

**The world without birds:
An experimental test of the ecological significance
of pollinating birds for plant communities**

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Thesis presented in fulfilment of the requirements for the degree of
Master of Science in the Faculty of Science at Stellenbosch University



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March 2017

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

March 2017

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Aan my ouers

Soos uit 'n kaf hang skigte lig
die kloof nog vol – dan
word dit onleesbaar stil, suikerkanne,
riet, die ingehoue val van voëlgeroep
asof iemand peins oor 'n gedig.

Uit *Luiperdgedig* deur Marlene van Niekerk

Uit die bundel *Kaar*. Human & Rousseau, 2013.

Abstract

Since pollinators are declining in many parts of the world, it is vital to understand the consequences of pollinator loss. How will plant communities be affected? If pollinators are keystone mutualists, then their loss may cause cascading extinctions with far-reaching consequences for communities. Alternatively, loss of a pollinator may have little effect, since many pollination systems are generalised and the typical structure of plant-pollinator interaction networks suggest flexibility and functional redundancy. Yet relatively few studies have assessed the effects of pollinator loss in the field.

This thesis considers the consequences of bird pollinator loss for plant communities in the megadiverse Cape Floristic Region of South Africa, where bird pollinators are particularly important. I constructed 20 x 20 m cages to exclude birds, but not insects, from six plant communities in the Jonkershoek Nature Reserve near Stellenbosch. Communities without birds were compared to neighbouring communities with birds. The scale of the experiment made it possible to assess the demographic and community-level consequences of pollinator loss.

Many Cape Proteaceae are pollinated by birds and reliant on seeds to regenerate after periodic fires. One such a species is the showy *Leucospermum lineare* (Proteaceae), the focus of Chapter 2. *Leucospermum lineare* was pollinated by Cape Sugarbirds. In the absence of birds, nectar volume and concentration increased, which seemed to amplify visitation by nectar thieving ants. Seed set was maintained without birds through autonomous selfing. However, seeds from bird-excluded plants had lower seed viability in laboratory trials. In the field, post-fire seedling recruitment was reduced significantly in plots where birds had been excluded, most likely as a result of inbreeding. *Leucospermum lineare* populations may thus decline without birds.

A number of other bird-pollinated plant species in the study communities also saw declines in fecundity when birds were excluded (Chapter 3). *Protea neriifolia* (Proteaceae), a dominant plant in many of the communities, was mostly pollinated by Cape Sugarbirds and its seed set declined by half in the absence of birds. *Erica plukenetii* (Ericaceae) was pollinated by Orange-breasted Sunbirds and its fruit set was reduced substantially without birds. In *Mimetes cucullatus* (Proteaceae) seed set collapsed in the absence of birds. In contrast, *Protea repens*, also a dominant species, and *Protea nitida* maintained their seed set, likely thanks to insect pollination. Seedling recruitment of *Protea* species in the presence of bird pollinators was well above adult replacement levels after a fire swept the six year old vegetation; *Protea* seedling recruitment in the absence of birds could not be assessed.

Overall my results suggest that extinction of bird pollinators may reduce the biomass and diversity of plant communities in the Cape Floristic Region.

Plant fecundity can also be reduced by animals that eat flowers. I quantified the effects of florivory by rodents and baboons on *Protea neriifolia*. Seed cone production was reduced substantially. Striped mice, *Rhabdomys pumilio*, climbed up to 1.6 m high into *P. neriifolia* plants to feed on inflorescences. Rodents also severed inflorescences by gnawing through subtending stems.

Opsomming

In die lig van die wêreldwye afname in die volopheid van bestuiwers is dit noodsaaklik om die gevolge van dié afname te verstaan. Hoe gaan plantgemeenskappe geaffekteer word? Enersyds, indien bestuiwers hoeksteen-mutualiste is, kan die verlies van bestuiwers tot trapsgewyse uitsterwings lei, met verreikende gevolge vir gemeenskappe. Andersyds kan die verlies van bestuiwers weinig impak maak, aangesien baie bestuiwingsisteme gegeneraliseerd is en die tipiese struktuur van plant-bestuier-interaksienetwerke dui op buigzaamheid en oortolligheid van funksie. Relatief min studies het egter tot dusver die effekte van bestuierverlies in die veld ondersoek.

Hierdie tesis handel oor die gevolge van die verlies van voëlbestuiwers vir plantgemeenskappe in die hiperdiverse Kaapse Floristiese Streek, waar voëlbestuiwers buitengewoon belangrik is. Hokke van 20 x 20 m is in die Jonkershoek Natuurreservaat buite Stellenbosch opgerig om voëls (maar nie insekte nie) uit te sluit. Gemeenskappe sonder voëls is vergelyk met gemeenskappe met voëls. Die skaal van die eksperiment het dit moontlik gemaak om die gemeenskapsvlak- en demografiese gevolge van bestuierverlies te ondersoek.

Baie Kaapse Proteaceae word deur voëls bestuif en is afhanklik van sade om na periodieke brande te regenerere. Die pronkerige *Leucospermum lineare* (Proteaceae) is 'n voorbeeld van só 'n spesie en is die fokus van Hoofstuk 2. *Leucospermum lineare* is deur Kaapse suikervoëls bestuif. In die afwesigheid van voëls het nektarvolume en -konsentrasie toegeneem, wat oënskynlik besoeke van nektar-stelende miere laat styg het. Danksy selfbestuwing het saadproduksie nie afgeneem sonder voëls nie. Die kiemkrag van sade afkomstig van plante waar voëls uitgesluit is, was egter laer in laboratoriumproewe. In die veld was saailingvestiging beduidend laer in plote waar voëls uitgesluit is, bes moontlik weens inteling. Gevolglik kan *Leucospermum lineare*-populasies kwyn sonder voëlbestuiwers.

Die fekunditeit van 'n aantal ander voëlbestuifde plantspesies in die studiegemeenskappe het ook afgeneem toe voëls uitgesluit is (Hoofstuk 3). *Protea neriifolia* (Proteaceae), 'n dominante spesie in baie van die gemeenskappe, is hoofsaaklik deur Kaapse suikervoëls bestuif en saadproduksie het met die helfte verminder sonder voëls. *Erica plukenetii* (Ericaceae) is deur Oranjoborssuikerbekkies bestuif en vrugproduksie het beduidend verminder in die afwesigheid van voëls. *Mimetes cucullatus* (Proteaceae) het sonder voëls byna geen sade geproduseer nie. Daarenteen kon *Protea repens*, ook 'n dominante spesie, en *Protea nitida* wel sade produseer sonder voëls, heel moontlik danksy insekbestuwing. In die aanwesigheid van voëlbestuiers was die saailingvestiging van *Protea*-spesies, nadat die sesjaar oue veld afgebrand het, aansienlik hoër as die vervangingsvlak vir volwassenes. *Protea*-saailingvestiging in plote sonder voëls kon nie gemeet word nie. In geheel gesien dui hierdie resultate aan dat die uitsterwing van voëlbestuiers die biomassa en diversiteit van plantgemeenskappe in die Kaapse Floristiese Streek kan verminder.

Plante se fekunditeit kan ook verminder word deur diere wat blomme eet. Die effekte van florivorie deur knaagdiere en bobbejane op *Protea neriifolia* is ondersoek. Die produksie van saadkoppe het beduidend verminder. Streepmuise, *Rhabdomys pumilio*, het tot 1,6 m hoog in *P. neriifolia*-plante geklim om op bloeiwyses te voed. Knaagdiere het ook bloeiwyses afgesny deur deur stingels te knaag.

Acknowledgements

First of all, I want to express my gratitude to my supervisor, Prof. Anton Pauw. I can hardly imagine a better supervisor and mentor. My thanks also to my co-supervisor, Prof. Cang Hui, for his input.

Various people helped with fieldwork. Thank you to Alheit du Toit, Jeroen van der Merwe, Robyn Kadis, Wilmarie Uys, Barbara Seele, Skye Butterson, Maria Loreto Castillo, Shereé Muller, David Marais, Ferdinand Botha, Christiaan Kriek, Anina Coetzee, Genevieve Theron, Stuart Hall and Ross Coppin. Special thanks to Christopher Bosc, Nanike Esterhuizen, Natasha Mothapo and Matthys Strydom for braving the black landscape to harvest *Protea* cones.

In the laboratory Vianca Van Rooyen, Celeste van Graan, Kyle Kulenkampff and Chrizelle Jordaan helped to count the many *Protea* seeds. Prof. Henk Geertsema kindly assisted with insect identifications, Dr Simon van Noort identified bees and wasps, and Natasha Mothapo helped with ant identification.

Project funding was provided by the National Geographic Society, the National Research Foundation and Stellenbosch University's Harry Crossley Bursary.

I acknowledge CapeNature for granting permission to conduct an unusual field experiment in the Jonkershoek Nature Reserve.

Thank you to my lab mates, Christopher Bosc, Barbara Seele, Ethan Newman, Jurene Kemp, Anina Coetzee, Marinus de Jager, Hannes Wiese, James Rodger, Willem Augustyn, Caroli de Waal and Janneke Aylward, for all the discussions, good cheer and field adventures.

I would like to convey my heartfelt gratitude to Nánike Esterhuizen, Jaco Janse van Vuuren, Tone Berg, Christiaan Kriek, Maria Loreto Castillo and David Marais for their friendship and support. My deepest gratitude goes to my parents, Jan and Louise, and my brothers, Jan and Ferdinand, for all their love and support.

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Chapter 1 General introduction

Mutualistic interactions like those between flowers and their pollinators are vital strands in the web of life (Bascompte & Jordano 2007; Vázquez et al. 2009). This came to be understood because the beauty and intricacies of plant-pollinator interactions have long fascinated people (e.g. Darwin (1862)). In recent years concern about that “much more insidious kind of extinction: the extinction of ecological interactions” (Janzen 1974) has also spurred research.

There is evidence from around the globe that disruption of pollination and seed dispersal threatens biodiversity and ecosystem function (Aslan et al. 2013; Valiente-Banuet et al. 2015). Declines in pollinator abundance in many parts of the world has caused much concern (Biesmeijer et al. 2006; Potts et al. 2010; Potts et al. 2016a). More than 80% of flowering plants are estimated to be pollinated by animals and many agricultural crops rely on pollinators (Aizen et al. 2009; Ollerton et al. 2011). The major interacting global change pressures behind pollinator declines are climate change, landscape alteration, agricultural intensification, non-native species, and the spread of pathogens (González-Varo et al. 2013; Potts et al. 2016b). Pollination is a variable and complicated phenomenon and pollination failure can occur at various different steps of the process and due to factors operating on a range of scales, from below the pistil to beyond the population (Wilcock & Neiland 2002).

What might the consequences of pollinator loss be? If pollinators are keystone mutualists their loss will lead to trophic cascades (Cox et al. 1991; Christian 2001; Anderson et al. 2011). Alternatively, pollination systems may be buffered against pollinator loss, considering their widespread generalisation (Waser et al. 1996); however, in certain regions, like the Cape of Africa, pollination systems are highly specialised (Pauw & Stanway 2015). Some of the typical structural properties of plant-pollinator interaction networks, such as nestedness, provide functional redundancy and predict that pollination networks will be resilient to species extinction in theory (Memmott et al. 2004; Bascompte & Jordano 2007; Kaiser-Bunbury et al. 2010). The few empirical field studies indicate that networks have been flexible to historical disturbance, but that rare, specialised interactions are particularly vulnerable and pollinator loss can have network-wide effects on plant reproduction and community composition (Pauw 2007; Aizen et al. 2012; Burkle et al. 2013; Brosi & Briggs 2013; Lundgren et al. 2016).

