An assessment of potential herbivory impacts of a reintroduced marsupial in a predator-free woodland sanctuary

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Data availability: All data and R code used in this study is available from https://github.com/lsoloughlin/MFWS_RapidFloristics_2021

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Summary

Fenced sanctuaries that exclude feral predators are critical for threatened species conservation. However, adaptive management of these sanctuaries requires careful consideration of the potential impact herbivore populations free from predation can have on the condition of native vegetation. The Mulligans Flat Woodland Sanctuary in the Australian Capital Territory comprises critically endangered box-gum grassy woodland, threatened orchids, abundant macropods, and a reintroduced population of the eastern bettong (*Bettongia gaimardi*, hereafter "Ngaluda"). To understand how Ngaluda, along with other herbivores, may be potentially impacting vegetation across the sanctuary we undertook an assessment of indicator plant species. We monitored 106 plots for 13 indicator species (10 species with tuberous roots that the burrowing Ngaluda would be preferentially targeting and three non-tuberous species). We found that most floristic indicators we investigated – including richness of indicator species and the abundance of lilies – were higher in the Goorooyarroo area of the sanctuary (where Ngaluda are absent and wallabies are rare) compared to the Mulligans Flat area of the sanctuary (where Ngaluda are present and wallabies are abundant), suggesting a negative impact of the overall herbivore assemblage of Mulligans Flat. However, within just Mulligans Flat, some indicators, including the abundance of a common orchid, were significantly lower in areas associated with high Ngaluda activity irrespective of other herbivore densities. We found no instance of Ngaluda presence or higher activity being associated with higher values for any floristic indicator we investigated. These results are consistent with known impacts of abundant herbivores and reintroduced digging marsupials in other predator-free sanctuaries in Australia. Our results highlight that Ngaluda herbivory may be outweighing any positive effect of their diggings on native vegetation, and indicates the need for careful risk mitigation when deciding how critically endangered animals and vegetation communities are managed together in sanctuaries.

Implications for Managers

- Native herbivores free from competition and predation are likely to require management to limit their negative effects on vegetation that can decrease ecosystem condition, particularly in predator-free fenced sanctuaries.
- While the reintroduction of burrowing species like bettongs will promote native forbs and geophytes through their diggings, these benefits can potentially be outweighed by negative effects of their herbivory in a predator-free sanctuary.
- Knowledge of the effects that reintroduced herbivores have on the recipient ecosystem of a sanctuary is critical for effective management for the benefit of both threatened animals and vegetation communities.

Introduction

Conservation management frequently requires decisions to be made despite high uncertainty about the predicted outcomes (Halpern et al. 2006; Wilson et al. 2007). This is particularly true for the management of rare and threatened species where data on their ecological niche, threats and function are likely limited (Scheele et al. 2017; Murphy et al. 2018; Neeson et al. 2018). Taking an adaptive management approach that explicitly recognises important areas of uncertainty and seeks to resolve them through integrated research and management is critical for improving the knowledge-base and conceptual models that underpin decision-making (Holling 1978; Lindenmayer et al. 2008; Chadès et al. 2017; Gillson et al. 2019). Adaptive management also provides a framework for considering the trade-offs, risks, and potential conflicts between multiple conservation objectives associated with a decision (Holling 1978; Driscoll et al. 2016; Fraser et al. 2017).

Considerations of risk, uncertainty, and balancing multiple conservation objectives are particularly important for the management of fenced wildlife sanctuaries. In Australia, fenced sanctuaries have played a key role in securing populations of critical-weight-range native mammals threatened by predation from the invasive Red Fox (*Vulpes vulpes*) and Feral Cat (*Felis catus*) (Dickman 2012; Kanowski et al. 2018). However, fenced sanctuaries are prone to ecosystem imbalance due to the absence of predators and competitors and the resulting overabundance of herbivores (Verdon et al. 2016; Linley et al. 2017; Moseby et al. 2018). For example, the successful reintroduction of digging marsupials (such as Burrowing

Bettong *Bettongia lesuer* and Bilby *Macrotis lagotis*) across multiple sanctuaries in semi-arid Australia has been associated with decreases in floristic values and vegetation condition, including lower plant species diversity, reduced frequency of important functional plant groups, and declines in seedling abundance and survival (Verdon et al. 2016; Linley et al. 2017; Moseby et al. 2018; Kemp et al. 2021; Michael et al. 2022). Ecologically, this is analogous to the predictable impacts of abundant large herbivores anywhere apex predators have been extirpated (e.g. Kangaroos *Macropus* spp. throughout south-eastern Australia; Morgan 2021) and why densities of such species are actively managed in sanctuary settings (Manning et al. 2011; Shorthouse et al. 2012). However, these examples of reintroduced species represent a management trade-off of accepting some floristic value loss in a widespread vegetation community to prevent the extinction of highly threatened herbivores.

In 2011, the Eastern Bettong (*Bettongia gaimardi*, hereafter referred to by their traditional Ngunnawal name "Ngaluda") was successfully reintroduced to a sanctuary dominated by Critically-Endangered yellow box (*Eucalyptus melliodora*) – Blakely's red gum (*E. blakelyi*) grassy woodland and derived grassland (Department of the Environment and Heritage 2006) in the Australian Capital Territory (Manning et al. 2019). The aim of the reintroduction was both to secure a species that had been extinct from the Australian mainland for a century and restore lost ecological function to the woodland community (Shorthouse et al. 2012; Manning et al. 2019). Ngaluda are digging marsupials – widely considered ecosystem engineers – whose soil disturbance benefits the ecosystem by facilitating nutrient cycling and seedling establishment (Rose and Johnson 2008; Eldridge and James 2009; Ross et al. 2020). While Ngaluda diggings and their positive effects have been widely acknowledged and researched at the sanctuary (Manning et al. 2019; Munro et al. 2019; Ross et al. 2019, 2020) and population-level negative effects on one species of geophyte observed (Ross 2020), any potential community-level negative effects that Ngaluda may have on plants from herbivory and soil disturbance remain unknown.

The aim of this study was to rapidly assess some key floristic values from across the sanctuary area where Ngaluda are present and compare to the floristic values of adjoining woodland reserves where Ngaluda are absent. Complicating our inference is that wallabies (Swamp Wallaby *Wallabia bicolor* and Red-Necked Wallaby *Notamacropus rufogriseus*, hereafter collectively "Baray") are highly abundant in the sanctuary but rare in the adjoining reserves. Undertaking this assessment was motivated by three time-sensitive factors. First, Ngaluda were soon to be translocated into a new predator-free sanctuary comprising the adjoining woodland reserves. Second, there were anecdotal observations suggesting that orchids and other geophytes were very abundant in areas of the new sanctuary compared to the established sanctuary where they appeared rare. Third, no systematic floristic monitoring of either area had been undertaken in the previous 10 years, meaning spring of 2021 was potentially the final opportunity to compare data on the presence and absence of Ngaluda between the two areas. We therefore developed a targeted "indicator" species survey that allowed us to quickly collect spatially extensive and comparative data within a single season.

To understand the relative influence of different drivers of floristic values we asked three questions: (1) to determine the effects of overall difference in herbivore assemblages on floristic value we asked *does the abundance of lilies, orchids and other indicator groups and species differ among the different sanctuary areas?* (2) to determine the effects of ground cover dominance independent of herbivory we asked *do ground cover and other vegetation attributes strongly influence plant indicator groups or species?* And (3) to determine the Ngaluda effects independent of Baray we asked *within just the sanctuary area where Ngaluda are present, do differences in overall macropod grazing influence richness and abundance plant indicator groups or species?* We hypothesised that (1) the abundance of lilies, orchids and other indicator groups and species would be greatest in the sanctuary area with the lower overall herbivory pressure (where Ngaluda and Baray are absent or rare), (2) the effect of overall herbivory pressure differences on our floristic values will be present independent of effects of ground cover and other vegetation attributes that will also be important, and (3) within just the area where Ngaluda are present, the richness and abundance of plant indicator groups and species will be higher where there activity is lower.

