Project report to Nature Foundation South Australia

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Project title:

"Assessment of pollination rates and colonization of revegetation areas of Cygnet Park"

Abstract

A variety of techniques were used to assess the development of the plant and pollinator communities within the revegetation on Cygnet Park Sanctuary. The species richness of floral visitor communities within the revegetation of different ages were assessed and compared to a remnant site. Species richness showed an increase with age of revegetation and was similar to the remnant site after 4 years. Floral visitor - plant interaction networks were calculated based on specimens collected and on observed interactions from the field. The interaction networks showed increased complexity with age of revegetation and were dominated by an invasive species *Apis mellifera*. The dependency on insect pollination of a variety of plant species that are to be used in future experiments was confirmed. Seeds production of plants within the revegetation and remnant site showed mixed results highlighting that plant responses to revegetation are not uniform.

Introduction

Revegetation for ecological outcomes is a growing industry (McDonald & Williams 2009). It is used to offset vegetation clearance and as a panacea for addressing landscape scale losses of biodiversity and ecosystem services. Ultimately newly established vegetation communities need to be self-sustaining and resilient (Clarke, Stokes & Wallace 2010). For this to occur, the individual plants need to not only survive, but reproduce (Dixon 2009; Fontaine et al. 2005; George & Zack 2001; Neal 1998). For many plants this requires the presence of suitable pollinators, yet pollinators may fail to colonize revegetation areas or lag behind the plants. Insects are the most common vector for pollination, pollinating approximately 70% of plant species (Aizen et al. 2003; Ollerton, Winfree & Tarrant 2011; Willmer 2011). This project aims to assess the development of insect pollinator communities in revegetation of ages 1-4 years and compare them to a remnant reference site. This information will then be used to create pollination networks for each revegetation site and in conjuction with measures of seed production, assess the functional success of the revegetation.

Study Site

The study site is Cygnet Park Sanctuary (UTM, 53H, 724000E, 6045000N) on Kangaroo Island South Australia. Sites were revegetated in 2008, 2010 and 2011 as part of the annual Kangaroo Island Planting Festival. The reference site is a patch of remnant vegetation within the Kangaroo Island Airport (see Fig. 1). This reference site was chosen for its proximity to Cygnet Park, known similarity in plant species and herbivore exclusion fencing similar to that at Cygnet Park. Within each site the study area had a 100m buffer from any edge.