Birds are prominent pollinators in various parts of the world, with over 920 species acting as pollinators (Şekercioğlu et al. 2016). Major groups include hummingbirds (Trochilidae) in the

Americas, honey-eaters (Meliphagidae) in Australia and the Pacific, sugarbirds (Promeropidae) in southern Africa, and sunbirds (Nectarinidae) in Africa, Asia and Australasia. Flowers pollinated by birds are often robust, reddish in colour, have large volumes of dilute nectar and lack scent, the pollination syndrome known as “ornithophily” (Van der Pijl 1961). Pollination interaction networks involving birds have the same properties as mutualistic networks in general (García 2016). As with pollination systems in general, bird pollination has been disrupted significantly in parts of the world (Şekercioğlu et al. 2016), e.g. southern Australia (Paton 2000) and New Zealand (Anderson et al. 2011).

Some of the most notable studies on pollinator loss have considered bird pollinators. Anderson and co-workers (2011) showed that functional extinction of bird pollinators can have cascading effects: the density of a bird-pollinated shrub decreased on the mainland of New Zealand, but not on islands where birds persisted. Likewise, the extirpation of bird pollinators on the island Guam caused the reproductive failure of two tree species (Mortensen et al. 2008).

My thesis is set in the Cape Floristic Region (CFR) of South Africa, a remarkable area renowned for its outsize biodiversity (Alsopp et al. 2014). The fynbos vegetation of these parts are fine-leaved, fire-prone shrublands which typically grow on nutrient-poor soils in a Mediterranean climate. Many unique and specialised pollination systems have evolved here and the region is a model system for research on pollinator-driven plant diversification (Anderson et al. 2014). Bird pollinators play a central role in the CFR: over 300 plant species are pollinated by only four specialist nectar-feeding bird species (Rebelo 1987). This represents approximately 5% of the plant species, which is comparable to other parts of the world such as New Zealand, but lower than Australia (15%) (Şekercioğlu et al. 2016). In the CFR, birds pollinate many of the dominant shrubs from the family Proteaceae, as well as numerous geophytes and 15% of the superbly species-rich genus *Erica* (Rebelo et al. 1985; Rebelo 1987; Anderson et al. 2014). There are four species of obligate nectar-feeding birds in the CFR: the iconic Cape Sugarbird (*Promerops cafer*) and three sunbirds, namely the Malachite Sunbird (*Nectarinia famosa*), Orange-breasted Sunbird (*Anthobaphes violacea*) and Southern Double-collared Sunbird (*Nectarinia chalybea*).

These charismatic and crucial birds face numerous threats. Earlier work has shown that habitat fragmentation, urbanization, road traffic, alien plant invasion and honeybee farming reduce the abundance of nectar-feeding birds (Fraser & Crowe 1990; Pauw 2004; Geerts & Pauw 2010; Geerts & Pauw 2011; Pauw & Louw 2012). In addition, nectar-feeding birds are likely to be sensitive to climate change because their abundance is strongly influenced by fires (Geerts et al. 2011) and the frequency of fires is expected to increase with climate

change (Altwegg et al. 2014). At present Cape Sugarbird and Orange-breasted Sunbird populations are quite large, but the range of Cape Sugarbirds has declined by 15% and that of Orange-breasted Sunbirds by 13% in the last two decades (Lee & Barnard 2015). However, it is not clear whether this decline is because of climate change (Lee & Barnard 2015). In terms of physiological limits, Cape Sugarbirds and Orange-breasted Sunbirds are not particularly vulnerable to climate change (Milne et al. 2015). However, climate envelope modelling suggests that the ranges of Cape Sugarbirds and Orange-breasted Sunbirds may decline by 27% and 20% respectively by 2050 as a result of climate change (Simmons et al. 2004).

Fire takes centre stage in fynbos ecology and plants have various ways of coping with the periodic blazes (Kraaij & Van Wilgen 2014). Considering the fire-dominated life history of the Cape Proteaceae, this important group is predicted to be particularly vulnerable to extinction by pollinator loss (Bond 1994). Many members of the family are strongly seed limited, since they have few, large seeds, rely on seeds to regenerate after fire and seedling densities are low. Many species also do not form persistent soil seedbanks (Bond 1994). Adding to the extinction risk is the fact that numerous species are specialised in terms of pollination, e.g. relying on bird pollinators (Johnson 2015; Schmid et al. 2015).

What consequences will pollinator loss have for plant demography? Pollinators may functionally replace each other if rewiring of pollination networks can take place (Kaiser-Bunbury et al. 2010). In the absence of pollinators, selfing may also provide reproductive assurance, but inbreeding depression can be substantial (Robertson et al. 2011). Not many studies have considered the longer term demographic effects of pollinator loss (but see Anderson et al. (2011); Van Etten et al. (2015)).

The field of pollination ecology also lacks a proper fieldwork-based understanding of the consequences of pollinator loss for plant communities. I set out to contribute to fill this gap by conducting an unprecedented, large scale field experiment in the CFR. I simulated the extinction of bird pollinators by erecting 20 x 20 m bird exclusion cages to exclude birds from entire plant communities. This community-level scale enabled me to answer questions that typical flower-level or plant-level exclusion studies cannot address. I conducted my study near the town of Stellenbosch in the Jonkershoek Nature Reserve, an area with a long history of ecological research (Van Wilgen et al. 2016).

For **Chapter 2** of this thesis, I took a demographic perspective by conducting an in-depth study of the consequences of bird pollinator loss for *Leucospermum lineare* (Proteaceae), a showy, bird-pollinated species that is representative of many Cape Proteaceae in terms of its life history and pollination mode. I address the following specific questions: 1) Does bird loss

affect nectar rewards and insect flower visits? 2) Can *L. lineare* produce seeds in the absence of bird pollinators? 3) Does the viability of seeds pollinated by birds differ from that of seeds resulting from insect or self-pollination? 4) Ultimately, is the re-establishment of *L. lineare* after fire affected by bird loss?

How will bird pollinator extinction affect plant communities? In **Chapter 3**, I investigate the effects of bird pollinator loss on the fecundity of communities of bird-visited plants. I consider the following questions: 1) How specialised in terms of pollinators are plants with bird-adapted flowers? 2) How does the exclusion of bird pollinators affect the fecundity of bird-visited plants? 3) What are the demographic consequences of bird pollinator loss and a short fire return interval for plants? 4) How will plant community composition be affected by bird pollinator extinction?

Flowers with their advertisements and rewards attract not only pollinators, but also animals that steal nectar and devour flowers (McCall & Irwin 2006). In **Chapter 4**, I describe the effects of florivory by marauding baboons and climbing rodents on the fecundity of the dominant shrub *Protea neriifolia*.

Chapter 5 of this thesis deals with general conclusions from my work.

Note: Since I have written the data chapters of this thesis as separate journal articles, there is necessarily some overlap between the introductory sections of the different chapters.

Chapter 2: Loss of bird pollinators reduces recruitment via inbreeding depression in the fynbos shrub *Leucospermum lineare* (Proteaceae)

Abstract

In light of global pollinator declines, it is vital to understand how pollinator loss affects plant demography. One possible outcome is that plant populations will decline because of pollinator loss. Alternatively, lost pollinator function may be replaced if plant-pollinator interactions can rewire, especially when floral rewards accumulate. In the absence of pollinators, autonomous selfing may offer reproductive assurance for some plants, but inbreeding depression can be substantial. Some studies have considered the effect of bird pollinator declines on plant demography, but not in controlled field experiments. Here we use a community-level field experiment to assess the consequences of bird pollinator extinction for *Leucospermum lineare* (Proteaceae), a showy, bird-pollinated shrub in the Cape Floristic Region of South Africa. We excluded birds, but not insects, from plant communities with 20 x 20 m exclusion cages and compared the birdless communities to open communities nearby, at three different study sites. We monitored the effects on nectar reward, flower visitors, seed set, seed viability in laboratory trials and post-fire seedling recruitment. Nectar accumulated in the absence of birds and seemed to increase visitation by nectar thieving ants. *L. lineare* maintained seed set without bird pollinators through autonomous selfing, but seed viability in laboratory tests declined somewhat. Post-fire seedling emergence was significantly lower inside the bird exclosures, likely as a result of inbreeding depression. This difference between treatments remained substantial, but reduced over time, likely due to density induced mortality in the open and shade treatments. Loss of bird pollinators may thus ultimately cause population declines in *L. lineare*. Many other Cape Proteaceae with similar pollination mode and life history may respond in the same way.

Introduction

The decline of pollinators in many parts of the world is cause for concern (Potts et al. 2016a). Understanding how pollination affects plant demography is thus vital. When will disruption of pollination lead to plant population declines? Plant species rely on pollinators to varying degrees, depending on the plant's breeding system and its demographic dependence on seeds (Bond 1994). Furthermore, the long term consequences of varying pollination success can depend on how density dependence affects plant demography, yet few studies consider this (Price et al. 2008).

The effect of losing a pollinator depends on how generalised a plant species is and whether pollinators can replace one another. Many plant species are apparently generalised (Waser et al. 1996), however, the relative importance of different flower visitors for seed production is not often assessed (but see e.g. Fang et al. (2012), Johnson & Pauw (2014)). When a pollinator is lost from a community, its function may in theory be replaced if some of the remaining pollinators change their visitation patterns, i.e. rewiring of the pollination network (Kaiser-Bunbury et al. 2010; MacLeod et al. 2016). Such shifts in visitation are especially likely if the removal of a pollinator increases the availability of floral rewards. Flower visitors may compete for nectar (Brown et al. 1981; Hanna et al. 2014), yet the effects of such competition among pollinators are relatively unknown (but see Brosi and Briggs (2013)).

When pollinators are scarce or absent, autonomous self-pollination can allow plants to maintain seed set, thus providing reproductive assurance (Eckert et al. 2010). However, selfing may come at a substantial demographic cost if inbreeding depression is high (Van Etten et al. 2015). Yet inbreeding depression is seldom assessed, pollination studies typically measure only seed set. Another shortcoming of considering just seed set is that population growth rate is not necessarily well correlated with seed set. Many species may be long-lived, have large seed banks, or have high levels of density dependent mortality (Saatkamp et al. 2014).

Some of the most notable studies of the demographic consequences of pollinator loss have been on birds. Anderson et al. (2011) showed that functional extinction of bird pollinators can have cascading effects: the density of an ornithophilous shrub decreased on the mainland of New Zealand, but not on islands where birds persisted. Similarly, the extirpation of bird pollinators on the island Guam caused the reproductive failure of two tree species (Mortensen et al. 2008).

Bird pollinators are particularly important in the megadiverse Cape Floristic Region (CFR) of South Africa (Anderson et al. 2014). At least 300 plant species are pollinated by only four specialist nectar-feeding bird species (Rebelo 1987). These birds are threatened by habitat fragmentation, urbanisation, invasive alien plants and increased fire-frequency (Fraser & Crowe 1990; Pauw 2004; Geerts et al. 2011; Pauw & Louw 2012). Many members of the Proteaceae, a dominant family of the region, are bird-pollinated (Rebelo 2001; Collins 1983; Schmid et al. 2015).

In this study, we assess the consequences of pollinator loss for *Leucospermum lineare* R. Br. (Proteaceae), a showy, ornithophilous species. It is predicted to have a high demographic dependence on seeds, since adults are killed by periodic fires, and is expected to produce few seeds and have low seedling densities (Rebelo 2001). The extent to which

seed production in *L. lineare* depends on bird pollinators is unknown: It may be pollinated by insects, and in addition might be able to self-pollinate, but inbreeding depression has not been assessed. In all these respects, *L. lineare* is representative of many of the Cape's Proteaceae (Rebelo 2001; Johnson 2015; Schmid et al. 2015; Treurnicht et al. 2016).