Methods

Study site: the Mulligans Flat Woodland Sanctuary

Our study was conducted at the Mulligans Flat Woodland Sanctuary, which is an area of two adjoining nature reserves enclosed by a predator-proof fence on the urban fringe of Canberra, south-eastern Australia. The sanctuary primarily contains critically endangered box-gum grassy woodlands and derived grasslands, with small areas of open forest and patches of natural temperate grassland (Lepschi 1993; McIntyre et al. 2010). These woodlands, dominated by *E. melliodora* and *E. blakelyi*, have been significantly impacted by land clearing, livestock grazing, invasive pest species, and loss of indigenous management following European occupation (Yates and Hobbs 1997; Lindenmayer et al. 2010). The Ngunnawal people are the Traditional Custodians of the land and waters in the ACT, and for tens of thousands of years actively managed woodlands in the region, shaping the structure and function of these ecosystems.

The full sanctuary comprises an original 458 ha sanctuary (est. 2009) in the Mulligans Flat Nature Reserve (hereafter “Mulligans Flat”) and an extended 801 ha sanctuary (est. 2018) made up of part of the Goorooyarroo Nature Reserve (hereafter “Goorooyarroo”, ~60% of the extended sanctuary) and three environmental offset areas (hereafter “Offsets”, ~40% if the extended sanctuary) (Figure 1). All areas of the sanctuary have similar recent histories of once being leasehold grazing land, with some areas of cropping and pasture improvement before being conserved and managed for their ecological values (McIntyre et al. 2010; Shorthouse et al. 2012). However, the areas differ in total time and intensity of agricultural impacts, the legacy effects of which determine floristic community composition (McIntyre et al. 2010, 2017). In general, Mulligans Flat was less impacted and has been managed for ecological values longer than Goorooyarroo (gazetted reserves in 1994 and 2003 respectively), with agricultural management of the Offsets ceasing in 2017. All areas are now managed for their conservation value with significant restoration efforts focussed on the Offsets to improve their poorer overall condition. Ngaluda were introduced to Mulligans Flat in 2012 and their introduction into the extended sanctuary is planned for 2023. Eastern Grey Kangaroo (*Macropus giganteus*, hereafter “Buru”) densities are similar across the two areas (around 1 Buru per ha, based on annual monitoring data from 2009) (ACT Government 2021; Supp. Material A). However, Baray (swamp wallaby and red-necked wallaby) are abundant in Mulligans Flat (around 0.8 per ha), having increased dramatically since fencing, and rare in Goorooyarroo (<0.1 per ha) (based on annual monitoring data from 2016, ACT Government *unpublished data*).

The sanctuary is also the site of the Mulligans Flat – Goorooyarroo Woodland Experiment: a manipulative field-based experiment that investigates the impact of a set of combined management actions to restore and enhance biodiversity (Manning et al. 2011; Shorthouse et al. 2012). Part of the experiment involves the reduction of grazing by large macropods (specifically established to exclude only Buru because at the time Baray were rare but grazing by both is reduced), achieved through fencing (two levels: Low Grazing (fenced) and High Grazing (unfenced)) (Figure 1). The fenced areas of Low Grazing within Mulligans Flat remain effective at reducing Buru densities by around 40% (Supp. Material A) unlike within Goorooyarroo where the fences are no longer effective (Supp. Material A). In 2012, an extra exclusion treatment that aimed to only exclude Ngaluda (smaller exclusion fences that would not exclude large macropods) was applied to a subset of sites in Mulligans Flat. However, they did not effectively exclude Ngaluda (which were able to climb over), and the large macropods almost completely avoid them (Evans et al. 2019). These extra fenced areas therefore represent a further reduction of large macropod grazing to almost zero, resulting in higher vegetation biomass. Anecdotal observations of diggings and sightings suggest these areas are in fact strongly preferred by Ngaluda (Authors, *pers. comm.*). In 2018, two of these fenced areas were modified to effectively exclude Ngaluda with the addition of a “floppy top” to prevent them climbing over. We also know that Ngaluda activity is significantly higher in the Low Grazing areas of Mulligans Flat compared to the High Grazing areas, likely because of a preference for more ground cover for foraging and shelter (Munro et al. 2019).

Floristic indicator monitoring

We undertook targeted species surveys of 12 indicator plant species at 106 plots across different areas of the sanctuary (Mulligans Flat *n* = 46, Goorooyarroo *n* = 30, and Offsets *n* = 30) (Figure 1; Table 1). Our 12 indicator species were selected based on (1) expectations that they would be targeted for herbivory by Ngaluda (i.e., an “indicator of Ngaluda herbivory”), (2) being relatively common enough to collect meaningful data, and (3) were floristic values that the sanctuary was being managed to enhance. We primarily targeted geophytes and species with tuberous root resources (10 species) to capture any effects of Ngaluda, which are expected to target and potentially have a negative impact on populations of these kinds of species (Ross 2020). Diet analysis of Ngaluda from extant populations in Tasmania determined plant material frequently occurred in faecal pellets (86-98% occurrence from spring to autumn) and most of that material was from roots and tubers (Taylor 1992). Similarly, diet analysis from the closely related Burrowing Bettong found fibrous plant parts (e.g., roots and root coverings) contributed the most by volume than any other dietary item (average of 34% of overall dietary composition (Bice and Moseby 2008)). Baray are generalist grazers that will preferentially consume forbs but will not specifically target below-ground structures (Davis et al. 2008; Di Stefano and Newell 2008). Therefore, we also targeted three control species without tuberous resources which would be responding to the overall herbivory pressure, not targeting by Ngaluda. Most plots (*n* = 82) were associated with the ACT Government's Herbage Mass Monitoring Program which collects vegetation structure and ground cover data to inform Buru and biomass management across the nature reserve network (Table 1) (ACT Government 2021). The other 24 plots were in the extra fenced areas in Mulligans Flat that either did effectively exclude Ngaluda thanks to the addition of a "floppy top" (*n* = 4 plots in two fenced areas) or promoted their presence and activity (*n* = 20 plots in 10 fenced areas) (Figure 1; Table 1). All plots were grouped in pairs at least 100 m apart in a mapped polygon (or "site") of relatively homogenous woodland vegetation (*n* = 53 polygons).

Surveyed plots were 400 m^2 circular quadrats (radius = 11.3 m), consistent with floristic plots already being monitored across the Offsets, and by other research in grassy ecosystems that aims to maximise the number of species observed (Armstrong et al. 2013). Monitoring at this scale increased our chances of capturing relatively rare and less abundant species in our surveys. Each plot was searched by one person for a minimum of 35 minutes (or by two people for a minimum of 20 minutes) with the presence or absence of the following targeted indicator species recorded: *Arthropodium* spp., *Bulbine bulbosa*, *Wurmbea dioica, Burchardia umbellata*, *Microseris lanceolata* (Dharaban), *Craspedia* spp., *Leptorhynchos squamatus, Chrysocephalum apiculatum*, *Stackhousia monogyna* and all members of the Orchidaceae family identified to genus (4 species observed). Abundance was then scored as count of individuals from species grouped together into four "indicator groups". The groups were (i) "lilies" (*Arthopodium, Bulbine, Wurmbea, Burchardia*; in reference to the common-name use of the term), (ii) “orchids”, (iii) “tuberous daisies” (Dharaban and *Craspedia*) and (iv) “control species” (*Leptorhynchos, Chrysocephalum*, *Stackhousia;* those without tuberous resources). Abundance was scored as either “0” (absent), “1” (1-9 individuals), “2” (10-99 individuals), “3” (100-999 individuals) or “4” (1000 or more individuals). Monitoring was undertaken between 26 October and 16 November 2021, in the second consecutive spring of above average rainfall.

Vegetation monitoring and other covariates

Ground cover and vegetation structure monitoring was undertaken at all plots that were not in extra fenced areas of Mulligans Flat (n = 82) (Table 1). Within the area of each monitoring plot, a step-point-intercept approach was used where either 75 (Offsets) or 100 (Mulligans Flat and Goorooyarroo) “steps” are taken at random and the ground cover vegetation at the point of the step recorded as either one of 25 different categories (ACT Government 2020; Snape et al. 2021). Those 25 detailed categories were summarised into 10 percent-groundcover variables of interest for our study: native grass, native shrub, other native species, rock, bare ground, leaf litter, dead grass thatch, exotic broadleaf, or exotic grass. These variables were standardised as a proportion of total steps (0-1) and the additional variable of “proportional nativeness of the perennial vegetated ground layer” was calculated (hereafter “proportional nativeness” = native perennial cover / (native perennial cover + exotic perennial cover)). Average grass height was also calculated from 10 measures of grass height (to nearest centimetre) taken randomly around the plot, meaning 12 vegetation variables were considered in total.

