan JL, Staniforth DW, Jordan CM (1982) Parental stress and prechilling effects on Pennsylvania Smartweed (*Polygonum pensylvanicum*) achenes. Weed Sci 30:243–248. https://doi.org/10.1017/S0043174500040467

Liyanage GS, Ooi MKJ (2017) Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank? Seed Sci Res 27:1–11

Marshall VM, Lewis MM, Ostendorf B (2012) Buffel grass (*Cenchrus ciliaris*) as an invader and threat to biodiversity in arid environments: a review. J Arid Environ 78:1–12. https://doi.org/10.1016/j.jaridenv.2011.11.005

McDonald CJ, McPherson GR (2011) Fire behavior characteristics of buffelgrass-fueled fires and native plant community composition in invaded patches. J Arid Environ 75:1147–1154. https://doi.org/10.1016/j.jaridenv.2011.04.024

McKinney AM, Goodell K (2010) Shading by invasive shrub reduces seed production and pollinator services in a native herb. Biol Invasions 12:2751–2763

Miller KE, Gorchov DL (2004) The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. Oecologia 139:359–375

Miller G, Friedel M, Adam P, Chewings V (2010) Ecological impacts of buffel grass (*Cenchrus ciliaris* L.) invasion in central Australia–does field evidence support a fire-invasion feedback? Rangel J 32:353–365. https://doi.org/10.1071/RJ09076

Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecol Lett 12:716–728. https://doi.org/10.1111/j.1461-0248.2009.01319.x

Morales-Romero D, Molina-Freaner F (2008) Influence of buffelgrass pasture conversion on the regeneration and reproduction of the columnar cactus, *Pachycereus pecten-aboriginum*, in northwestern Mexico. J Arid Environ 72:228–237

Nurse RD, DiTommaso A (2005) Corn competition alters the germinability of velvetleaf (*Abutilon theophrasti*) seeds. Weed Sci 53:479–488. https://doi.org/10.1614/WS-04-185R1

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. Trends Ecol Evol 19:18–24. https://doi.org/10.1016/j.tree.2003.09.010

Ooi MKJ, Auld TD, Whelan RJ (2004) Comparison of the cut and tetrazolium tests for assessing seed viability: a study using Australian native *Leucopogon* species. Ecol Manag Restor 5:141–143

Ooi MKJ, Auld TD, Denham AJ (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. Glob Change Biol 15:2375–2386. https://doi.org/10.1111/j.1365-2486.2009.01887.x

Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Glob Change Biol 18:1725–1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Ritz C, Pipper CB, Streibig JC (2013) Analysis of germination data from agricultural experiments. Eur J Agron 45:1–6

Rossiter NA, Setterfield SA, Douglas MM, Hutley LB (2003) Testing the grass-fire cycle: alien grass invasion in the tropical savannas of northern Australia. Divers Distrib 9(3):169–176

Schlesinger C, White S, Muldoon S (2013) Spatial pattern and severity of fire in areas with and without buffel grass (*Cenchrus ciliaris*) and effects on native vegetation in central Australia. Austral Ecol 38:831–840. https://doi.org/10.1111/aec.12039

Seabloom EW, Harpole WS, Reichman OJ, Tilman D (2003) Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proc Natl Acad Sci 100:13384–13389

Segura F, Vicente MJ, Franco JA, Martinez-Sánchez JJ (2015) Effects of maternal environmental factors on physical dormancy of *Astragalus nitidiflorus* (Fabaceae), a critically endangered species of SE Spain. Flora 216:71–76. https://doi.org/10.1016/j.flora.2015.09.001

Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD (2014) The role of the testa during development and in establishment of dormancy of the legume seed. Front Plant Sci 5:351. https://doi.org/10.3389/fpls.2014.00351

Steadman KJ, Ellery AJ, Chapman R, Moore A, Turner NC (2004) Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). Aust J Agric Res 55:1047–1057. https://doi.org/10.1071/AR04083

Stevens JM, Fehmi JS (2009) Competitive effect of two non-native grasses on a native grass in southern Arizona. Invasive Plant Sci Manag 2:379–385. https://doi.org/10.1614/IPSM-09-022.1

Sun K, Hunt K, Hauser BA (2004) Ovule abortion in *Arabidopsis* triggered by stress. Plant Physiol 135:2358–2367. https://doi.org/10.1104/pp.104.043091

Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. Trends Ecol Evol 21:208–216. https://doi.org/10.1016/j.tree.2006.01.006

Vallam P, Qin XS (2017) Projecting future precipitation and temperature at sites with diverse climate through multiple statistical downscaling schemes. Theor Appl Climatol. https://doi.org/10.1007/s00704-017-2299-y

van Klinken RD, Friedel MH (2018) Unassisted invasions: understanding and responding to Australia's high-impact environmental grass weeds. Aust J Bot 65:678–690. https://doi.org/10.1071/BT17152

Vilà M, Weiner J (2004) Are invasive plant species better competitors than native plant species?—evidence from pair-wise experiments. Oikos 105:229–238. https://doi.org/10.1111/j.0030-1299.2004.12682.x

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological

impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x

Ward JP, Smith SE, McClaran MP (2006) Water requirements for emergence of buffelgrass (*Pennisetum ciliare*). Weed Sci 54:720–725. https://doi.org/10.1614/WS-05-163R1.1

Weber H, Borisjuk L, Wobus U (2005) Molecular physiology of legume seed development. Annu Rev Plant Biol 56:253–279. https://doi.org/10.1146/annurev.arplant.56.032604.144201

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.