To simulate the extinction of bird pollinators, we conducted an unprecedented field experiment. We erected large cages to exclude birds from entire plant communities that contained *L. lineare*. This community-level scale enables us to answer questions that typical flower-level or plant-level exclusion studies cannot address. By assessing not only seed set, but also seed viability and post-fire recruitment, we attempt to study the longer-term consequences of pollinator loss for plant populations. The close proximity of different treatments in our experimental design also allows us to draw better comparisons than the studies of "natural experiments" on islands; e.g. plant communities in different treatments at a site were very similar and the vegetation type was the same across sites.

The aim of our study was to determine the ultimate effects of pollinator loss on the demography of *Leucospermum lineare*. Specifically, we asked: 1) Does bird loss affect nectar rewards and insect flower visits? 2) Can *L. lineare* produce seeds in the absence of bird pollinators? 3) Does the viability of seeds resulting from bird pollination differ from that of seeds resulting from insect or self-pollination? 4) Ultimately, is the re-establishment of *L. lineare* after fire affected by bird loss?

Methods

Study sites and species

We conducted our study in the Jonkershoek Nature Reserve near Stellenbosch, South Africa. The study area has a long history of ecological research (Van Wilgen et al. 2016). Three study sites were established in 4-year old Boland granite fynbos (Rebelo et al. 2006) on the valley's southwest-facing slopes, at altitudes of 404 – 467 m (Site 1: 33°59'18.60"S, 18°58'20.87"E; Site 2: 33°59'24.92"S, 18°58'26.97"E; Site 3: 33°59'28.39"S, 18°58'44.04"E). The study area burned in February 2009 and March 2015. The granite-derived soil supported dense stands of *Protea neriifolia* R. Br., *Protea coronata* Lam. and *Leucospermum lineare*. Plant species that co-flowered with *L. lineare* included *Mimetes cucullatus* (L.) R. Br., *Thesium* sp., *Ursinia paleacea* (L.) Moench., *Pseudoselago* sp., *Polygala* sp. and *Erica cerinthoides* L.

At each of the three sites, we established three different kinds of treatments in October 2013 (Fig. 2.1E). We excluded birds from one treatment by erecting a cage of 20 x 20 x 2.2 m, made from black plastic netting (Alnet Trawl 400/30) with a mesh size of 2 x 2 cm. The

netting of this “bird exclusion” treatment prevented all birds from entering, but allowed large insects to pass through. A second plot of 20 x 20 m was demarcated, but not enclosed in any way and served as “open” control. In order to separate the possible shading (and other) effects of the cages from the bird exclusion effect, we also established two “shade control” plots per site, which consisted of 2.2 m high roofs of netting with completely open sides to allow access to birds. These shade roofs were smaller (10 x 7 m) than the bird enclosures, so that birds would readily fly underneath them. Treatments were selected to have the same vegetation composition and were located at least 15 m from each other. At the initiation of the experiment, the caged and open plots contained estimated averages of 86 and 57 *L. lineare* plants respectively and the smaller shade plots contained an estimated average of 10 plants each. These plants had established from seed in 2009. Most of them flowered for the first time in September 2012, based on general age-to-flowering of the species (Rebello 2008).

Leucospermum lineare lineare (hereafter referred to as *L. lineare*) is endemic to a small part of the CFR and occurs on granite-derived clay soils at altitudes of 300 to 1000 m (Rebello 2001; Johnson 2015). The species is listed as “Vulnerable” in the Red List of South African Plants due to the effects of invasive alien species, flower harvesting and habitat loss (Rebello et al. 2015). *Leucospermum* flowers are hermaphrodite. Styles first serve a male function by acting as pollen presenters and thereafter serve the female function when stigmas become receptive. Seeds are released 6 weeks after flowering and dispersed by ants into their underground nests. Ant dispersers bury *Leucospermum* seeds at depths of up to 12 cm (Christian & Stanton 2004).

Nectar measurements

We measured the nectar of *L. lineare* at all three study sites between 8 and 10 November 2013, visiting one site per day. Nectar measurements were taken between 9:00 and 14:00. To control for possible time of day differences, the order in which data was collected from different plot types was varied across sites. We randomly selected six plants per plot (in the smaller shade plots with fewer than six open inflorescences we measured all open inflorescences) and measured the nectar of one inflorescence per plant. We randomly chose two open flowers (dehisced anthers and released style) on each inflorescence; old flowers towards the outside of the inflorescence were avoided. Inflorescences were selected to be of similar age. Nectar was extracted and the volume determined using graduated 40 microliter capillary tubes (Drummond Scientific Company, Broomall, PA, USA). We determined the sugar concentration of each sample with a 0 – 50% handheld refractometer (Eclipse, Bellingham & Stanley, Basingstoke, UK). The amount of sugar (mg) in a flower was calculated by multiplying the volume (μL) and concentration (%/100).

Flower visitor observations

To assess the effect of bird exclusion on the pollinator fauna of *L. lineare* we observed visits by insects and birds in our study plots between 22 – 28 October, 8 – 13 November and 23 – 25 November. Inflorescence abundance was determined by counting the number of open inflorescences of each species in each plot once during each observation period. An inflorescence was considered “open” as soon as some of its flowers were in anthesis, i.e. when pollen presenters separated from the perianths. An inflorescence was “finished” when all its flowers had opened and the central, youngest flowers no longer offered pollen. Flower visitors were observed simultaneously in the different plot types at a study site by two or three different observers. All flower visitors were recorded, but only visitors touching the reproductive structures of a flower were considered pollinators. Visitation rates were calculated as number of visits / number of inflorescences observed / hours of observation. We did not conduct pollinator observations on rainy or windy days. Birds were observed in the early morning (6:30 to 9:30) and late afternoon (16:30 to 18:00), when they were most active. Insects were observed during 20 minute observation periods, between 10:00 and 16:00. Insect flower visitors were caught in vials filled with 70% ethanol for identification. Voucher specimens of Hymenoptera are housed at the Iziko Museum in Cape Town and other insects at the Department of Botany and Zoology, Stellenbosch University. *Leucospermum lineare* was observed for bird visits for a total of 24.5 hours on 9 different days and for insect visits for a total of 8.5 hours on 4 different days.

We sampled ants on all the open inflorescences of randomly selected *L. lineare* plants in all study plots. Ants were located by systematically parting the styles, almost as one would look for ticks in a dog's fur. We counted the number of ants in each inflorescence, including ants fleeing down the inflorescence stem, and collected ants in 70% ethanol. During the first sampling period on 23 and 24 November 2013 we sampled 369 inflorescences from 250 different plants, catching a representative sample of ants for identification. In the second sampling period (29 November and 2 December 2013) we surveyed 184 inflorescences from 171 plants, collecting all ants. We gathered data once at each study site per sampling period, always sampling all plots at a site on a single day and alternating the time of day a particular plot type was sampled.

Pollination experiments

We assessed autogamy and pollen limitation by subjecting inflorescences in the “bird exclusion”, “shade control” and “open” plots at each site to two different pollination treatments in 2013. These inflorescence treatments were fully nested within the community treatments of “bird exclusion”, “shade control” and “open”. We applied each inflorescence treatment to one inflorescence on seven different plants. In a few cases, where there were

not enough flowering individuals in a plot, we used two or three different inflorescences per individual, applying a different treatment to each. Inflorescences were tagged with their treatment.

To test for autogamy, we excluded all pollinators by covering inflorescence buds in green organza mesh bags. To test for pollen limitation we hand-supplemented inflorescences with outcross pollen. Pollen was collected from inflorescences across all three sites. We picked one inflorescence per plant from 10 plants, about 75 m distant from the study plots. Inflorescences were kept indoors in water for 1 to 5 days to dehisce before the pollen was mixed together in a petri dish. We applied pollen with a paintbrush to the stigmas of open flowers of tagged inflorescences in the field. Since the flowers of *Leucospermum* inflorescences open sequentially, we hand pollinated each inflorescence three times over a period of about 14 days. All open flowers on an inflorescence were pollinated each time, so that older flowers received pollen twice or three times.

In order to determine the natural seed set in the community treatments of “bird exclusion”, “shade control” and “open”, we tagged unmanipulated inflorescences on separate plants as controls for the bag and hand-pollination treatments. Once flowering had finished we covered all tagged inflorescences with organza mesh bags on 3 December in order to catch the released seeds (technically achenes, hereafter “seeds”). We collected the inflorescences on 26 December 2013 and counted the number of seeds in each inflorescence.

To increase our sample size for the community treatments we also assessed inflorescences in 2014, but only control inflorescences, we did not add pollen or exclude pollinators with mesh bags. We tagged 8 to 16 inflorescences (total $n = 112$) in each community treatment at each site and counted the number of flowers per inflorescence (range 55 – 221, mean = 110, SD = 27, median 105, $n = 95$). We harvested seeds on 14 and 30 December 2014, in the same way as the previous year, and counted the number of seeds produced by each inflorescence. Since *Leucospermum* species have one ovule per flower, proportional seed set could be calculated as (number of seeds) / (number of flowers). The few inflorescences for which flowers were not counted were excluded from proportional seed set analyses.

To assess *L. lineare*'s ability to produce seeds via selfing, we calculated the Autofertility Index (Lloyd & Schoen 1992) as (seed set from autogamous selfing) / (seed set from hand outcross pollen addition), using the average values for each enclosure type at each site. We also calculated an autofertility value (AV) as (seed set from autogamous selfing)/(seed set from natural pollination).

Germination trials

Prior to the germination trials, the seeds were stored in paper envelopes in cool, dark, dry conditions for 6 months (2014 seeds) or 18 months (2013 seeds). We sorted the seeds by hand and excluded any with obvious external defects (e.g. hollow when squeezed) from the germination trials. Seeds harvested from different inflorescences were kept separate throughout, with ten seeds randomly selected from each inflorescence. If an inflorescence had fewer than ten seeds, we used all the seeds. Seeds were treated following the established protocol for *Leucospermum* (Brits 1990; Brits et al. 2014; Brits et al. 2015; G.J. Brits pers. comm.). We scarified seeds in 98% H₂SO₄ for 7 minutes while stirring slowly with a glass rod. Seeds were washed thoroughly under running water and allowed to air dry. Thereafter seeds were disinfected in water at 50 degrees Celsius for 30 minutes, before being soaked in 1% H₂O₂ for 24 hours. The pericarp and any remaining pieces of elaiosome were removed by gently rubbing seeds between fingers, before air-drying at room temperature. To prevent fungal attack, seeds were shaken in a plastic bag with Benomyl wettable powder 50% active ingredient until covered with a thin film of powder. We placed seeds in petri dishes on Whatman no. 1 filter paper, watering them with dH₂O as needed. Seeds were incubated in the dark at a temperature cycle of 8 h at 21 degrees C, 16 h at 10 degrees C (Brits et al. 2014). Germination was assessed weekly for ten weeks, and a seed was scored as germinated when the radicle protruded 1 mm.

Post-fire seedling recruitment

Our entire study area burned on 9 March 2015. Experimental exclosures were not rebuilt and remaining parts of structures were removed. One year after the fire, we counted the number of *Leucospermum lineare* seedlings that had recruited, also noting dead seedlings. We divided each 20 x 20 m plot into sixteen 5 x 5 m subplots and randomly selected four subplots per plot in which to count the seedlings. In shade plots we counted seedlings in the entire 10 x 7 m plot. To account for differences in plot sizes we divided seedling counts by the area surveyed. The number of seedlings per adult was calculated as (seedlings per m²) / (pre-fire adults per m²). We estimated the number of pre-fire adults per plot as (total number of open inflorescences) / (mean number of open inflorescences per plant). The mean number of open inflorescences per plant was calculated from samples of 72 – 103 plants at each site. Since ant dispersers move seeds over many meters, it was justified to use plot level data for the inflorescence counts (and thus pre-fire adult estimates) but 2.5 x 2.5 m subplots for the seedling counts. We also counted the seedlings of *Protea neriifolia* and *P. coronata*, in order to test for interspecific competition with *L. lineare* seedlings.