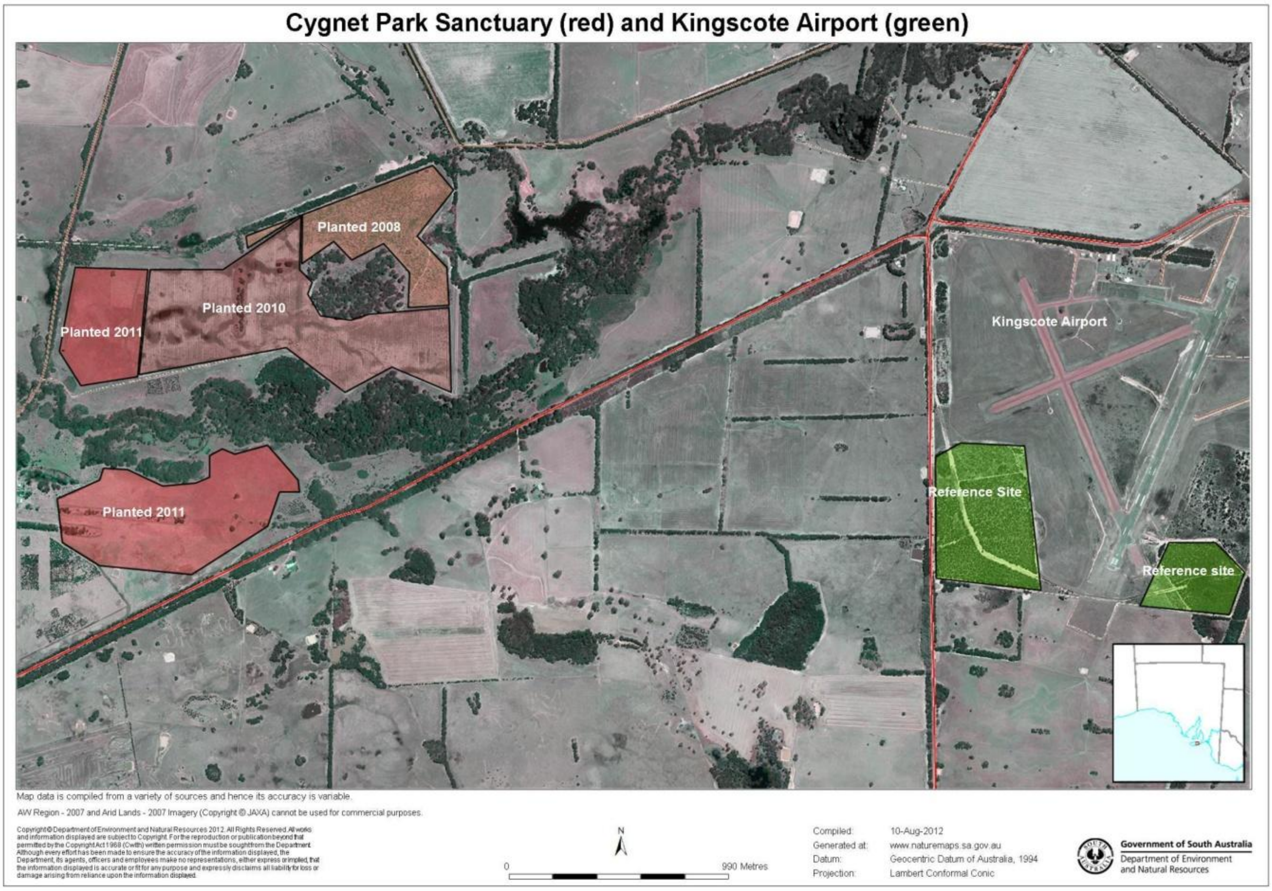

Fig. 1) Aerial photograph of the trial sites and the remnant reference site. Revegetation is shaded reds and remnant site shaded green.

Materials and methods

Development of pollinator communities

Six random walks, each of 1-2 h duration were performed in each of the study sites. The researcher walked randomly through the study site from one flowering plant to the next. During these walks data on: total floral visitor number per plant and individual insect's floral visitation rate were recorded and floral visitor specimens were collected. Total floral visitor number per plant was measured either by taking an instantaneous count of floral visitors (identified to order) for plants with more abundant flowers or by watching the plant for a given time period and recording the number of visitors (identified to order) during that time period. Individual insects floral visitation rate was recorded by watching individual insects, starting a stop watch, then counting how many flowers they visit until they were no longer able to be followed or >10 flowers had been visited, then recording the number of flowers and the time taken. Specimens were collected opportunistically during the random walks. Specimen collection aimed to maximise the coverage of species present rather than relative abundance, hence they were not taken randomly. All specimens collected were sorted into morphospecies based on obvious morphological characteristics. Results presented here are based on these morphospecies. Specimens are in the process of having their CO1 mitochondrial DNA sequenced and all analysis will be repeated with this additional information once complete. However, any differences detected based on the morphospecies are only likely to be amplified if cryptic species are found to exist within these morphospecies.

Plant – floral visitor interaction networks

Plant – floral visitor networks were created from the specimen and visitation count data. Networks were created using the Bipartite package within the statistical program R. For the specimen network, the insect morphospecies and plant it was collected on were used to assign the network connections. The visitation count network was calculated using the observed floral visitations within the field (with insects sorted to order with the exception of *Apis mellifera*) and the interaction strength calculated on the number of observed interactions.

Reproductive outcomes of plants

The reproductive outcome for the plants was measured via seed production. To assess the importance of insect pollinators to a group of chosen plant species, mesh pollinator exclusion bags were placed over random branches on a sample of each species prior to flowering. Once flowering had ceased, identical bags were placed over a similar branch on the same plant. To assess the difference in seed production between the different trial sites, after flowering, branches of selected species were bagged in the same way as for the pollinator exclusion experiments. Once the seeds had matured, the branches, with bags still on, were cut from the plant and taken back to the laboratory for processing. In the laboratory, seeds were thrashed from their pods, sifted and then aspirated until clean. Seeds were then spread over a white piece of card and photographed and the numbers of seeds counted using particle counting software.