Three additional categorical variables were associated with our observations. They were: (1) “Condition State” that was derived from vegetation condition mapping undertaken by ACT Government. Each plot was identified as either a “Woodland” (mature trees present with a native dominated ground layer), “Derived Grassland” (mature trees absent with a native dominated ground layer), or “Exotic Woodland” (mature trees present or absent with an exotic dominated ground layer); (2) “Experimental Grazing Treatment” was identified as either “High Grazing” or “Low Grazing” as per the Buru exclusion areas that exist throughout the sanctuary for the restoration experiment (Manning et al. 2011). Extra fenced areas were identified as “Extra Fence (High Grazing)”, “Extra Fence (Low Grazing)”, or “Extra Fence (Floppy Top)” depending on their context; and, (3) “Dominant Grass

Species" was the most common species across 10 grass height measures in the vegetation monitoring, associated with our observations as being either *Themeda triandra*, *Rytidosperma* spp., *Austrostipa* spp., "Other Native" (mix of less common native grasses), or "Exotic" (combined non-native grasses).

Data analysis

All analyses were performed using R version 4.1.3 (R Core Team 2022).

We collected data sufficient to model the abundance of all four indicator groups and the occurrence of seven plant indicator species (*Arthropodium* spp., *Bulbine bulbosa*, *Wurmbea dioica, Microtis* spp., *Craspedia* spp., *Leptorhynchos squamatus* and *Chrysocephalum apiculatum*). We also summarised our data to model the response of "indicator group richness" (count of plant indicator groups present (0-4)), "indicator species richness" (count of plant indicator species present (0-13)), "highest indicator group abundance" (highest count for any of the plant indicator groups present), and "total indicator group abundance" (sum of abundance scores for plant indicator groups present). This provided us with a total of 15 plant indicator responses to investigate.

We performed a principal component analysis on the 12 ground cover and vegetation variables to identify potential co-linearity among variables and broad gradients in environmental variation (Nichols 1977). Principal components were calculated using the 'pca' function in the "vegan" package (Oksanen et al. 2022). This analysis reduced our 12 variables to four new axes that accounted for approximately 73% of total variation in those variables. The first axis PC1 (Eigenvalue = 4.1, 37% variance explained) had a high negative loading for average grass height and exotic grass cover, and a high positive loading for proportional nativeness and native grass cover. The other three axes accounted for 10-15% of additional explained variance each and represented co-linearity between the cover of thatch and the cover of bare ground, leaf litter and shrubs on PC2, thatch cover and exotic broadleaf cover on PC3, and rock cover and exotic broadleaf cover on PC4.

Each plant indicator response was the subject of three Generalised Linear Mixed Models (GLMM, Bolker et al. 2009) that correspond to the three questions of this study. Models were fit using the 'glmmTMB' function in the "glmmTMB" package (Brooks et al. 2017). To address Question 1 (do plant indicator responses differ among the different areas of the sanctuary?), all observations were included (n = 106) with each response fitted with a GLMM that included the additive effects of Sanctuary Area (“Mulligans Flat”, “Goorooyaroo”, or “Offsets”), condition state, experimental grazing treatment and dominant grass species. To address Question 2 (are plant indicator responses influenced by ground cover and vegetation structure?), the subset of observations with vegetation covariate data (n = 82) were fitted with a GLMM that included the additive effects of Sanctuary Area and the four principal components. To address Question 3, (do plant indicator responses differ among grazing treatments where Ngaluda are present?), the subset of observations from Mulligans Flat (n = 46) were fitted with a GLMM that included only the effect of experiment grazing treatment.

We used mixed models over linear or generalised linear models for two reasons. First, abundance scores and richness response variables were modelled with Poisson error distributions and log-link functions, while occurrence variables were modelled with binomial error distributions and logit link functions. Second, the random effect of “polygon” was included in all models to account for any spatial autocorrelation associated with the paired nature of our plots. Tests for overdispersion were undertaken to assess whether there was additional variance in the data than assumed by the error distributions and verify homogeneity and expected properties of residuals. If models were overdispersed, a random observation was included as a random effect to correct for the unexplained variance (Zuur et al. 2009). For the models used to investigate Questions 1 and 2, we used Akaike’s Information Criterion corrected for small sample sizes (AICc) to rank subsets of the full model and determine the best (lowest AICc) and whether there were any supported (ΔAICc < 2) models (Burnham and Anderson 2002). Models were compared using the ‘dredge’ and ‘AICc’ functions in the “MuMIn” package (Barton 2020). Where multiple models were supported, the model that included “Sanctuary Area” and had the simplest structure was used to make inference. All numerical predictor variables were scaled to a mean of zero and a standard deviation of one, prior to modelling, to allow direct comparison of regression coefficients. Explained variance (R^2) of each model was calculated using the ‘r2_nakagawa’ function in the “performance” package (Lüdecke et al. 2021).

Results

We observed at least one indicator species in 76 of the 106 plots surveyed across the sanctuary (72%). *Microtis* (onion orchids) were the most common orchid genera, occurring in 42% of plots, followed by *Thelymitra* (sun orchids) present in 13% of plots. *Arthropodium* spp. and *Wurmbea dioica* were the most common of the lilies indicator group, occurring in 30% and 28% of plots respectively. *Burchardia umbellata, Microseris lanceolata* (Dharaban) and *Stackhousia monogyna* were rare in the sanctuary, each recorded in <5% of plots. The complete absence of any indicator species was relatively rare within the Mulligans Flat and Goorooyarroo (13% and 26% of plots respectively) compared to the Offsets where most plots recorded zeros (70%).

Average grass height differed between the experimental grazing treatments within Mulligans Flat (Low Grazing = 10.7 cm [9.2 – 12.2 cm 95% CI]; High Grazing = 6.1 cm [5.9 – 6.3 cm]) but not within Goorooyarroo (Low Grazing = 14.9 cm [12.9 – 16.9 cm]; High Grazing = 13.1 cm [12.5 – 13.8 cm]).

Question 1: Does the abundance and occurrence of plant indicators differ among the different areas of the sanctuary?

Sanctuary Area featured in the top-ranked or a supported model for explaining variation in 10 of the 14 plant indicator responses (Table 1). There was a positive effect of Goorooyarroo (where Baray are rare and Ngaluda are absent) on indicator group richness, indicator species richness, the sum of indicator abundance scores, the abundance of the lilies and control species groups, and the occurrence of *Arthropodium* spp. and *Wurmbea dioica* compared to Mulligans Flat (Table 1). Of those 10 response models that featured Sanctuary Area, nine also featured one or both of Condition State and Dominant Grass in their top-ranked or supported model (Table 1).

The positive effects of Goorooyarroo on various plant indicators was most evident when Condition State was also considered (Figure 2). Most indicator species were rarely recorded in Exotic Woodland irrespective of Sanctuary Area (Figure 2). For indicator group richness, indicator species richness, and lilies abundance score (Figure 2a,b,e), Woodland in Goorooyarroo was associated with significantly higher values than Woodland in Mulligans Flat (Figure 2). For example, an average Woodland plot in Mulligans Flat would include 2.5 indicator species and count less than 10 lilies compared to an average Woodland plot in

Gooorooyarroo that would include five indicator species and count either more than 10 or more than 100 lilies (Figure 2). The average abundance score for the control species indicator group was higher in Goorooyarroo compared to Mulligans Flat irrespective of Condition State (Figure 2f).

Question 2: Do ground cover and vegetation structural attributes strongly influence plant indicator groups or species among areas of the sanctuary?

Sanctuary Area still featured in the top-ranked or a supported model for explaining the variation observed in eight of the 13 plant indicator responses that could be modelled from the subset of plots with corresponding covariate data (n = 82) (Table 2). All best models also featured the significant positive effect of PC1 on all plant indicator responses except for *Craspedia* spp. (Table 2). This represents a general response of all indicators being lowest where grass height and exotic cover was high, and highest where proportional nativeness and total native cover was high.

The positive effects associated with PC1 were generally greater in Goorooyarroo compared to Mulligans Flat (Figure 3). Mulligans Flat was predicted to record on average two indicator species and count less than 10 lilies and control species in plots with the greatest native ground cover, whereas the equivalent values were predicted to occur in Goorooyarroo where proportional nativeness and total native ground cover were much lower (Figure 3). The highest indicator abundance score recorded per plot, and the abundance scores of the orchids and tuberous daisies, were all positively associated with PC1 and were not related to Sanctuary Area at all (Figure 3).