Statistical methods

All data analyses were done in R version 3.3.0 (R Core Team 2016). Linear mixed models (LMMs) and generalised linear mixed models (GLMMs) were fit using the functions “lmer” and “glmer” in the package lme4 (Bates et al. 2015), unless indicated otherwise. In general, we report measured values as mean \pm standard deviation (SD), unless indicated differently. In box-and-whisker plots bold lines are medians, bars show upper and lower quartiles, whiskers indicate ranges and dots are outliers.

Nectar volume and nectar sugar amount were analysed using GLMMs with a gamma distribution and inverse link. To satisfy the gamma distribution we removed zero values by adding 1 to all nectar volume values and 0.001 to all nectar sugar values. Sugar concentration percentage values were logit transformed (Warton & Hui 2011) before fitting a LMM. We estimated test statistics and degrees of freedom for the LMM using Kenward-Roger’s approximation (Kenward & Roger 1997) in the package pbkrtest (Halekoh & Højsgaard 2014). The fixed effect in all three models was enclosure type (treatment) and site and plant individual were included as random effects.

Bird visitation rate was compared between open plots and shade control plots with a Wilcoxon signed-rank test, since observations were paired in time between open and shade control plots and the data were non-normal.

Ant count data were zero inflated and overdispersed relative to a Poisson distribution, hence we fitted zero-inflated negative binomial GLMMs, using the package glmmADMB (Skaug et al. 2016). We fitted mixture models, which treat zero counts as a mixture of structural zeros and sampling zeros (Martin et al. 2005). We included enclosure type as fixed effect and site and plant individual as random effects. Plant individual was included as random effect, since we sampled multiple inflorescences per plant. The two sampling periods were analysed separately.

We collected seed set and seed viability data across two years, 2013 and 2014. Where possible we analyse and present data from the two years together, but this was not possible in all cases, since inflorescence pollination treatments were only applied in 2013. Results were typically consistent across years; for clarity we highlight the instances where they were not.

Seed set data were analysed with a GLMM with poisson errors and a log link, with enclosure type as fixed effect, and site and year as random effects. Proportional seed set was fitted to a GLMM with binomial errors and a logit link, with enclosure type as fixed factor and site as random factor. For analysis of seed set under different pollination treatments in 2013 we

used a poisson GLMM with the concatenation of “inflorescence treatment”-“exclosure type” as fixed effect and site as random effect. Analyses for 2013 are for Site 2 and Site 3 only, since Site 1’s data could not be collected. We removed a lone zero outlier from the “control”-“bird exclosure” treatment concatenation in the 2013 seed set data, since it was the only zero in the entire data set and deemed to most likely have been caused by an extraneous factor, e.g. insect larva in the inflorescence stem.

Proportion seed viability data were overdispersed, i.e. the variance was greater than expected for a binomial distribution. To account for the overdispersion we fit a GLMM with a beta-binomial error distribution (Harrison 2015) and logit link, with exclosure type as fixed effect and site and year as random effects, using the package glmmADMB. For a given mean, the variance of the beta-binomial distribution is greater than that of the binomial distribution (Hughes & Madden 1993). For analysis of seed viability under different inflorescence treatments in 2013, we used a beta-binomial GLMM with a logit link, with the combination of inflorescence treatment and exclosure type as fixed effect and site as random effect. Analyses for 2013 are for Site 2 and Site 3 only, since Site 1’s data could not be collected.

Post-fire seedling emergence data were analysed using a GLMM with a Gaussian error distribution and a log link, with exclosure type as fixed factor and site as random factor. Post-fire seedling survival was analysed in the same manner, but using the package glmmADMB.

Results

Nectar

The mean nectar volume of *Leucospermum lineare* flowers was significantly higher in bird exclusion plots ($6.2 \pm 2.8 \mu\text{L}$, $n = 41$) than in open plots ($2.8 \pm 1.4 \mu\text{L}$, $n = 25$) and shade control plots ($1.9 \pm 1.2 \mu\text{L}$, $n = 29$) across all study sites (Table 2.1). Sugar concentration was also higher in bird exclusion plots ($27.9 \pm 9.5\%$, $n = 40$) than in open plots ($18.3 \pm 7.0\%$, $n = 24$) and shade control plots ($18.8 \pm 6.0\%$, $n = 22$) (Table 2.1). Unsurprisingly, thus, the amount of sugar in milligrams per flower was also significantly higher in the absence of birds than in open and shade control plots (Table 2.1) (Fig. 2.2). The same patterns were evident when comparing plots within each study site (analyses not shown).

Flower visitors

Leucospermum lineare was pollinated by the Cape Sugarbird, *Promerops cafer*, (92% of bird visits, $n = 183$). Cape Sugarbirds consistently perched on top of inflorescences and probed from above (99% of Cape Sugarbird visits, $n = 168$), thus contacting pollen presenters and gathering pollen on their heads. Orange-breasted sunbirds, *Anthobaphes violacea*,

occasionally visited inflorescences (8% of bird visits, $n = 183$), mostly probing from below (67% of visits, $n = 15$), but sometimes acting as legitimate pollinators by probing from above (33% of visits, $n = 15$). The mean visitation rate of bird pollinators was 0.8 ± 2 (SD) pollinating visits per inflorescence per hour, $n = 114$ observation periods. We found no difference in overall bird visitation rate between open plots and shade control plots (Wilcoxon signed rank test, $Z = -0.28$, $p = 0.79$).

Few insects visited the flowers of *Leucospermum lineare*. In 12 out of 21 observation periods there were no insect visitors except for ants. We observed only ten pollinating insect visits during focused observations of 8.5 hours over 4 different days. Overall the mean rate of insect pollinator visitation was 0.42 ± 1 (SD) visits per inflorescence per hour. Three insect species contacted pollen presenters while collecting pollen and thus likely effected pollination: a colletid bee (Colletidae: *Hylaeus* sp. 1), 8 observations on 6 different days over the course of a month; the Cape honey bee, *Apis mellifera capensis* Esch., 5 observations; and a halictid bee (Halictidae: *Lasioglossum* sp. 1), 1 observation. Some other insects visited inflorescences, but did not act as pollinators. Two beetle species [Mordellidae: Mordellidae sp. 1, Mordellidae sp. 2 (*Anaspis* sp.)] and the fly *Scatophaga stercoraria* L. (Diptera: Scatophagidae) were each observed once. The brown protea beetle, *Trichostetha capensis* L. (Coleoptera: Scarabaeidae), visited inflorescences in large numbers at times, “digging” in inflorescences as if trying to reach the nectar and likely also eating flower parts, since styles wilted after their visits.

Ants often visited *L. lineare* inflorescences to thief nectar, almost never contacting the pollen presenters. Amplified nectar availability in bird enclosures seemed to increase ant visitation. The number of ants per inflorescence was significantly higher in the bird enclosure treatment than in the open and shade control treatments during the first sampling period (Fig. 2.3A) (Table 2.2). However, in the second sampling period a week later there were no differences among treatments (Fig. 2.3B) (Table 2.2). The most abundant ant species by far in inflorescences was *Camponotus niveosetosus* Mayr, while *Anoplolepis custodiens* F. Smith and another *Camponotus* sp. were also frequently recorded. Less common species were *Tetramorium frigidum* Arnold, *Tetramorium* sp. 1, *Pheidole* sp. 1, *Lepisiota* sp. 1, *Lepisiota* sp. 2 and a *Monomorium* sp.

Not many plant species flowered at the same time as *L. lineare*. The only species co-flowering in all plots at all sites was *Diosma hirsuta*. See Table S2.3 for a list of insect flower visitors.

Seed set

The number of seeds produced by *Leucospermum lineare* inflorescences did not differ between bird exclusion (12.0 ± 4.9 , $n = 46$) and open plots (12.6 ± 5.1 , $n = 44$), but was significantly lower in shade control plots (10.3 ± 4.2 , $n = 38$) than in open plots and bird exclusion plots (2013 and 2014 seasons combined, Fig. 2.4A) (Table 2.3). The same pattern was evident for proportional seed set in 2014, with no difference between open plots ($10.5 \pm 3.3\%$, $n = 29$) and bird exclusion plots ($10.8 \pm 3.3\%$, $n = 27$), while shade control plots were significantly lower ($8.8 \pm 2.2\%$, $n = 21$) (Table S2.1). Proportional seed set for 2013 is not reported due to lack of data on the number of ovules per inflorescence, but we assume there were no differences among groups, since the number of seeds produced were similar across groups (Fig. 2.5, Table 2.5). Overall, differences in seed production were inconsistent across treatments and of small magnitude.

In open plots, the exclusion of all pollinators from inflorescences using bags significantly reduced the number of seeds produced (11.1 ± 2.4 , $n = 12$) relative to control inflorescences (15.2 ± 5.2 , $n = 12$) (Table 2.5) (Fig. 2.5). In bird exclusion plots and shade control plots there were no differences between bagged inflorescences and control inflorescences (Table 2.5) (Fig. 2.5). Hand pollen addition did not increase the number of seeds produced relative to control or bagged inflorescences in any of the enclosure types (Table 2.5) (Fig. 2.5).

The Autofertility Index (bag/hand) was high in all cases: open plots (Site 2 = 0.88; Site 3 = 0.78) and shade plots (Site 2 = 0.96; Site 3 = 1.25). Autofertility values (bag/control) were also high: Site 2 Open = 0.69, Site 2 Shade = 0.84; Site 3 Open = 0.75, Site 3 Shade = 1.37. Data were not available for Site 1.

Seed viability

Seed viability, as assessed in laboratory germination trials, was lower for inflorescences from bird exclusion plots (median = 28.6%, IQR = 55.6%, $n = 43$) than open plots (median = 40%, IQR = 53.4%, $n = 44$) or shade control plots (median = 40%, IQR = 56.7%, $n = 37$), but differences among enclosure types were not statistically significant (Table 2.6) (Fig. 2.4B). Within each of the enclosure types, seed viability was similar for bagged inflorescences, control inflorescences and hand pollinated inflorescences (Table 2.7) (Fig. 2.6).

Post-fire seedling recruitment

The number of seedlings emerging per adult plant was significantly lower in bird enclosure plots than in open and shade control plots (Fig. 2.4C) (Table 2.8). Seedling emergence was not predicted by the pre-fire percentage cover of *L. lineare* in 2.5 x 2.5 m subplots (linear regression, $R^2 = 0.016$, $p = 0.3$). There was also no relationship between *L. lineare* seedling emergence and pre-fire percentage cover of other Proteaceae sp. in 2.5 x 2.5 m subplots

(linear regression, $R^2 = 0.07$, $p = 0.05$). We found a positive relationship between seedling emergence and pre-fire percentage cover of Restionaceae at the 2.5 x 2.5 m level (linear regression, $y = 0.7x + 1.8$, $R^2 = 0.22$, $p < 0.001$)¹.