In addition to the assessment of the reproductive outcomes of the *in situ* plants, potted plants will be used to control for differences in age of plants, soil type, soil nutrients, soil moisture etc. This will allow for differences in seed production to be more narrowly attributed to differences in pollination rates at different sites. Forty plants of each of seven plant species (*Davesia asperula, Eremophila behriana, Eutaxia diffusa, Lasiopetalum baueri, Leptospermum continetale, Pultenaea daphnoides* and *Solanum simile*) have been grown out in large pots. Plant species were chosen to cover a variety of flower types. These potted plants will be placed in each of the trial sites during flowering, then returned to the nursery as soon after as possible to minimise differences in rainfall, sunshine etc between sites. Due to the time to grow the plants to a flowering stage, only two plant species were able to be deployed to the trial sites in 2012. These were *Lasiopetalum baueri* and *Solanum simile*.

Results

Development of pollinator communities

250 insect samples were collected from 21 plant species. Despite the uniform sampling effort, different numbers of specimens were collected from the different trial sites; likely due to differences in the absolute abundance of insects at each site. As there is usually a direct relationship between species diversity and sample number, floral visitor diversity at the different sites was compared using rarefaction. The rarefied species accumulation curves showed a clear increase in floral visitor diversity with age of revegetation (see Figure 1). They also showed that the 4 year old planting has a similar diversity to the remnant site.