Question 3: Within the Mulligans Flat area where Ngaluda are present, do differences in overall macropod grazing influence plant indicator groups or species?

Indicator group richness, indicator species richness, and highest indicator abundance score recorded per plot were negatively associated with the Low Grazing treatment compared to High Grazing within Mulligans Flat (Figure 4a-c). Low Grazing plots were characterised by, on average, a single indicator species with less than 10 individuals, while High Grazing plots were characterised by 2.5 indicator species with at least one group having 10 to 100 individuals present (Figure 4a-c).

The abundance score of the orchid indicator group – and the occurrence of *Microtis* which was almost exclusively responsible for that score – was significantly lower in Low Grazing plots, Extra Fence (Low Grazing) and Extra Fence (High Grazing) plots (which do not exclude Ngaluda) than in High Grazing plots (Figure 4d). Those grazing treatments recorded on average 0-9 individuals compared to an average 10-99 individuals recorded for the unmanipulated High Grazing plots (Figure 4d). Orchid abundance or occurrence in Extra Fence (Floppy Top) plots (which do exclude Ngaluda) was not different from the High Grazing treatment (Figure 4d).

Discussion

We assessed floristic values from across a woodland sanctuary with areas of different overall herbivory pressures to try and understand any potential effects of a reintroduced digging herbivore – Ngaluda (Eastern Bettong). We found that most floristic indicators we investigated, including the richness of indicator species, total abundance score, and the abundance of lilies, were greater in woodland where Ngaluda were absent (Goorooyarroo) compared to equivalent woodland where Ngaluda were abundant (Mulligans Flat). We also found that within Mulligans Flat the experimental grazing contexts that were associated with higher Ngaluda activity had some lower floristic values, including significantly lower abundance of a common orchid, irrespective of whether the densities of other large macropods were high or low. While the differences observed between Mulligans Flat and Goorooyarroo could be explained by either Ngaluda presence and/or Baray numbers (swamp wallaby and red-necked wallaby that are abundant in Mulligans Flat but rare in Goorooyarroo), the differences observed within Mulligans Flat more clearly suggest a negative effect of Ngaluda. Our assessment highlights the impacts of both Ngaluda in concert with Baray and of Ngaluda on its own, which is consistent with the negative effects of overabundant herbivores generally, and reintroduced digging marsupials on native vegetation observed in other Australian sanctuaries specifically (Verdon et al. 2016; Linley et al. 2017; Moseby et al. 2018; Kemp et al. 2021; Michael et al. 2022).

Evidence of overall higher herbivory pressures in Mulligans Flat impacting floristic values

Goorooyarroo plots contained on average higher richness of our indicator species than Mulligans Flat plots. Woodland forb diversity is influenced by multiple interacting processes, including soil nutrification, overgrazing and weed invasion that suppresses native forbs (Prober et al. 2002, 2016; McIntyre and Lavorel 2007) and favourable seasonal conditions, cool burning, and a heterogenous native ground layer that promote them (Smallbone et al. 2007; Prober et al. 2009; McIntyre et al. 2017). Past studies of the sanctuary when the herbivory pressures were more uniform found no difference in species richness between Mulligans Flat and Goorooyarroo, with native forbs in general increasing consistently in both areas in response to more favourable conditions between 2007 and 2011 (McIntyre et al. 2014, 2017). The richness patterns in the indicator species we observed is counter to these results. If abundant Baray and Ngaluda were not having a negative effect, we would have expected indicator species richness to be either similar between Mulligans Flat and Goorooyarroo as indicated by previous monitoring, or potentially greater in Mulligans Flat where there are fewer areas of high soil nutrification owing to a less-intense agricultural land-use history. Therefore, these results strongly suggest an impact of the overall higher herbivory pressure that exists within Mulligans Flat compared to Goorooyarroo and not an impact any legacy-effect differences that exist between the two areas.

We found some evidence that the occurrence of four indicator species (of the seven species with sufficient data) was greater in Goorooyarroo than Mulligans Flat. These include the lilies *Arthropodium* spp. and *Wurmbea dioica* that have below-ground tubers, but also the control species *Leptorhynchos squamatus* and *Chrysocephalum apiculatum* that do not have specific below-ground resources Ngaluda may target. The occurrence of these common species was not significantly different between the more degraded Offsets area and Mulligans Flat, suggesting that soil condition and other factors associated with different land-use histories are not responsible for these occurrence patterns. Instead, these occurrence patterns suggest the greater overall herbivory pressures at Mulligans Flat (due to abundant Ngaluda and Baray) is limiting for these species, compared to the overall lower herbivory pressures at Goorooyarroo (where Ngaluda are absent and Baray rare).

The relative abundance of lilies and control species was also significantly higher in Goorooyarroo compared to Mulligans Flat. These results again highlight the impact of the overall higher herbivory pressure of abundant Ngaluda and Baray, but also suggest a synergistic effect of Ngaluda-targeted herbivory. Control species were ~1-order of magnitude less abundant in Mulligans Flat which can be interpreted as the impact of overall general herbivory, whereas the lilies group were ~1-2-orders of magnitude less abundant inferring an amplification of the general herbivory effect (1-order) from likely targeted herbivory. If Ngaluda were not having a specific impact on these tuberous species, as they are known to have on *Wurmbea* specifically (Ross 2020), then we would have expected the same level of impact from general herbivory of Baray and Ngaluda as observed for the control species. While these tuberous plants would be consumed by a broad range of animals, including some birds and insects, it is unlikely the effects of these differ between the two sanctuary areas. For example, beetles are more abundant across the sanctuary in areas that had previously been fertilised for pasture improvement (Ross et al. 2017), meaning if root and tuber consuming beetles were impacting lilies in one area over the other, we would expect to observe that impact in Goorooyarroo, which has a greater legacy of pasture improvement (McIntyre et al. 2010). Our comparison of floristic values between Mulligans Flat and Goorooyaroo clearly shows a negative impact of a predator-free sanctuary with higher herbivory pressures (of Ngaluda and Baray combined) on floristic values, which is not likely the result of some other legacy-effect or ecological process.

Evidence of woodland condition and ground cover impacting floristic values along with herbivory

The ecological condition of the woodland vegetation as measured by the dominance of native species in the ground layer explained much of the variation observed in our floristic indicators. Indicator species were rare and often completely absent in woodland or derived grassland with a predominantly exotic ground layer, and there was a negative effect of increasing exotic cover and average grass height on indicator richness and the abundance score of all four indicator groups. This is consistent with widely demonstrated impacts that past land-use, soil nutrification, weed invasion, and biomass accumulation have on native plant species establishment and persistence (Prober et al. 2005; Blumenthal 2006; Prober and Wiehl 2012; Bernard-Verdier and Hulme 2019; O’Reilly-Nugent et al. 2019). Conversely, we found the highest richness and abundance of all indicator groups were observed in plots that had the most intact native ground layer. Variation in some indicators (e.g. orchid abundance score that was almost exclusively *Microtis*) was best explained by woodland condition and/or ground cover and not Sanctuary Area at all, suggesting overall differences in herbivory between Mulligans Flat and Goorooyarroo at a reserve-scale were not influencing these values more than ground cover processes at the patch-scale. These results highlight the importance of management interventions that decrease weed dominance and biomass accumulation in maintaining opportunities for the native forbs we targeted in our survey.

The ecological condition of the woodland vegetation did not fully explain the differences in floristic values observed between Goorooyarroo and Mulligans Flat. While the negative effect of exotic cover and grass height on floristic indicators was consistent among the sanctuary areas, the positive effect of native dominance of the ground layer was amplified in Goorooyarroo relative to Mulligans Flat. This means that any like-for-like comparison of woodland along that continuum of increasing native dominance has Goorooyarroo containing higher floristic values than Mulligans Flat. For each indicator where both Sanctuary Area and PC1 were significant predictors, the response curve of Mulligans Flat was the same as the Offsets area. The Offsets were expected to have lower floristic value even in the areas where native species dominate the ground layer owing to the more recent history of livestock grazing and agricultural management. These results further indicate the impacted floristic values of Mulligans Flat compared to Goorooyarroo are most likely the product of herbivory difference between the two areas, not differences in ground layer condition.