Seedlings surviving one year after the fire, i.e. the number of live seedlings per adult, was still significantly lower in bird enclosure plots (median = 2.5, IQR = 1.8, $n = 35$) than shade control plots (median = 8.3, IQR = 8.5, $n = 24$), but not significantly lower relative to open plots (median = 6, IQR = 7.8, $n = 36$) (Fig. 4D) (Table 9). Thus, the difference between bird enclosure and open plots had decreased over time due to higher seedling mortality in open plots ($39 \pm 30\%$, $n = 36$) than in bird enclosure plots ($23 \pm 27\%$, $n = 35$) and shade enclosure plots ($23 \pm 18\%$, $n = 24$) (Table S2.2). We found no relationship between *L. lineare* seedling mortality and the seedling densities of *Protea neriifolia* (linear regression, $R^2 = 9e^{-7}$, $p = 0.99$) or *P. coronata* (linear regression, $R^2 = 0.02$, $p = 0.3$). Density dependence of seedling mortality was not evident on the 2.5 x 2.5 m scale (linear regression, $R^2 = 0.05$, $p = 0.09$). In contrast, seedling density was a significant parameter in a statistical model of the relationship between seedling mortality, enclosure type and seedling density (Table S2.2).

Discussion

When bird pollinators are lost, the bird-adapted *Leucospermum lineare* maintains seed set by autonomous selfing. However, seed viability is reduced by inbreeding, ultimately causing a decline in seedling recruitment after fire.

Leucospermum lineare was pollinated mainly by the Cape Sugarbird and received few insect visits. Insect visitors rarely contacted pollen presenters and thus did not transfer pollen, except for bees that occasionally collected pollen.

Nectar accumulated in *L. lineare* inflorescences in the absence of birds and nectar sugar concentration increased (Table 2.1; Fig. 2.2), likely due to evaporation. The nectar concentration of *L. lineare* in our open control plots was comparable to that of bird-pollinated Proteaceae like *L. conocarpodendron* and *Mimetes fimbriifolius* (Johnson 2015), while nectar volume was lower in *L. lineare*. The volume and concentration of *L. lineare* nectar was similar to that of plants pollinated by specialised bird pollinators (i.e. sunbirds and hummingbirds) in general (Johnson & Nicolson 2008).

¹ Christian & Stanton (2004) observed that roots of the hemiparasitic plant *Mastersiella digitata* (Restionaceae) attach their roots to dormant seeds of *Leucospermum truncatulum*. We found a positive, rather than negative, association between Restionaceae % cover and *L. lineare* seedling emergence, thus Restionaceae roots did not affect *L. lineare* seed germination. No *Mastersiella* species occurred in our study area.

Elevated nectar volume and concentration in the absence of birds increased ant visitation (Fig. 2.3; Table 2.2), but this result was not consistent across sampling periods. Ants rarely touched pollen presenters and thus did not facilitate pollination, therefore they were nectar thieves and did not compensate for the removal of birds. In *L. conocarpodendron* ant flower visitors also do not act as pollinators (Lach 2008).

Although *Leucospermum lineare* has showy bird-adapted flowers, loss of its bird pollinators did not cause a decline in seed set (Fig. 2.4A; Tables 2.3 and 2.4). Seed set was maintained by autonomous self-pollination (Fig. 2.5; Table 2.5), as indicated by high autofertility values. These results suggest that *Leucospermum lineare* has a mixed mating system, although genetic data are needed to confirm this. Similar seed set for open inflorescences and bagged inflorescences in the bird exclosures indicate that insect pollinators did not contribute to seed set (Fig. 2.5; Table 2.5). In open plots, seed set was lower for bagged inflorescences than control inflorescences, indicating that seed set can decline due to the absence of bird pollinators, but seed set was still substantial, indicating high levels of autonomous selfing (Fig 2.5, Table 2.5). Overall the sample sizes of analyses indicating no decline in seed set in the absence of birds were also much larger than in the case of the above comparison of bagged and control inflorescences in open plots. The high degree of selfing overall is contrary to previous work that found low levels of selfing (autofertility value of 0.1) in *L. lineare* (Johnson 2015), however, that study's sample size was only 10 inflorescences. Nevertheless, our results correspond to Johnson's (2015) finding of an association between selfing and bird-pollination in the genus *Leucospermum*. In general plants that are specialised in terms of pollination are often able to self-pollinate and this is thought to be a means of reproductive assurance in the face of unpredictable pollination by specialist pollinators (Bond 1994; Kalisz & Vogler 2003; Fenster et al. 2007).

Our results suggest that seed production in *L. lineare* is not pollen limited (Fig. 2.5; Table 2.5). This is the case for many Proteaceae, including e.g. *L. conocarpodendron* (Lach 2008; Johnson 2015; Schmid et al. 2015). However, our finding may be due to pollen viability being lost in the days between pollen collection and application, as well as the long interval between applications. *Leucospermum* flowers open sequentially and stigmas may only be receptive for a few days (Brits & Van den Berg 1991).

Selfing may come at a substantial cost for plants due to pollen and seed discounting and inbreeding depression (Barrett 2003). One of the life history stages that may be affected by inbreeding is seed germination (Heschel & Paige 1995). In *L. lineare* inbreeding depression reduced seed viability and seedling emergence in the absence of bird pollinators (Fig. 2.4). Seed viability diminished when birds were lost, but not significantly so (Fig. 2.4B, Table 2.6),

and seed viability did not differ among different pollination treatments (Fig. 2.6; Table 2.7). However, reduced seed viability is the best explanation for the decline in seedling emergence in the absence of birds. It is possible that seeds behaved differently under field conditions than in laboratory trials. In addition, some inbred seeds that germinated in the field may have failed to emerge if their hypocotyls could not reach the soil surface. Seed viability was lower in seeds that had been stored for 18 months than in seeds stored for 6 months, consistent with marked declines in seed viability after one year of storage in another *Leucospermum* (Brits et al. 2015). In fynbos bird-pollinated *Protea*, bird exclusion reduces seed set of many species, but does not affect seed mass or seed germination (Schmid et al. 2015). In the grassland species *Protea caffra*, selfing does not reduce seed germination or early seedling survival (Steenhuisen et al. 2012). Depression of seed germination due to inbreeding has been found in some bird-pollinated Australian Proteaceae (Forrest et al. 2011).

The ultimate demographic effects of altered seedling emergence will depend on seedling survival and how it is affected by density. Density dependent seedling mortality in the first year of life is evident in some fynbos *Protea* species, but not in others (Maze & Bond 1996) and is high in Australian *Banksia* (Lamont et al. 1993). In *Leucospermum lineare*, the first-year seedling survival of 61 – 76% was similar to other fynbos Proteaceae (Maze & Bond 1996). The post-fire seedling emergence of *L. lineare* was significantly lower in the absence of birds (Fig. 2.4C), but when considering only the surviving seedlings, the difference between open plots and bird excluded plots was no longer statistically significant (Fig. 2.4D). This was due to higher first-year seedling mortality in open plots than in bird exclusion and shade control plots (Table S2.2). This seedling mortality pattern was contrary to what would be expected if inbreeding depression determined seedling mortality. Our multivariate analysis indicates that seedling mortality was influenced by conspecific seedling density (Table S2.2), but our sampling scale of 2.5 x 2.5 m was not small enough to detect this by simple regression. *Leucospermum lineare* seedlings often emerged in clumps, as expected for an ant-dispersed species, thus density dependent effects will likely be important. As seedlings increase in size over time, density dependent effects will become stronger, and it is likely that only a single adult plant can ultimately occupy the space of a seedling clump. Although enough seedlings survived in the bird enclosure plots one year after the fire to replace the pre-fire adults (seedling to adult ratio was 2.5), density dependent mortality in the following years may well reduce plant density to below replacement levels. If the much higher seedling to adult ratios of 8 or 6 in control plots is an indication of the seedling densities required to replace the adult population, then the reduced seedling numbers in the absence of birds will cause ultimate declines in *L. lineare* population size.

The overall reduction in the seed bank of *L. lineare* due to bird exclusion may have been greater than what we observed with seedling emergence, considering the following. Ant-dispersed fynbos plants form soil seed banks that germinate after fire. In *Leucospermum*, seed dormancy is broken by heat desiccation-scarification of the testa (Brits et al. 1993) and as a result seedling density increases with fire intensity (Bond et al. 1990). Vegetation density and thus fuel loads were similar among plots at a site, therefore fire intensity did not differ between open and bird enclosure treatments in our study. *Leucospermum* seeds may persist underground for long periods and most, but not all seeds in the soil seed bank will germinate in the year after a fire (Brits et al. 2015). Younger seeds with more intact testas may only germinate in later years or even only after the next fire (Brits et al. 2015).

Another factor that may have reduced the effect size of bird exclusion is the fact that *L. lineare* had flowered for one year before the bird enclosures were erected. However, this reduction may not have been large, since not all plants flower in the first year of flowering and the number of inflorescences per plant increase non-linearly as plants age. Seed input from the first year would thus be small relative to later years.

The ultimate demographic effects of bird loss on *L. lineare* will depend not only on density dependent effects, but also on how inbreeding manifests in later life stages. Late-acting inbreeding depression can reduce flowering and survival. In the case of two bird pollinated trees from New Zealand, such effects were so strong that self-pollination was demographically futile (Robertson et al. 2011). Similarly to *L. lineare*, self-pollination in *Protea caffra* did not reduce germination and early seedling survival, but population genetic analyses suggested that inbred seedlings do not survive to adulthood (Steenhuisen et al. 2012). In addition, the ultimate demographic effects of bird loss will also be determined by how inbreeding accumulates over multiple generations (e.g. Forrest et al. (2011)).

If our findings for *Leucospermum lineare* are applicable to other members of the genus, the extinction of bird-pollinators will have far-reaching effects: half of the 48 *Leucospermum* species are bird-pollinated (Johnson 2015). These species are morphologically specialised for pollination by birds by having long styles, as well as long nectar tubes when adapted for pollination by long-billed birds like the Cape Sugarbird (Johnson 2015). Very different suites of flower traits in insect-pollinated and rodent-pollinated members of the genus suggest that it will not be easy for bird-pollinated species to switch to other pollinators on an ecological time scale (Johnson 2015). An exception may be the long-proboscid flies, a prominent pollinator guild in the Cape (Anderson et al. 2014). The single *Leucospermum* taxon pollinated by long-proboscid flies, *L. tottum* var. *tottum*, has long nectar tubes and is also pollinated by Cape Sugarbirds (Johnson et al. 2014) In addition, the taxon's "high" nectar

concentration and low nectar volume, deemed to be an adaptation for long-proboscid fly pollination, are very similar to that of open control *L. lineare* flowers. The amplified nectar concentration we measured in *L. lineare* in the absence of birds is thus high enough for insect pollinators (Johnson et al. 2014). The question remains though why long-proboscid flies have not been observed on other bird-pollinated *Leucospermum* species, perhaps because long-proboscid flies may require other traits, like horizontal, rather than vertical, flower orientation (Johnson et al. 2014).

Our findings are relevant to Cape Proteaceae more generally, since *Leucospermum lineare* is representative of many members of the family in terms of its pollination mode and life history (Rebelo 2001; Johnson 2015; Schmid et al. 2015; Treurnicht et al. 2016). Our results suggest that the loss of avian pollinators will threaten the persistence of bird-pollinated Cape Proteaceae through the insidious effects of inbreeding and not only by reducing seed set, as found in certain species (Collins 1983; Wright et al. 1991; Schmid et al. 2015).

Our experimental demonstration of reduced seedling recruitment due to bird pollinator extinction, adds to the evidence from “natural experiments” and habitat fragmentation studies that pollinator loss can cause declines in plant fecundity and ultimately in plant populations (Pauw 2007; Mortensen et al. 2008; Anderson et al. 2011).

Even though species like *L. lineare* may persist without bird pollinators by selfing, and possibly also through limited pollination by pollen-collecting bees, there will be a loss of genetic diversity within populations in the absence of birds. This will transpire since bird pollinators are vital for gene flow (e.g. Reisch et al. 2010), due to the short distances of ant seed dispersal (Slingsby & Bond 1985). Indeed the mobility of bird pollinators can protect plants against increased selfing in the face of habitat fragmentation (Breed et al. 2015). Smaller effective population size and loss of genetic diversity without bird pollinators could diminish the ability of species to adapt to global change.