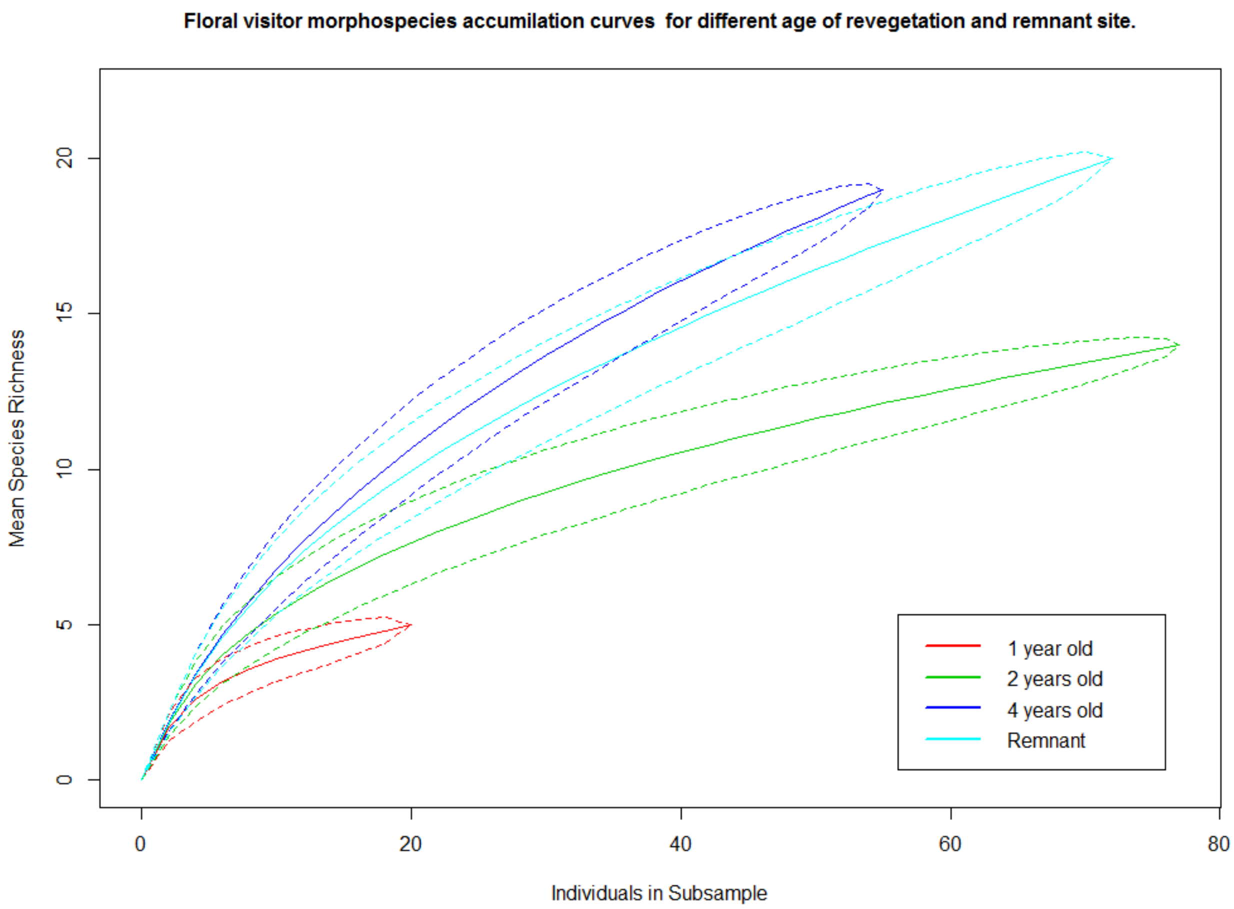

Fig. 2) Floral visitor morphospecies accumulation curves for the different aged revegetation and the reference site. Note the similarity between the 4 year old site and remnant. Errors around lines are the S.E. of the iterations, not true S.E. of the means.

Plant-floral visitor interaction networks

The specimen based plant – floral visitor interaction networks showed increased network complexity with age (see Fig. 3). Network statistics showed a general trend towards increased complexity with age of revegetation, especially plant 'Vulnerability' which decreased dramatically with age of vegetation (increased score = decreased vulnerability) (see Table 1). This indicates the increase in complexity was mainly attributable to increased network size and the increase in floral visitor species with age, rather than systematic differences in the types of interactions.

Table 1) Network statistics calculated for the above Floral visitor – plant interaction networks. Increased network complexity is indicated by increased Number of floral visitor morphospecies, Vulnerability and Robustness.

Network statistic	1 Year old	2 Years old	4 Years old	Remnant
Number of Floral visitor Species	5	14	19	20
Number of flowering plant species	6	8	5	8
Floral visitor to plant species ratio	0.83	1.75	3.8	2.5
Vulnerability (Effective floral visitors per plant species)	2.4	3.54	8.32	4.48
connectance	0.33	0.27	0.30	0.21
links per species	0.91	1.36	1.21	1.21
Extinction slope plants (higher score = higher rate of plant extinction per floral visitor extinction)	1.7	1	1	1
Robustness floral visitors (Closer to 1 = more robust floral visitor community is to plant extinctions)	0.64	0.63	0.59	0.57
Robustness plants (Closer to 1 = more robust plant community is to floral visitor extinctions)	0.62	0.77	0.74	0.74

The plant – floral visitor networks based on the observed interaction count data showed dramatic increase in network size (total number of observed interactions) with age of revegetation. This reflects the increased flower production as the plants grow and also a coinciding increase in floral visitors. These networks also clearly show that *Apis mellifera* are the dominant floral visitors both within the revegetation and remnant sites. Interestingly, the network sizes of the two and four year old revegetation surpassed that of the remnant site. This phenomenon was probably due to the high relative abundance of *Melaleuca gibbosa* and *Leptospermum continentale* within the revegetation sites compared to the remnant site. Due to the timing of the observations, late in the season, these two species were the main species still flowering. Hence, this phenomenon would not be expected at other times when different plant species are flowering.