Evidence of Ngaluda impacts on floristic values at Mulligans Flat

The lower floristic values observed at Mulligans Flat compared to Goorooyarroo are driven largely by the overall greater herbivory pressures experienced at Mulligans Flat that contains both abundant Ngaluda and Baray. However, analysis of floristic differences within Mulligans Flat does suggest that specific Ngaluda herbivory impacts are also occurring, based on floristic differences among experimental grazing treatments within Mulligans Flat, and the known responses of Ngaluda to those. Ngaluda activity is similar (low or absent) in High Grazing and the Extra Fence (Floppy Top) plots (see Munro et al. 2019) and it is these two types of plots in which the abundance of the onion orchid *Microtis* spp. was highest, despite the activity of larger macropods being highly dissimilar across them (high vs absent). This indicates grazing by larger macropods is not a major determinant of *Microtis* abundance as the highest values were found under completely different grazing pressures. Low Grazing areas and the other Extra Fences are the contexts where Ngaluda activity is highest (see Munro *et al.* 2019) and where the abundance of *Microtis* is significantly lower. As our orchid response contrasts exactly to Ngaluda activity (low where high, high where low) but is disconnected from larger macropod activity (high where Buru or Baray activity is either low or high) we conclude that it is herbivory by Ngaluda, not any direct or indirect effect of Buru and Baray, that is most likely responsible for this pattern. While *Microtis* abundance was the clearest result, three other richness and abundance indicators were significantly reduced in Low Grazing areas of decreased Buru and Baray densities and higher Ngaluda activity, further supporting the hypothesis that targeted herbivory by Ngaluda has a significant additional impact following general herbivory by all macropods.

The Ngaluda population in Mulligans Flat grew quickly and since autumn 2016 has been at the relatively stable and density-dependent size of between 150-200 individuals (Manning et al. 2019). This asymptotic population size is thought to be the carrying capacity of the sanctuary based on available food resources limiting juvenile survival (Manning et al. 2019). Whether or not this density-dependent population represents an overabundant population in terms of having detrimental ecological effects remains untested. At the time of our study Ngaluda had been present in Mulligans Flat for nine years and at an abundant population size for the preceding five years, meaning their effects on the woodland community should be evident. For example, a decline in palatable plant cover was observed as soon as the population of reintroduced Burrowing Bettongs began to grow at Arid Recovery (Moseby et al. 2018) and increased plant species richness was evident within two years of excluding reintroduced digging marsupials from areas at Scotia Sanctuary (Michael et al. 2022). If Ngaluda herbivory was not having a net negative effect on these species we would expect them to be at least be as abundant in Mulligans Flat as in Goorooyarroo, or potentially more abundant if Ngaluda diggings were have a net positive effect of facilitating establishment (as hypothesised in Ross et al. 2020).

Study limitations and implications for adaptive management

We are confident that our assessment has identified that a predator-free sanctuary with increased herbivory pressures from the combined herbivore assemblage has clear impacts on the floristic values of the endangered box-gum grassy woodland community. Our results also strongly suggest an additional impact of targeted herbivory on specific floristic values by a reintroduced digging herbivore. While Buru densities between Mulligans Flat and Goorooyarroo remain similar (ACT Government 2021), since the establishment of the original sanctuary the densities of Baray (wallabies) and Wilay (brushtail possum *Trichosurus vulpecula*) have increased at Mulligans Flat and therefore limits our inference from reserve-level comparisons of Ngaluda presence or absence. We can largely dismiss Wilay as a key driver of our results as forbs typically are an extremely minor component of their diet (How and Hillcox 2000; Sweetapple et al. 2004), but that is not the case for Baray, which are generalist grazers that will preferentially consume forbs (Davis et al. 2008; Di Stefano and Newell 2008). While it is difficult to conclusively attribute floristic differences between sanctuary areas to Ngaluda and/or Baray because of a lack of independent replicates (there is only one sanctuary area of abundant Ngaluda and Baray), our spatially extensive survey combined with the analytical way we have considered alternative hypotheses (such as woodland condition, experimental grazing manipulation, or ground cover) has largely overcome this logistical constraint (Oksanen 2001). Ultimately more research with greater replication (e.g., other areas where Ngaluda are reintroduced) and better controls (e.g., establishing an experimental treatment that excludes Ngaluda but not the larger macropods which currently does not exist) is needed to confidently identify the relative contribution of Baray and Ngaluda to the clear impacts of overall herbivory pressure.

The floristic patterns we have observed in the sanctuary are consistent with the ecology of the herbivores present, are consistent with the impacts of reintroduced digging marsupials in other sanctuaries, and suggest Ngaluda have decreased some floristic values at Mulligans Flat. Unlike other examples of reintroduced marsupials decreasing floristic values of sanctuaries that are relatively small areas within otherwise continuous and non-threatened vegetation communities (e.g., Arid Recovery and Scotia Sanctuary), Mulligans Flat is a largely isolated remnant of Critically Endangered ecological community within a peri-urban landscape. Ngaluda numbers and recovery objectives may need, in this case, to be balanced against woodland floristic values to achieve the ecosystem-level objective of the sanctuary, which is to conserve a thriving grassy woodland through restoring lost ecological function (Mulligans Flat Woodland Strategy 2022). The adaptive management implications of this assessment are two-fold. First, it highlights that both Ngaluda and these plant species are values that need active management to achieve broader objectives of the sanctuary. Second, it demonstrates that there is still uncertainty when it comes to knowing what the balance of Ngaluda effects are in this sanctuary context. Our assessment suggests restoring the ecological function of Ngaluda in the absence of top-down regulation of their population risks the detrimental effects of their herbivory being greater than the beneficial effects of their diggings for key species of this endangered woodland community, and that interventions to manage numbers are likely to be required (such as relocation of individuals to other sanctuaries).

Managing sanctuaries to achieve more diverse ecosystem objectives than simply safeguarding threatened predator-susceptible mammals will remain an ongoing challenge. At Mulligans Flat, further research is needed to quantify density-impact relationships between Ngaluda and these floristic values to determine at what population size the actions of Ngaluda will be of net benefit to native plant diversity, which is a key ecological outcome the reintroduction seeks to achieve (Manning et al. 2019; Ross et al. 2020). Reintroducing other lost ecological functions to the woodland sanctuary could potentially be used to balance these impacts, including top-order predation (to decrease the effects of Ngaluda) as well as the restoration of traditional Indigenous management (such as cool burning to directly enhance floristic value). Determining the effectiveness of any change in management will require robust monitoring to track outcomes, resolve uncertainty, and adapt practise into the future. In the meantime, increasing our knowledge of the effects that reintroduced species have on the recipient woodland ecosystem at the sanctuary will help ensure the sanctuary is managed to benefit both critically endangered animals and critically endangered vegetation. Our findings will be of interest to a wide range of groups including sanctuary managers, government agencies with decision-making responsibility around translocations, conservation planners, and conservation and translocation policy makers.

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Tables and Figures

Table 1. Number of plots monitored within any particular sanctuary area and experimental grazing treatments that exist within that area. The "Extra Fence" treatments within Mulligans Flat are either nested within a high grazing (HG) or low grazing (LG) context, or have a floppy top (FT) which excludes all species. *denotes that while still present, the experimental fencing within Goorooyarroo is no longer effective at lowering Buru densities (see Results and Supplementary Material A).

Sanctuary area	Overall herbivore assemblage	Experimental grazing treatments within area	Treatment-specific change in herbivore assemblage	Number of plots	Associated herbage mass monitoring
Original: 458 ha					
Mulligans Flat	Buru with	High grazing	Lower Ngaluda activity	12	Yes
(n=46)	abundant	Low grazing	Lower Buru/Baray density	10	Yes
	Baray and	Extra fence (HG)	Buru/Baray excluded	12	No
	Ngaluda	Extra fence (LG)	Buru/Baray excluded	8	No
		Extra fence (FT)	Buru/Baray/Ngaluda excluded	4	No
Extended: 801 ha					
Goorooyarroo	Buru, Baray	High grazing	None	20	Yes
(n=30)	rare and	Low grazing	Lower Buru density*	10	Yes
Offsets	Ngaluda	High grazing	None	30	Yes
(n=30)	absent				

Table 2. Model coefficients (effect sizes) for explanatory variables from the top-ranked Question 1 GLMM for each of 14 plant indicator and species occurrence response variables that could be modelled. Bold values indicate a statistically significant relationship ($P < 0.05$) where 95% confidence intervals do not intercept zero. "Sanctuary Area" refers to Goorooyarroo and Offsets that are compared to Mulligans Flat. "Condition State" refers to Derived Grassland and Exotic Woodland or Grassland that are compared to Woodland. Dominant Grass refers to *Rytidosperma*, *Austrostipa* and Exotic species that are compared to *Themeda*. Marginal R^2 (R^2m) provides the variance explained only by fixed effects and conditional R^2 (R^2c) provides the variance explained by the entire model. R^2c could not be calculated for models that included a random observation effect to correct overdispersion. NA indicates where meaningful effects could not be calculated. "Arthop." refers to *Arthropodium*. "Chryso." refers to *Chrysocephalum*.