Pollination studies should not stop short at assessing only seed set, but should consider seed viability and recruitment and where possible ideally also later life-stages (Price et al. 2008). Our study shows that the ultimate effects of pollinator loss on the persistence of plant populations are likely to be underestimated since often only seed set is considered.

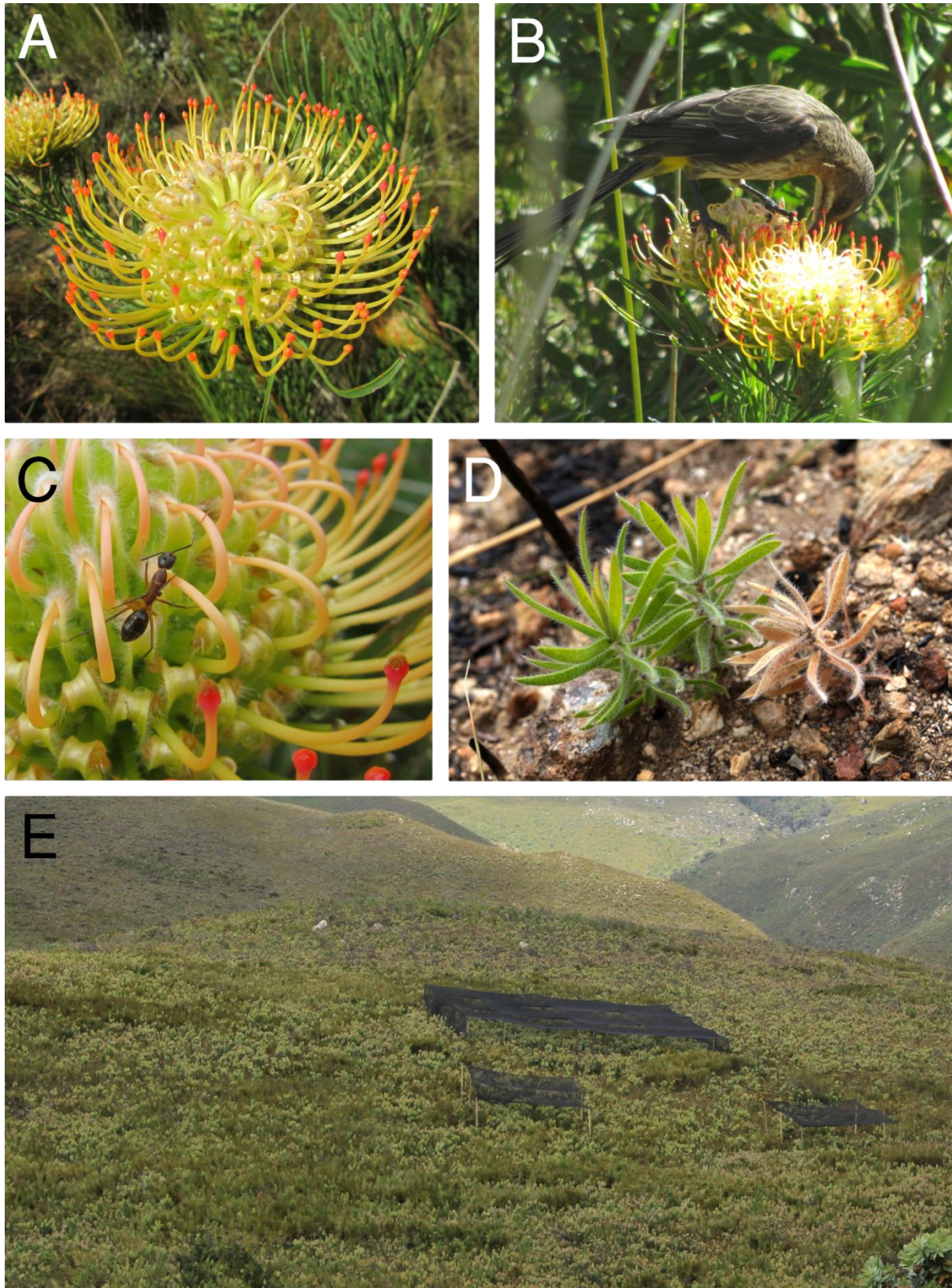


Figure 2.1. A) *Leucospermum lineare* inflorescence; B) Cape Sugarbird pollinating *L. lineare*; C) The ant *Camponotus maculatus* thieving nectar from *L. lineare*; D) Seedlings of *L. lineare*; E) Experimental setup: bird exclusion cage and shade control roofs at study site number 2. Photo credits: D) by Anton Pauw and E) by Nanike Esterhuizen.

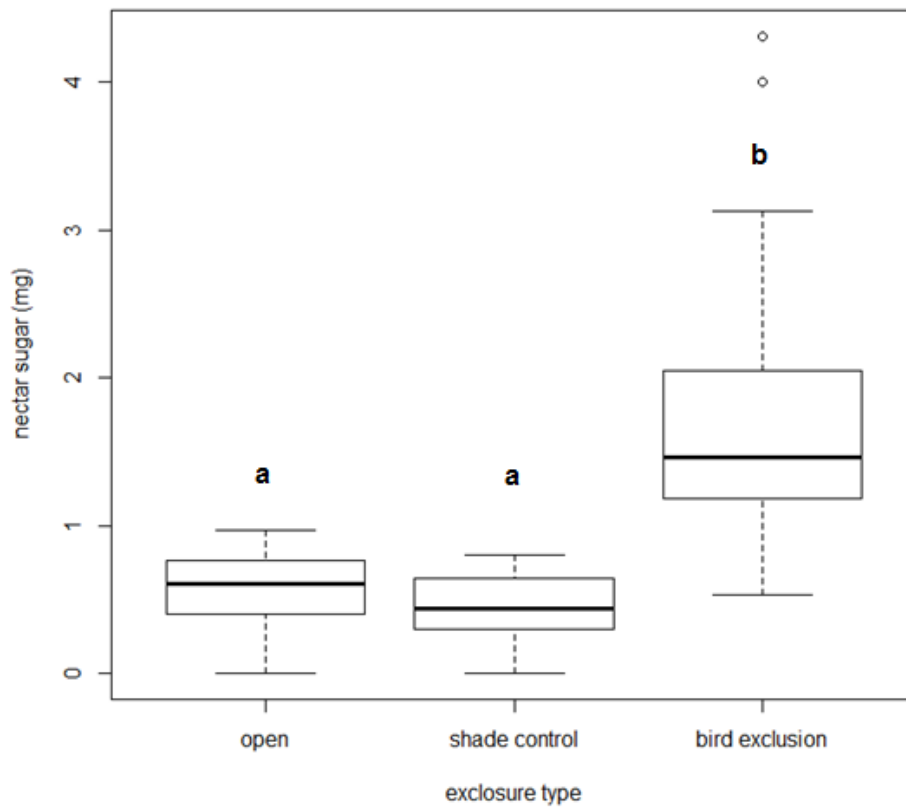


Figure 2.2. The amount of sugar in nectar of *Leucospermum lineare* flowers under different pollinator exclusion treatments. Different letters indicate significant differences (GLMM, Table 2.1).

Table 2.1. Results of statistical models comparing nectar properties among different pollinator enclosure treatments. Estimates and standard errors of volume and sugar amount are on the inverse (1/x) scale, while those of sugar concentration is on the logit scale. Est. = Estimate, Var. = Variance.

Effects	<u>Nectar volume</u>					<u>Sugar concentration</u>					<u>Nectar sugar amount</u>				
	Est.	SE	t	p	n	Est.	SE	t	p	n	Est.	SE	t	p	n
<u>Fixed effects</u>															
Exclosure type															
Intercept (Bird excl.)	0.16	0.020	8.13	< 0.001	95	-0.96	0.16	-5.96	0.020	83	0.59	0.092	6.35	< 0.001	92
Open	0.12	0.029	4.19	< 0.001		-0.44	0.092	-4.77	0.032		1.26	0.38	3.33	< 0.001	
Shade control	0.20	0.032	6.37	< 0.001		-0.49	0.096	-5.13	0.027		2.07	0.52	4.00	< 0.001	
Intercept (Open)	0.28	0.026	10.74	< 0.001	95	-1.40	0.17	-8.43	0.002	83	1.85	0.37	5.02	< 0.001	92
Bird exclusion	-0.12	0.029	-4.19	< 0.001		0.44	0.092	4.77	0.013		-1.26	0.38	-3.33	< 0.001	
Shade control	0.082	0.036	2.27	0.023		-0.054	0.10	-0.52	0.63		0.81	0.63	1.29	0.20	
	Var.	SD				Var.	SD				Var.	SD			
<u>Random effects</u>															
Plant individual	0.0015	0.039			55	0.039	0.20			48	2.37 e ⁻¹⁸	1.54 e ⁻⁰⁹			55
Site	0	0			3	0.068	0.26			3	1.90 e ⁻¹⁸	1.38 e ⁻⁰⁹			3

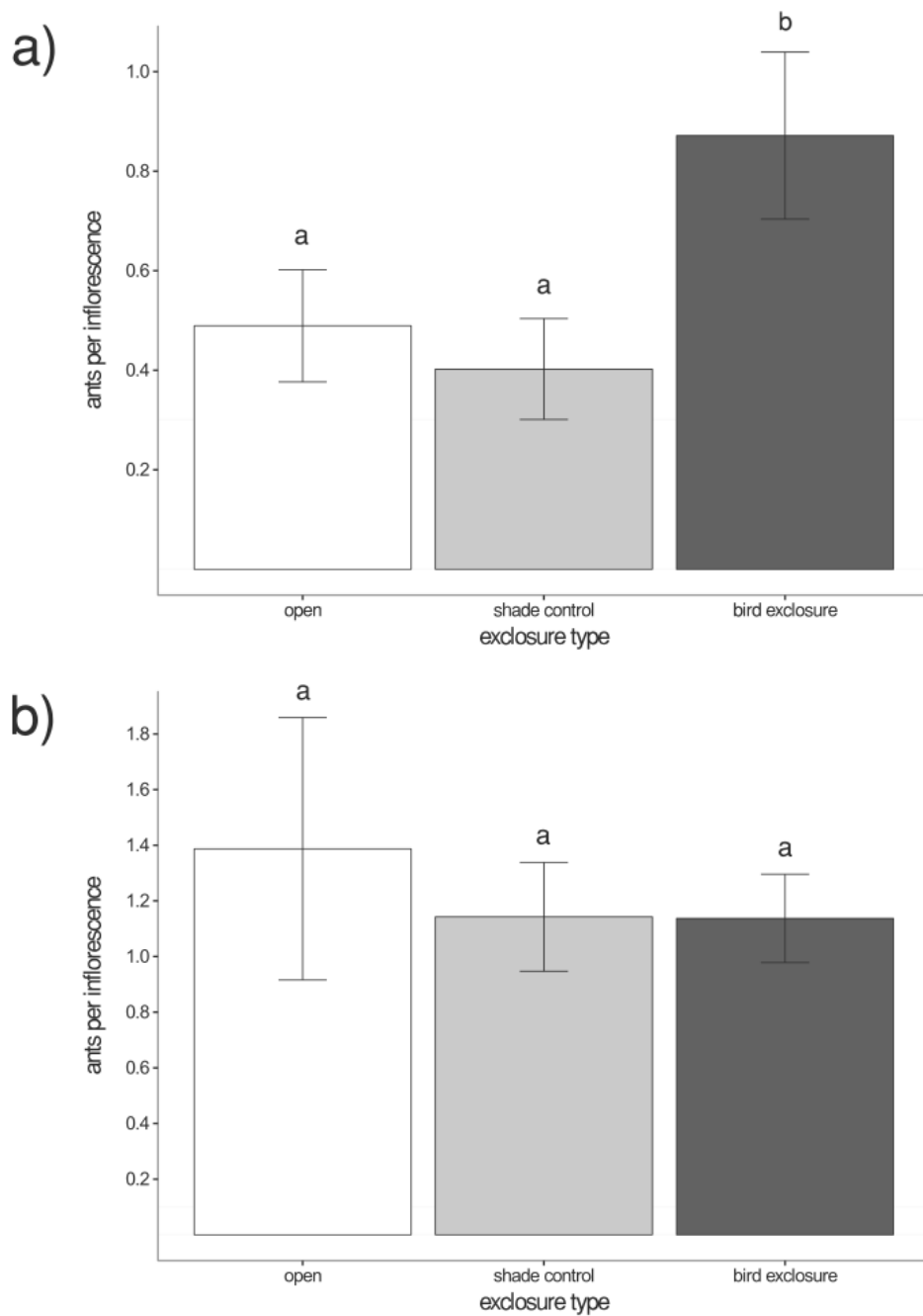


Figure 2.3. Mean number of ants in *Leucospermum lineare* inflorescences under different pollinator treatments during the first sampling period (a) and the second sampling period (b). Different letters indicate significant differences (GLMMs, Table 2.2). Bars are standard errors.