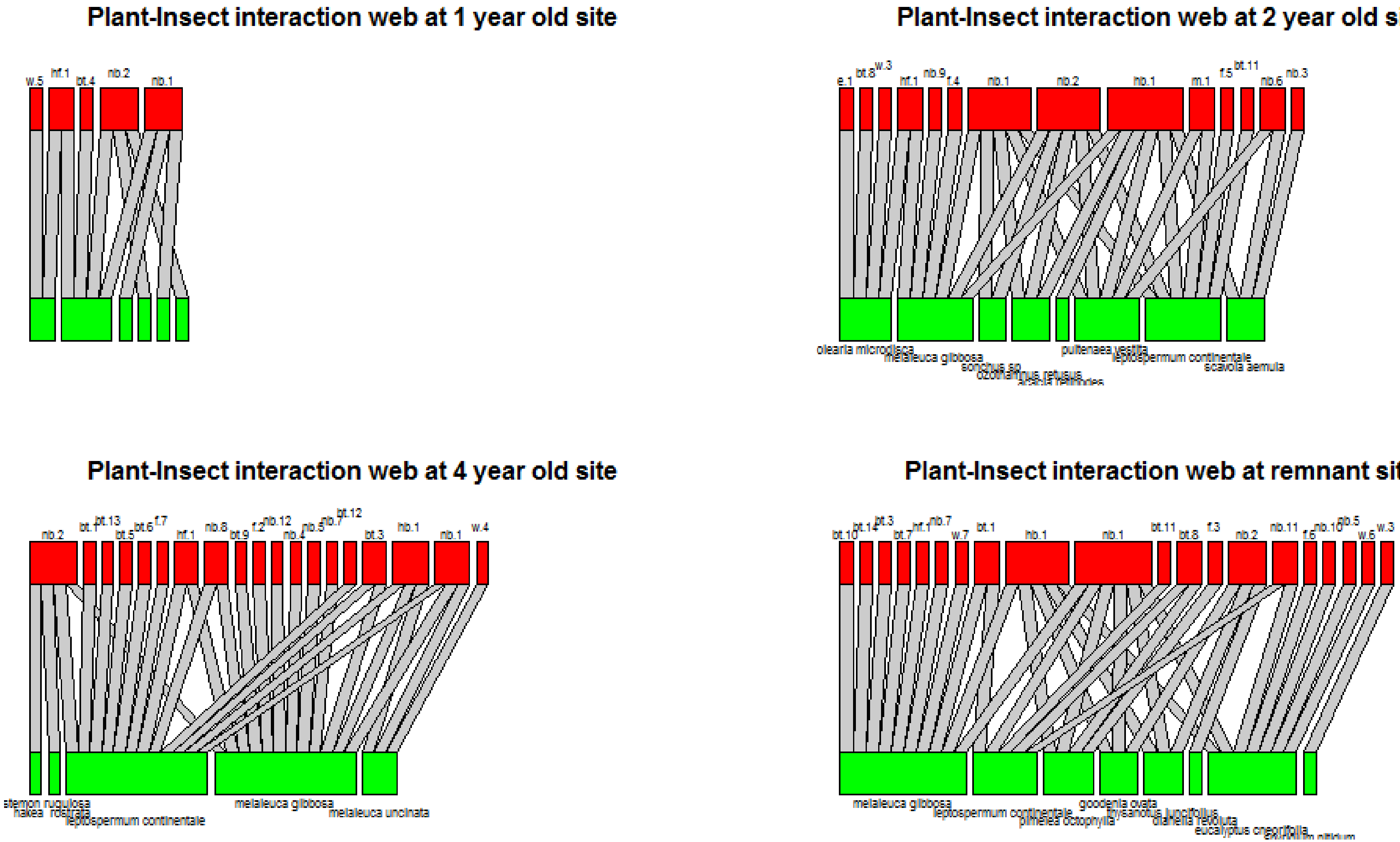

Fig. 3) Floral visitor – plant interaction networks for the 1, 2 and 4 year old revegetation and the remnant site based on the specimens collected and the plant they were collected from. Each link represents a unique interaction and box size indicates number of species that species interacts with. Only unique interactions were included as the sampling method was biased towards unique interactions rather than relative abundance.