	Sanctuary Area		Cond. State		Dominant Grass					
			Derived	Exotic						
Response	Gooroo	Offsets	Grass.	Wood.	Rytid.	Stipa	Exotic	AICc	R^2m	R^2c
Indicators										
Group Rich.	**0.53**	-0.18	-0.21	**-2.31**	-0.16	**-1.27**	**-0.89**	321.8	0.66	-
Spp. Rich.	**0.73**	-0.25	-0.16	**-2.41**	-0.01	**-1.24**	**-0.95**	363.2	0.71	-
High. Score	0.22	**-0.72**	0.09	**-2.47**				312.7	0.62	-
Sum Scores	**0.69**	-0.48	-0.14	**-2.49**	-0.07	**-0.96**	**-0.93**	413.4	0.75	-
Lilies	**1.08**	-0.46	-0.63	NA	<0.01	-0.75	**-1.14**	223.3	0.98	1.00
Orchids	<0.01	-0.52	0.26	NA	<0.01	-1.03	**-1.07**	261.9	0.97	0.98
Tub. Daisies					-1.64	NA	**-1.05**	149.3	0.95	0.96
Control spp.	**1.34**	**-1.95**						209.3	0.32	0.62
Spp. Occ.										
Arthrop.	**1.69**	-0.71	-1.30	NA				124.9	0.93	0.94
Bulbine	1.30	-0.54						91.0	0.81	0.86
Wurmbea	**2.90**	2.56			0.07	**-3.77**	**-5.26**	114.9	0.43	0.70
Microtis			0.12	0.00	6.97	**-15.92**	**-16.35**	111.4	0.45	0.99
Craspedia			**15.80**	10.98	NA	NA	**-11.63**	91.6	0.53	0.99
Chryso.					9.87	-6.73	7.39	90.7	0.05	0.99

Table 3. Model coefficients (effect sizes) for explanatory variables as they feature in the top-ranked Question 2 GLMM for each of 13 plant indicator and species occurrence response variables that could be modelled. Bold values indicate a statistically significant relationship ($P < 0.05$) where 95% confidence intervals do not intercept zero. “Sanctuary Area” refers to Goorooyarroo and Offsets that are compared to Mulligans Flat. Principle Components (PC1, PC2, PC3, PC4) represent multiple broad environmental gradients in ground layer vegetation cover and structure. Marginal R^2 (R^2m) provides the variance explained only by fixed effects and conditional R^2 (R^2c) provides the variance explained by the entire model. R^2c could not be calculated for models that included a random observation effect to correct overdispersion. “Arthop.” refers to *Arthropodium*. “Leptoryn.” refers to *Leptorhynchos*.

	Sanctuary Area		Principle Components					
Response	Gooroo	Offsets	PC1	PC2	PC3	AICc	R^2m	R^2c
Indicators								
Group Rich.	**0.78**	-0.13	**0.87**		0.27	223.3	0.59	0.60
Spp. Rich.	**0.95**	-0.09	**0.94**		**0.26**	252.4	0.63	0.66
High. Score			**1.00**	**-0.18**		213.7	0.60	-
Sum Scores	**1.13**	0.18	**1.13**	**-0.23**	**0.37**	290.8	0.64	0.76
Lilies	**1.12**		**0.95**			168.9	0.47	-
Orchids			**1.86**	**-0.44**		157.7	0.73	0.75
Tub. Daisies			**0.60**			124.8	0.12	0.37
Control spp.	**1.65**	-0.66	**1.18**			146.2	0.58	0.64
Spp. Occ.								
Arthrop.	**2.65**	1.36	**1.56**			84.5	0.50	-
Microtis			**67.24**	**-16.79**		42.1	0.46	1.00
Craspedia				-0.84		74.8	0.12	0.41
Leptoryn.	**3.10**	-2.12	**3.06**			58.5	0.97	0.98
Chryso.	**1.97**	-0.05	0.90			74.5	0.38	-

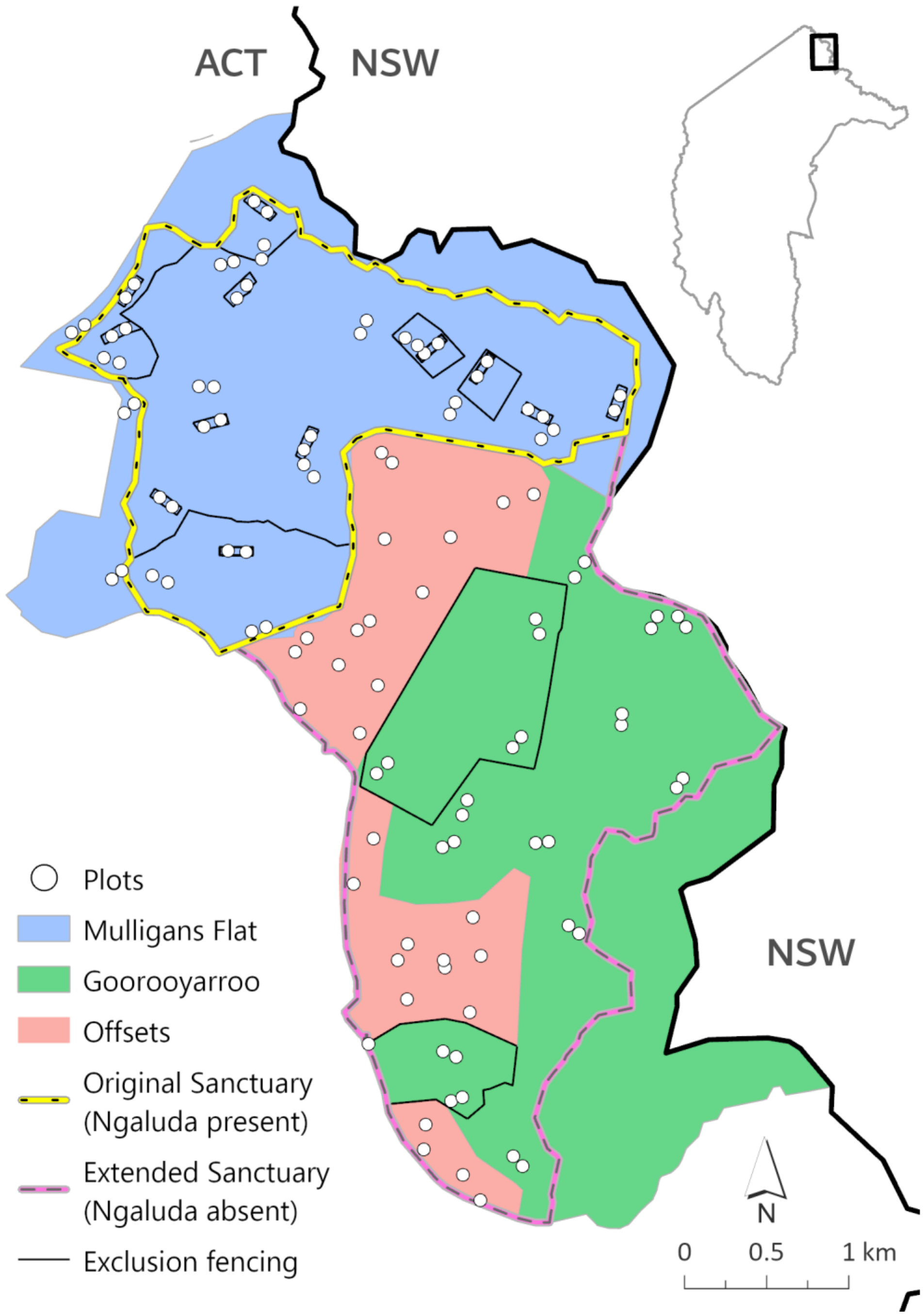


Figure 1. Map of the Mulligans Flat Woodland Sanctuary in the Australian Capital Territory (ACT), south-eastern Australia. Displayed are the locations of the 106 plots monitored for this study, the three different areas that comprise the sanctuary (Mulligans Flat, Goorooyarroo and Offsets), the boundaries of the original and extended sanctuaries where Ngaluda are present or absent, and the exclusion fencing that are either “Low Grazing” areas of reduced Buru densities (larger areas) or Extra Fences (smaller rectangle areas).