Table 2.2. Results of GLMMs comparing the number of ants in *Leucospermum lineare* inflorescences among different pollinator exclusion treatments, for two separate sampling periods. Estimate and SE values are on the log scale.

Sampling period 1					
Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	-0.72	0.18	-4.0	< 0.001	368
Open	-0.63	0.25	-2.5	0.012	
Shade control	-0.72	0.30	-2.4	0.016	
Intercept (Open)	-1.35	0.21	-6.4	< 0.001	368
Bird exclusion	0.63	0.25	2.5	0.012	
Shade control	-0.091	0.32	-0.30	0.77	
Variance SD					
<u>Random effects</u>					
Plant individual	1.12	1.06			250
Site	1.63 e ⁻⁷	0.0004			3
Sampling period 2					
Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	0.0083	0.22	0.04	0.97	184
Open	-0.098	0.28	-0.35	0.73	
Shade control	-0.024	0.29	-0.08	0.93	
Intercept (Open)	-0.089	0.28	-0.32	0.75	184
Bird exclusion	0.098	0.28	0.35	0.73	
Shade control	0.074	0.32	0.23	0.82	
Variance SD					
<u>Random effects</u>					
Plant individual	0.37	0.61			162
Site	0.026	0.16			3

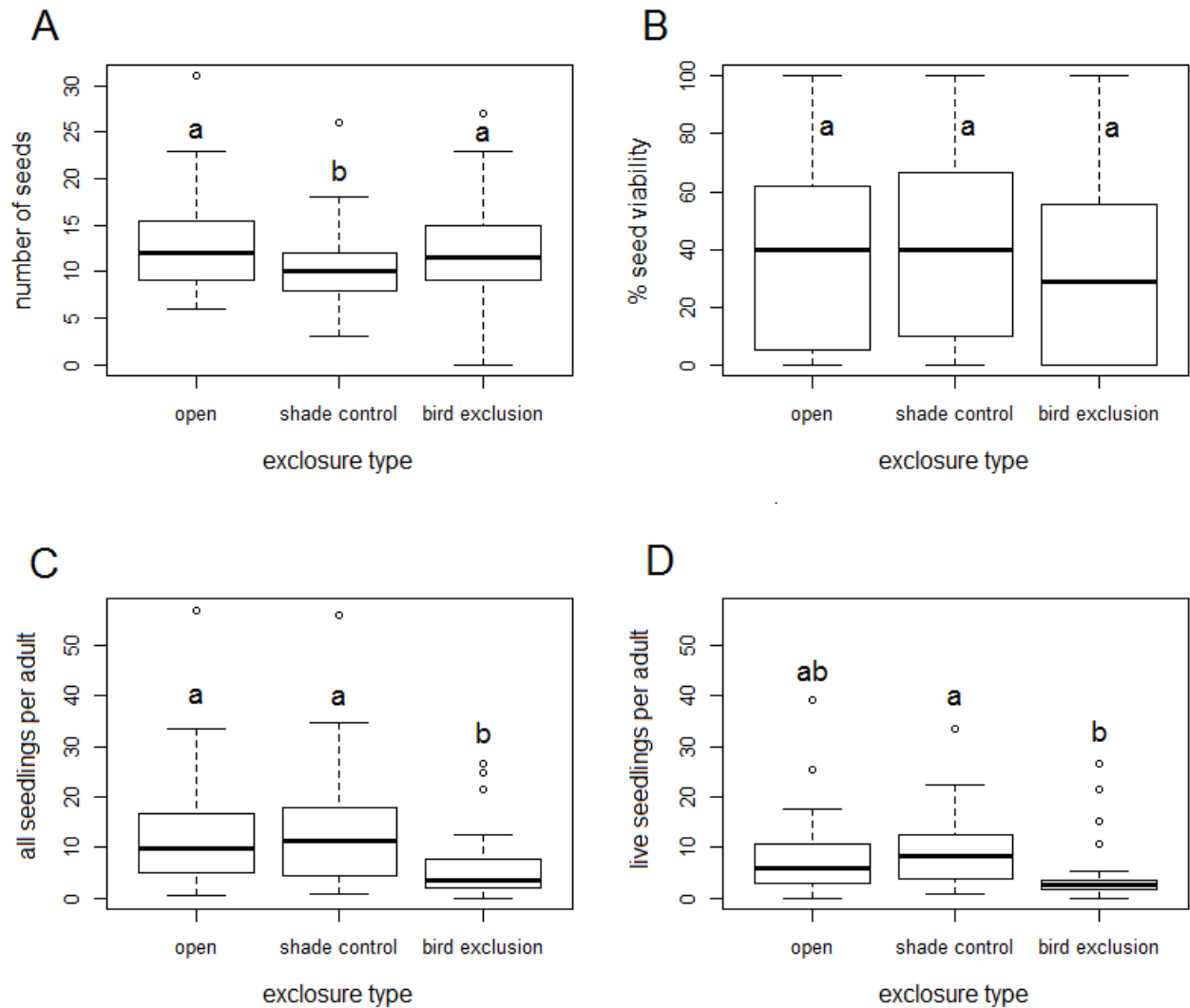


Figure 2.4. Effects of bird exclusion on the reproduction of *Leucospermum lineare*. A) Seed set; B) Seed viability in laboratory germination trials; C) Post-fire seedling emergence; D) Seedlings surviving one year after fire. Different letters indicate significant differences (GLMMs: Tables 2.3, 2.6, 2.8, 2.9). Seed set and seed viability graphs are for 2013 and 2014 data combined.

Table 2.3. Results of GLMM comparing seed set of *Leucospermum lineare* among different pollinator exclusion treatments in 2013 and 2014. Estimate and SE values are on the log scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	2.50	0.09	27.8	< 0.001	127
Open	0.028	0.06	0.46	0.65	
Shade control	-0.16	0.07	-2.35	0.019	
Intercept (Open)	2.53	0.09	27.8	< 0.001	127
Bird exclusion	-0.028	0.06	-0.46	0.65	
Shade control	-0.184	0.07	-2.75	0.006	
	Variance	SD			
<u>Random effects</u>					
Site	0.005	0.07			3
Year	0.008	0.09			2

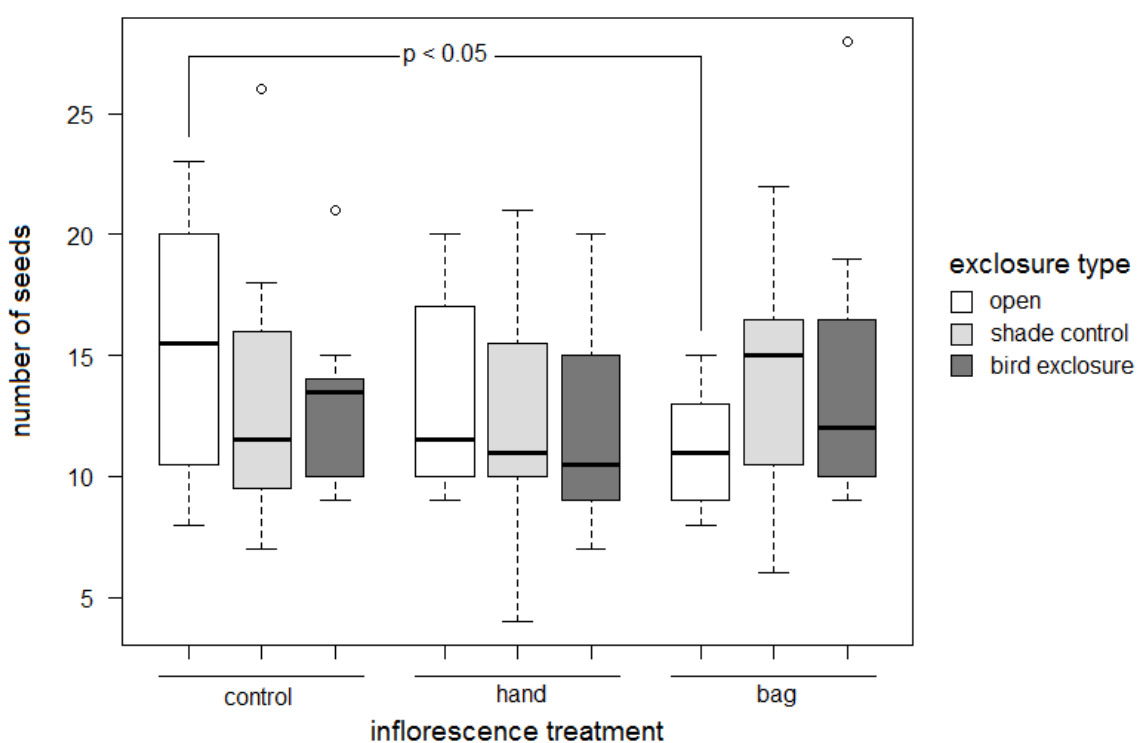


Figure 2.5. Seed set of *Leucospermum lineare* for different inflorescence treatments (control, hand cross pollen supplementation and full pollinator exclusion) in different pollinator exclosure plots in 2013. Pairwise significant differences indicated (GLMM, Table 2.5).

Table 2.5. Results of GLMM comparing seed set of *Leucospermum lineare* among different combinations of enclosure type and inflorescence treatment in 2013. Significance at $p < 0.05$ indicated by boldface. Estimate and SE values are on the log scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type + Inflorescence treatment					
Intercept (Open + Control)	2.72	0.087	31.16	< 0.001	101
Shade control + Control	-0.15	0.11	-1.41	0.16	
Bird exclusion + Control	-0.13	0.11	-1.18	0.24	
Open + Hand pollen addition	-0.13	0.11	-1.15	0.25	
Shade control + Hand pollen addition	-0.20	0.11	-1.84	0.066	
Bird exclusion + Hand pollen addition	-0.23	0.11	-1.93	0.054	
Open + Bag	-0.30	0.11	-2.67	0.0077	
Shade control + Bag	-0.091	0.11	-0.82	0.41	
Bird exclusion + Bag	-0.092	0.11	-0.86	0.39	
<u>Random effects</u>					
Site	Variance	SD			2
	0.0042	0.065			

Table 2.6. Results of GLMM comparing proportion seed viability for *Leucospermum lineare* inflorescences from different enclosure types in 2013 and 2014. Estimate and SE values are on the logit scale. Beta-binomial dispersion parameter = 1.69 ± 0.29 (SE).