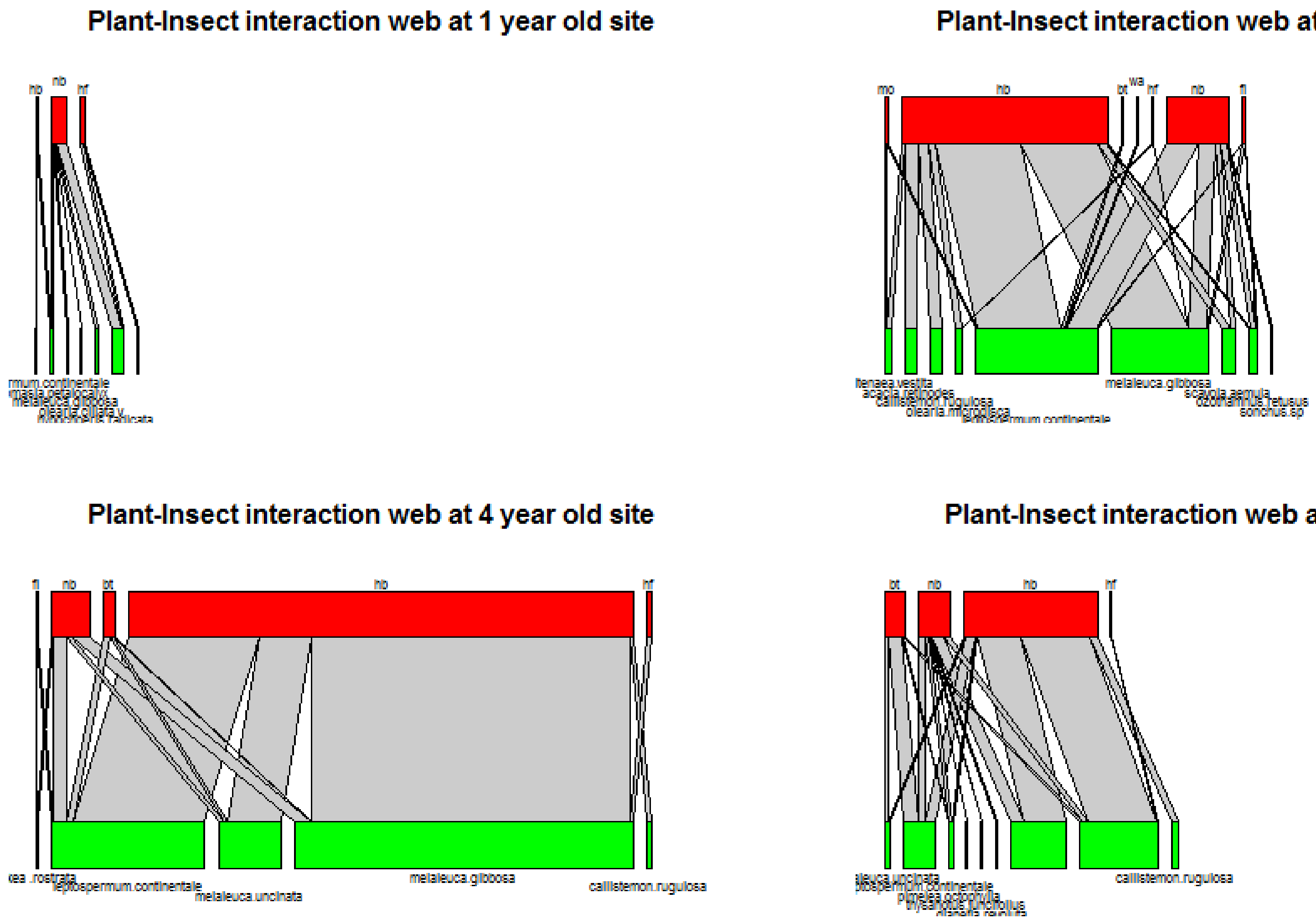

Fig. 4) Floral visitor – plant interaction networks for the 1, 2 and 4 year old revegetation and the remnant site based on field observations of interactions. Unlike the networks based on the specimens, these networks incorporate a measure of the abundance of interactions, but at the expense of insect identification precision. Note the increase in network size with age of revegetation and the dominance by *Apis mellifera* ("hb" on graphs).

Reproductive outcomes of plants

The pollinator exclusion experiments showed that *Pultenaea daphnoides, Eutaxia diffusa, Lasiopetalum baueri*, *Pultenaea tenuifolia,* all require insect pollination to maximize yields. *Solanum simile* and *Pomaderris halmaturina* showed no decrease in yield with pollinator exclusion. Results for *Olearia microdisca, Melaleuca uncinata, Leptospermum continentale* are still to be analysed.

Table 2) Results of the pollinator exclusion experiments. All yields are seeds number accept Solanum simile which is fruit numbers. ** indicates significance at 0.5 level.