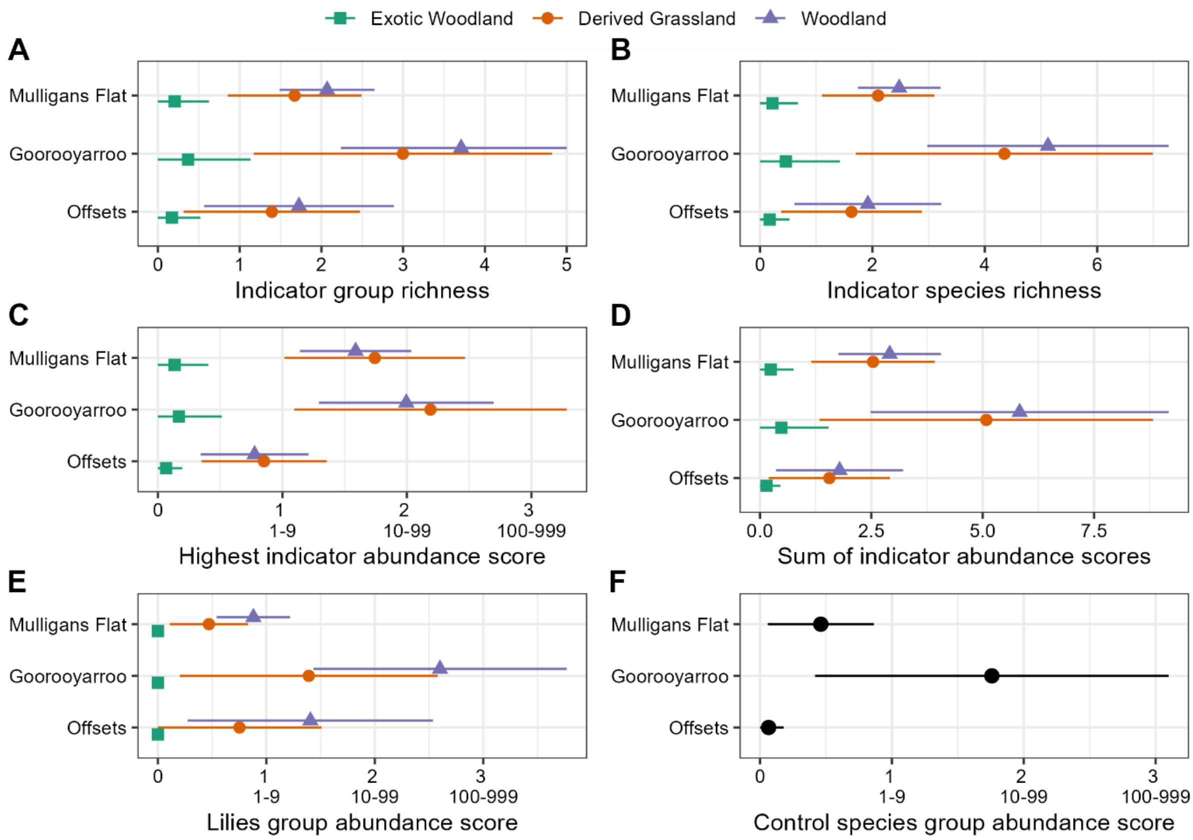


Figure 2. Predicted average (± 95% CI) plant indicator responses in different Sanctuary Area (Mulligans Flat, Goorooyarroo or Offsets) and Condition State (Woodland, Derived Grassland, Exotic Woodland) contexts at the sanctuary. Predicted values are for the top-ranked Question 1 GLMM where predictions were made by varying both "Sanctuary Area" and "Condition State" and holding "Dominant Grass" as "*Themeda*". The exceptions are highest indicator abundance score (**C**) which did not include dominant grass in it's top-ranked model, and Control species group abundance score (**F**) that only included Sanctuary Area. There were significant singular effects of "Sanctuary Area" on each variable that were either a positive effect of Goorooyarroo (**A**, **B**, **D**, **E**), negative effect of Offsets (**C**), or both (**F**) compared to Mulligans Flat (Table 1). All significant effects of "Condition State" were a negative effect of Exotic Woodland compared to the Woodland reference level.

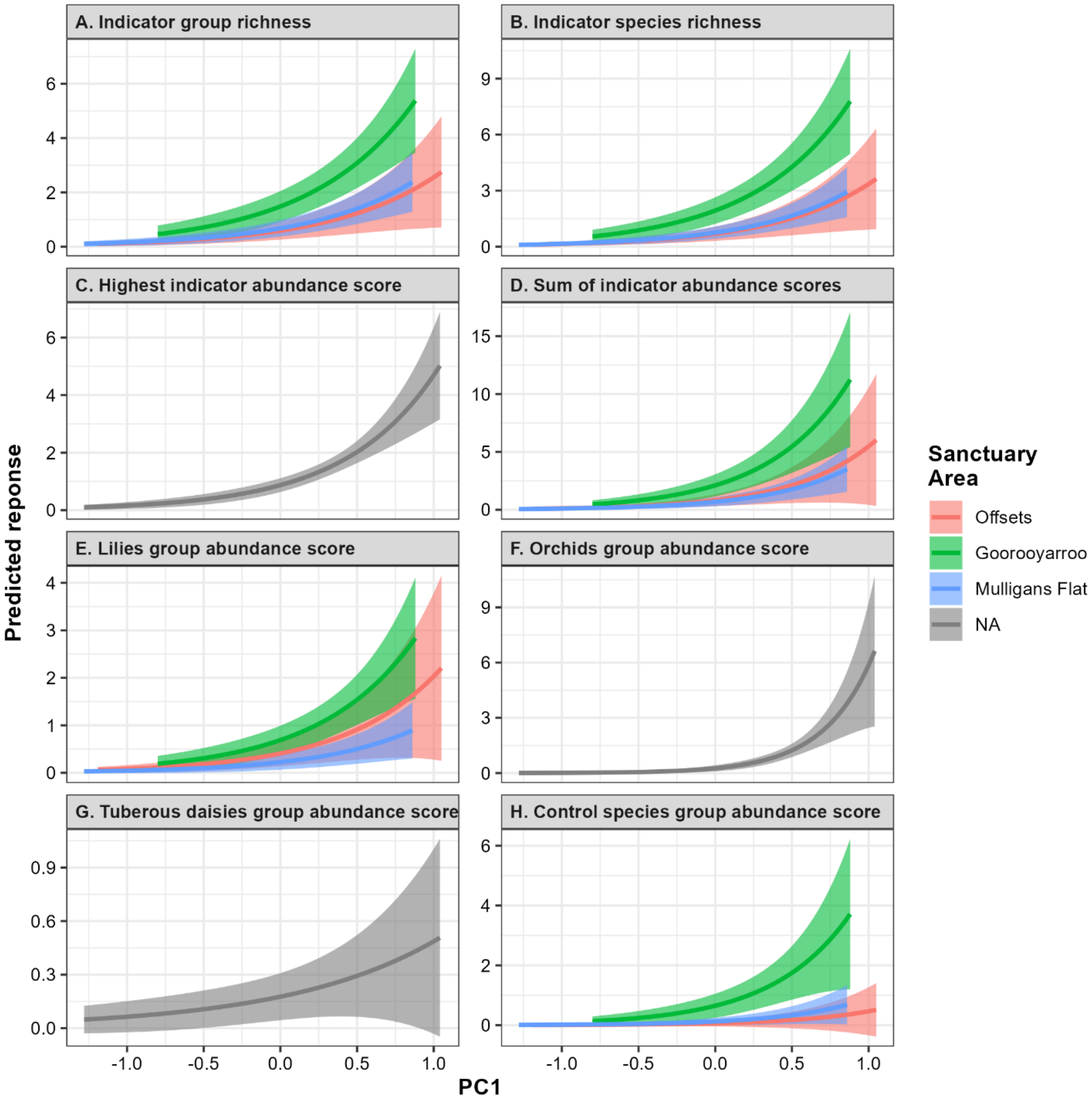


Figure 3. Relationships between Principal Component 1 (PC1) and plant indicator responses in different Sanctuary Areas. PC1 is an environmental gradient that broadly represents increasing grass height and non-native cover in the negative direction, and increasing proportional nativeness and native grass cover in the positive direction. Predicted values (solid lines) and 95% confidence intervals (shaded areas) were generated from the top-ranked Question 2 GLMM for each variable where different "Sanctuary Area" and "PC1" combinations were set while holding other variables in the model (PC2, PC3 or PC4) at their average values. Grey predictions are where Sanctuary Area did not feature in the top-ranked model (Sanctuary Area = "NA"). Where Sanctuary Area did feature, there was a consistent positive effect of Goorooyarroo and no effect of Offsets on each indicator compared to the Mulligans Flat reference level.