Species	Yield exclusion	Yield Open Pollinated	P value	Statistical test
Pultenaea daphnoides	38.4 ± 32.6	525.8 ± 111	4.019e-05**	Paired - T
Eutaxia diffusa	2 ± 2.6	49.75 ± 38.8	0.03**	Paired - T
Pultenaea tenuifolia	0.25 ± 0.6	34.75 ± 21.96	0.007**	Paired - T
Pomaderris halmaturina	750 ± 379.3	656.2 ± 334.8	0.62	Paired - T
Lasiopetalum baueri	0.168 ± 0.35	0.335 ± 0.5	0.048**	Paired - T
Solanum simile	14.286±3.53	16.143±4.88	0.47	Paired - T

Comparisons of yields between revegetation and remnant site showed mixed results. No differences in yield between revegetation and remnant sites were detected for *Acacia pycnantha* or *Lasiopetalum baueri*. However, *Pultenaea tenuifolia* showed a higher seed yield in the revegetation than remnant and *Eutaxia microphylla* had a much higher seed yield in the remnant site than the two year old revegetation.

Table 3) Comparison of seed yields between plants in the remnant site and revegetation. ** indicates significance at 0.5 level, *indicates significance at 0.1 level.

Plant species	**Location**	**Yield Open Pollinated**	**95% CI**	**P value**			**Statistical test**
Acacia pycnantha	4 year old	10.84	9.08 - 12.6	0.99		0.99	TukeyHSD
	2 year old	10.99	7.49 - 14.5		0.99		
	Remnant	10.98	8.47 - 13.48			0.99	
Eutaxia microphylla	2 year old	2.07	0.25 - 6.54	0.05**			ANOVA
	Remnant	215.1	110.37 - 418.39				
Pultenaea tenuifolia	4 year old	34.75	12.8 - 56.71	0.07*			ANOVA
	Remnant	16.5	6.18 - 26.82				
Lasiopetalum baueri	4 year old	0.33	-0.16 - 0.83	0.66			ANOVA
	Remnant	0.266	0.134- 0.4				

Of the two species of potted plants placed within the revegetated and remnant sites (*Lasiopetalum baueri* and *Solanum simile)*, the exclusion experiments showed that *Solanum simile* did not require insect pollination and also was damaged by the unusually hot and dry spring, so was not used for analysis. *Lasiopetalum baueri* showed a significant difference (TukeyHSD, P adjusted = 0.013) between the seed production from plants placed in the one year old revegetation and the remnant site. No other significant differences between any other combinations of sites were observed, despite a general trend of increased seed production with age (see Fig. 5).

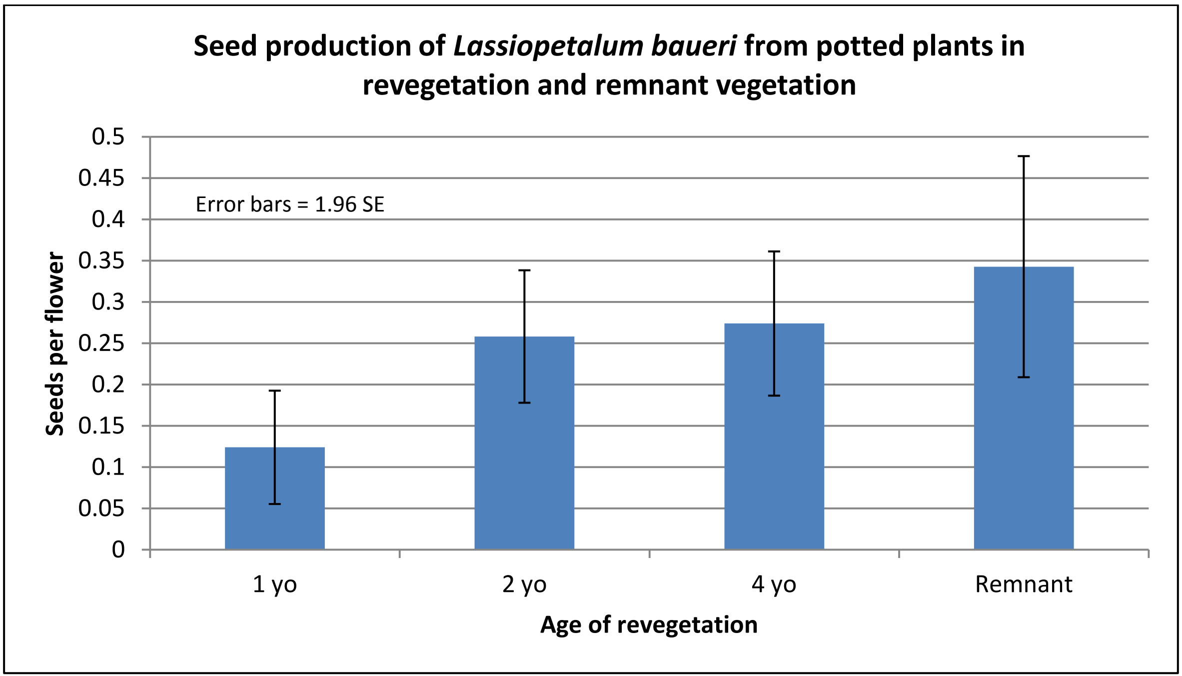

Fig. 5) Comparison of *Lasiopetalum baueri* seed yield per flower between potted plants placed in the different aged revegetation and the remnant site.

Discussion

The increase in floral visitor species richness with age of revegetation is a good sign for self-sustainability and resilience of the sites. More floral visitor species means more niches are being filled and potentially more pollination is occurring. Also, higher species richness means higher potential for niche overlap and resilience within the pollinator network. Interestingly, the floral visitor species richness of the 4 year old revegetation was very similar to the remnant site. This indicates that at Cygnet Park the floral visitor community is developing to an ecologically appropriate level quite quickly.

The specimen based floral visitor – plant interaction networks showed an increase in complexity, which was attributed mainly to the increased floral visitor species richness. This resulted in an increase of both the vulnerability and robustness measures with age of revegetation which indicates increased resilience with age of revegetation. The interaction networks based on the field observation graphically shows a large increase in network size with increased revegetation age reflecting the increased total abundances of the floral visitors and flowers. It also highlights the dominance of the floral visitor communities by *Apis mellifera* at all but the 1 year old site. The effect on the revegetation from this domination of the floral visitation community by an invasive species is unknown, but is likely to alter the development and trajectory of the plant community from that which would have occurred in its absence.

The pollinator exclusion experiments reiterated the importance of insects as pollinators of many plant species. It also served to verify the necessity of insect pollinator for the selected species to be used in the potted plant experiments. The mixed results in the seed yields between the revegetation and remnant site show the importance of investigating a variety of plant species. It is expected that different plant species will respond differently to the developing conditions of the revegetation. If the plant species diversity of the revegetation is to be maintained, it is important that the pollination requirements of all or most of it constituent plant species are met, otherwise those species with poor seed production may 'drop out' of the system. The 'poor performers' within a system may give considerable information as they can highlight what is missing or what may require extra intervention.

The potted plants experiment showed a significant difference in yield between plants placed in the 1 year old revegetation and those placed in the remnant site. This result gives proof of concept for the continuation and scaling up of the potted plant component of the project. The overall yields of *Lasiopetalum baueri* were quite low in the order of 0.1 – 0.4 seeds per flower (each flower is capable of producing multiple seeds). *Lasiopetalum baueri* has a specialist flower morphology indicating it requires 'buzz' pollination. This low seed yield may be indicative of a general lack of its required specialty pollinators.

The combined results from the project indicate that the plant and pollinator communities within the revegetation at Cygnet Park Sanctuary are developing quite quickly and likely to meet or exceed many of the ecological characteristics of the remnant site. However, as the characteristics measured are highly variable and seasonally dependant, repetition of the trials over multiple years is necessary before firm conclusions can be drawn. Also, repetition of the trials over multiple years will allow the comparison of the same sites at different ages as well as different sites of different ages, allowing greater assessment of the generalisation of the findings.

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Acknowledgments

This project is made possible by the generous funding of the Nature Foundation SA as well further funding from the University of Adelaide, the Native Vegetation Council and an ANZ Charitable Trusts: Holsworth Wildlife Research Endowment Grant.