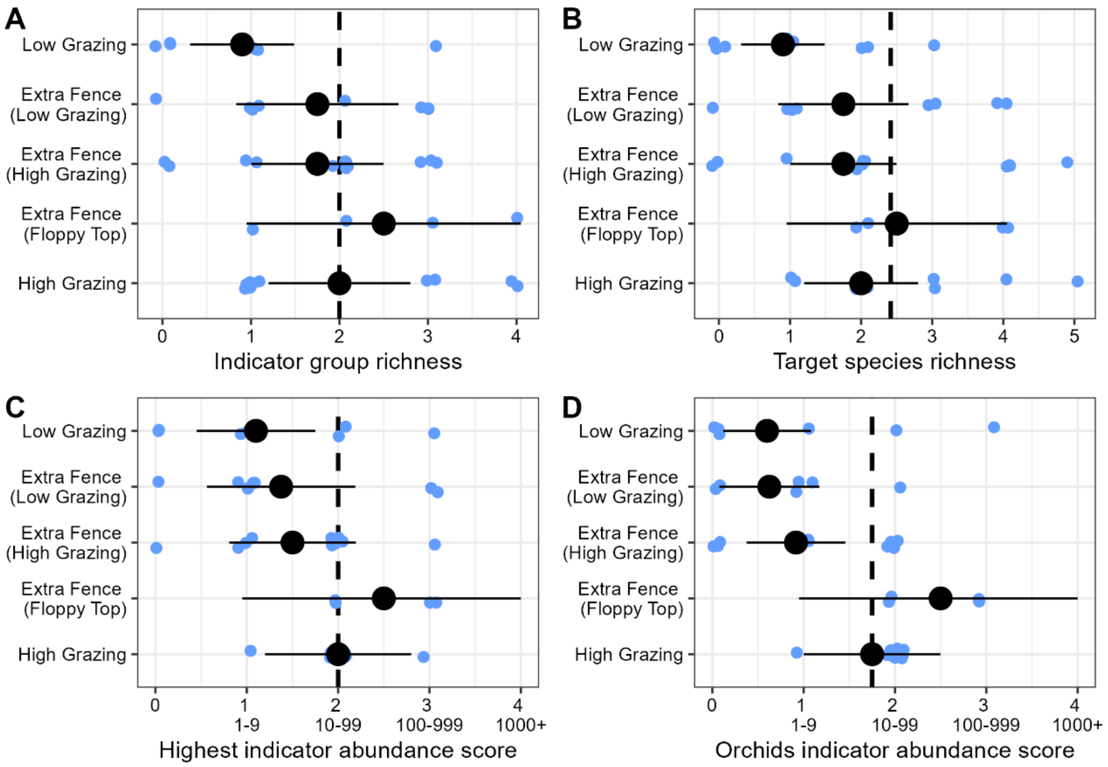
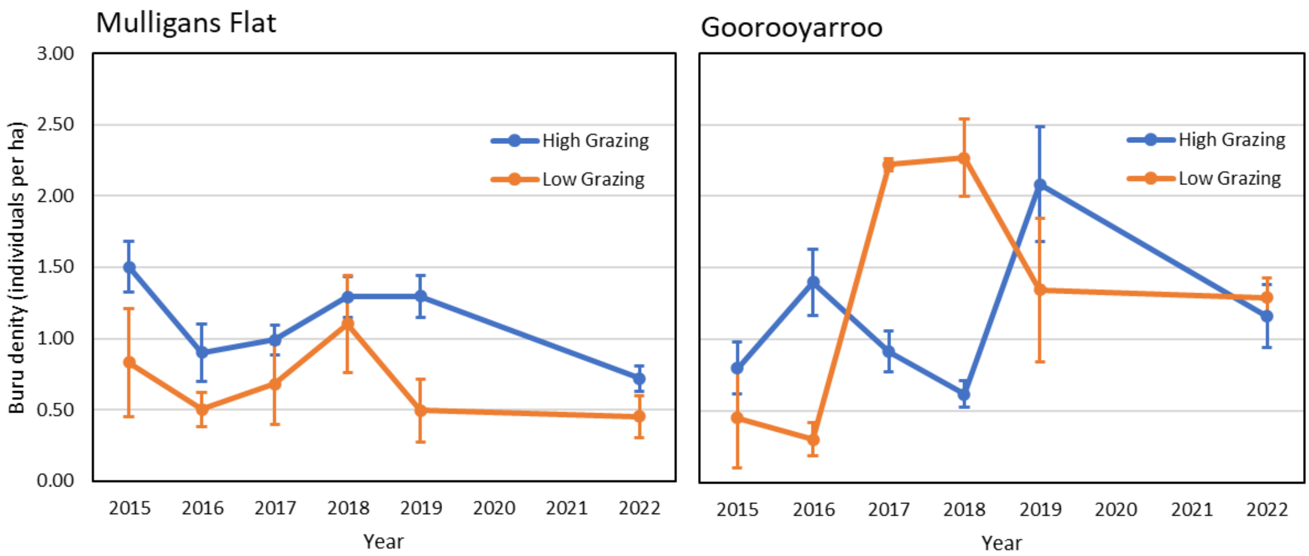


Figure 4. Predicted average (± 95% CI) plant indicator responses to different experimental grazing contexts within the Mulligans Flat area of the sanctuary. Small blue circles are the jittered raw values from the plot data (*n* = 46). The dashed line represents the model intercept reference condition. Effects of grazing treatments are considered significant where confidence intervals do not intercept the dashed line. High Grazing refers to no manipulation and where Buru and Baray are abundant. Low Grazing refers to large areas that have been fenced where Buru and Baray are less abundant, although Ngaluda activity is higher. Extra Fences reduce Buru and Baray grazing to effectively none, and either effectively exclude ("Floppy Top") or amplify (other contexts) Ngaluda presence and activity.

Supplementary Material A

O'Loughlin, L.S., Baines, G., Carlson, E., Wimpenny, C. & Cooney, R. An assessment of potential herbivory impacts of a reintroduced marsupial in a predator-free woodland sanctuary. *Ecological Management and Restoration.*

Average (± 1 standard error) Buru (Eastern Grey Kangaroo *Macropus giganteus*) densities within the "Low Grazing" and "High Grazing" areas of the Mulligans Flat-Goorooyarroo Woodland Experiment (Manning et al. 2011) in the "Mulligans Flat" sanctuary area where the treatment effect of decreased Buru densities in "Low Grazing" is effective, and the "Goorooyarroo" sanctuary area where it is not. Buru densities monitored as part of the ACT Government's Kangaroo Management Program (ACT Government 2021) where fenced-area-specific data was not collected in 2020 and 2021 due to COVID-19 related constraints.



References

ACT Government (2021) Eastern Grey Kangaroo Conservation Management Advice 2021. Environment, Planning and Sustainable Development Directorate, ACT Government, Canberra.

Manning A. D., Wood J. T., Cunningham R. B., McIntyre S., Shorthouse D. J., Gordon I. J., and Lindenmayer D. B. (2011) Integrating research and restoration: The establishment of a long-term woodland experiment in south-eastern Australia. Australian Zoologist 35, 633–648.