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	-0.82	0.39	-2.10	0.036	124
Open	0.19	0.30	0.65	0.52	
Shade control	0.39	0.32	1.23	0.22	
Intercept (Open)	-0.63	0.38	-1.66	0.097	124
Bird exclusion	-0.19	0.30	-0.65	0.52	
Shade control	0.20	0.30	0.65	0.52	
<u>Random effects</u>					
Site	Variance	SD			3
	0.074	0.27			
Year	0.13	0.36			2

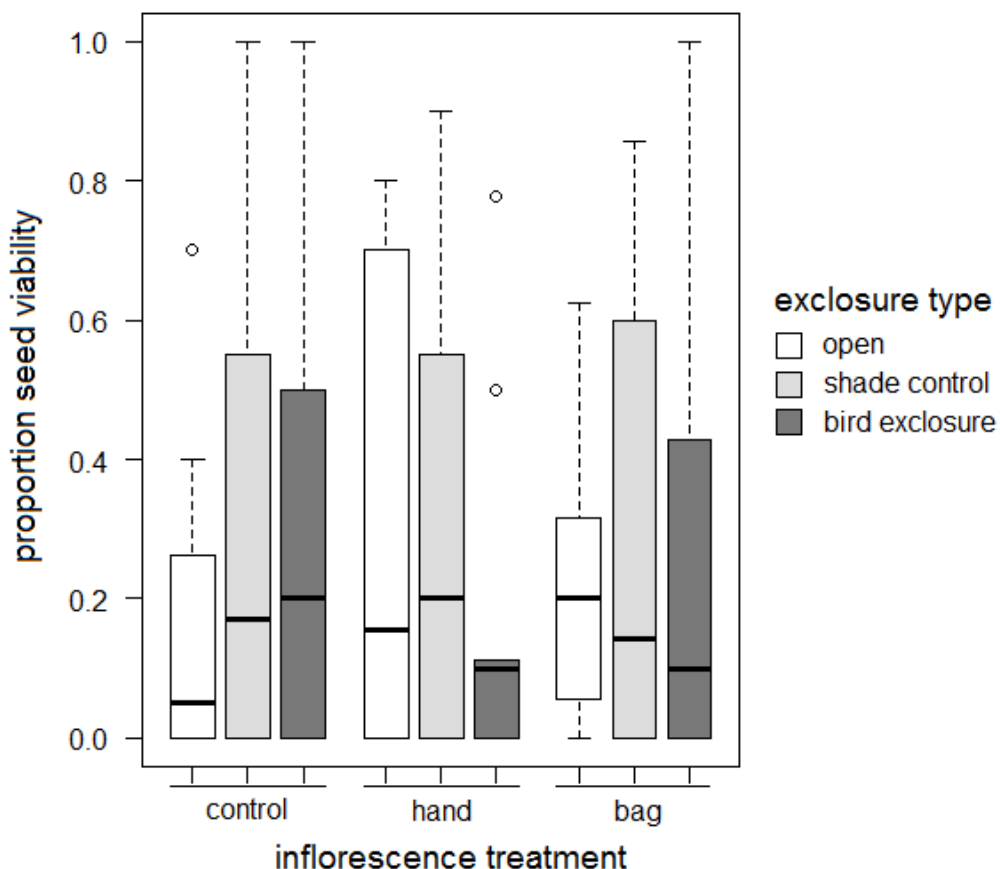


Figure 2.6. Proportion seed viability of *Leucospermum lineare* inflorescences for different inflorescence treatments in different enclosure types in 2013. There were no significant differences among treatments (GLMM, Table 2.7).

Table 2.7. Results of GLMM comparing proportion seed viability of *Leucospermum lineare* inflorescences among different combinations of enclosure type and inflorescence treatment in 2013. Estimate and SE values are on the logit scale. Beta-binomial dispersion parameter = 1.43 ± 0.28 (SE).

Effects	Estimate	SE	z	p	n
Fixed effects					
Exclosure type + Inflorescence treatment					
Intercept (Open + Control)	-1.56	0.46	-3.39	< 0.001	100
Shade control + Control	0.73	0.62	1.19	0.23	
Bird exclusion + Control	0.64	0.66	0.97	0.33	
Open + Hand pollen addition	0.79	0.64	1.23	0.22	
Shade control + Hand pollen addition	0.76	0.63	1.20	0.23	
Bird exclusion + Hand pollen addition	0.15	0.68	0.22	0.83	
Open + Bag	0.58	0.62	0.93	0.35	
Shade control + Bag	0.69	0.60	1.14	0.26	
Bird exclusion + Bag	0.36	0.65	0.56	0.58	
Random effects					
Site	Variance	SD			2
	$1.13 e^{-7}$	0.00034			

Table 2.8. Results of GLMM comparing the post-fire seedling emergence of *Leucospermum lineare* among different exclosure types. Estimate and SE values are seedlings per adult and are on the log scale.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	1.86	0.28	6.66	< 0.001	96
Open	0.65	0.26	2.49	0.013	
Shade control	0.71	0.27	2.64	0.0083	
Intercept (Open)	2.51	0.21	12.20	< 0.001	96
Bird exclusion	-0.65	0.26	-2.49	0.013	
Shade control	0.060	0.19	0.32	0.75	
<u>Random effects</u>	Variance	SD			
Site	9.59	3.10			3

Table 2.9. Results of GLMM comparing the number of *Leucospermum lineare* seedlings surviving one year after a fire among different exclosure types. Estimate and SE values are seedlings per adult and are on the log scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	1.47	0.28	5.20	< 0.001	96
Open	0.49	0.29	1.71	0.088	
Shade control	0.76	0.28	2.70	0.0069	
Intercept (Open)	1.97	0.22	8.95	< 0.001	96
Bird exclusion	-0.49	0.29	-1.71	0.088	
Shade control	0.27	0.21	1.28	0.20	
<u>Random effects</u>	Variance	SD			
Site	0.063	0.25			3

Chapter 3: Variable effects of bird exclusion in communities dominated by bird-pollinated plants

Abstract

Pollinator declines around the world raise a crucial question: How will pollinator loss affect plant communities? If a pollinator is a keystone mutualist its loss may cause cascading extinctions. Alternatively communities may suffer little if pollinators can replace one another, as suggested by widespread generalization of pollination systems. Few studies have considered the community-level effects of pollinator loss. We built large cages to exclude bird pollinators from entire plant communities in the hyperdiverse Cape Floristic Region of South Africa, where bird pollinators are particularly important. We observed flower visitors and assessed the effects of bird loss on the fecundity of all the bird-pollinated plant species. The Cape Sugarbird and Orange-breasted Sunbird were the main bird pollinators and small beetles were common visitors to *Protea* species (Proteaceae). The seed set of the most dominant shrub *Protea neriifolia* and two other species, *Mimetes cucullatus* (Proteaceae) and *Erica plukenetii* (Ericaceae) declined significantly without birds. *Protea repens*, also a dominant species, and *Protea nitida* maintained seed set under bird exclusion, likely because of insect pollination. Seedling recruitment of *Protea* species was well above adult replacement levels after a fire swept the six year old vegetation, but this result did not include the effect of bird pollinator loss. Overall our results suggest that extinction of bird pollinators will reduce biomass and diversity of plant communities in the Cape Floristic Region.

Introduction

The decline of pollinators in many parts of the world (Potts et al. 2016a) is concerning, since more than 80% of flowering plants are animal-pollinated (Ollerton et al. 2011) and plant reproduction is often limited by pollinator service (Knight et al. 2005). Loss of pollinators may have far-reaching consequences. The keystone mutualist hypothesis posits that loss of a pollinator or other mutualist can precipitate a cascade of extinctions that affects entire communities or ecosystems (Cox et al. 1991; Christian 2001). Alternatively, pollinator extinction may have little effect. Widespread generalisation of pollination systems (Waser et al. 1996) suggests that other pollinators in a community can compensate for extinction (Kaiser-Bunbury et al. 2010; Burkle et al. 2013). Ultimately, the likelihood of plant extinction as a result of pollinator loss will depend on the degree of specialisation, the plant's breeding system, its demographic reliance on seeds (Bond 1994) and its community context.

Plants have long been thought to be specialised in terms of pollinators, with suites of flower traits adapted to different pollen vectors, i.e. pollination syndromes (Faegri & Van der Pijl 1966). More recently, generalisation has been considered to be common, with a continuum from broad generalisation to specialisation on single pollinator species (Johnson & Steiner 2000). The concept of pollination syndromes, although criticised, is often still a useful way to organise pollinators into functional groups (Ollerton et al. 2009; Fenster et al. 2004). The bird-pollination syndrome, known as “ornithophily”, is one of the well-recognised syndromes: many bird-pollinated plants have robust, reddish flowers, high volumes of dilute nectar and lack scent (Van der Pijl 1961). However, such floral traits do not always predict the ecological importance of bird pollinators for plants (Pauw 1998). Plants apparently adapted for bird pollination can also be pollinated by insects (Whelan et al. 2009). To determine which pollinators are important for maintaining plant populations, pollinator observations and selective exclusion experiments must be conducted.

Birds are prominent pollinators in many regions around the world (Şekercioğlu et al. 2016). In the hyperdiverse Cape Floristic Region (CFR) of South Africa, bird pollinators are particularly important: only four specialist nectar-feeding bird species pollinate over 300 plant species (Rebello 1987; Anderson et al. 2014). However, these crucial pollinators are threatened by habitat fragmentation, invasive alien plants and increased fire frequency (Pauw 2004; Fraser & Crowe 1990; Geerts et al. 2011; Chalmandrier et al. 2013). Birds pollinate many members of the dominant family Proteaceae and the species-rich genus *Erica*, two of the defining elements of the CFR’s fynbos shrublands (Bergh et al. 2014). Among the Proteaceae, the important genera *Protea*, *Leucospermum* and *Mimetes* contain numerous bird-pollinated species (Rebello 2001). It is estimated that 15% of the 680 *Erica* species in the CFR are bird-pollinated (Rebello et al. 1985; Oliver & Oliver 2002).

The breeding systems of flowering plants are diverse. Some plants self and do not need pollinators at all, some have mixed mating systems, while others rely completely on pollinators to reproduce (Barrett 2003). The CFR is renowned for intricate, specialised pollination systems and a wide range of breeding systems (Anderson et al. 2014). The Cape Proteaceae that are visited by nectar-feeding birds rely on bird pollination to varying degrees. Some *Protea* species suffer sharp declines in seed set when birds are excluded (Wright et al. 1991; Wright 1994; Schmid et al. 2015). Although many bird-pollinated *Protea* are self-compatible, they produce few seeds by autonomous selfing (Hargreaves et al. 2004; Schmid et al. 2015). In a number of *Protea* species with apparently ornithophilous flowers that are frequently visited by birds, seed set is not reduced in the absence of birds, since insects provide sufficient pollination (Coetzee & Giliomee 1985; Steenhuisen et al. 2012; Schmid et al. 2015).

A plant's extinction risk in the wake of pollinator loss will also depend on how important seeds are in its demography (Saatkamp et al. 2014). Many of the Cape Proteaceae are killed by the periodic fires that are typical of the region and thus rely completely on seeds to regenerate. Pollinators will be particularly important for the survival of such "reseeder" species (Bond 1994). Some *Erica* species also survive fires only as seeds, but others can resprout (Segarra-Moragues & Ojeda 2010). Plants that cannot produce sufficient seed banks before a fire due to lack of pollinators may become locally extinct (Van Wilgen 1981; Kraaij & Van Wilgen 2014).

Pollinators can play important roles in the assembly and function of plant communities (Sargent & Ackerly 2008; Pauw 2013), yet relatively few studies have assessed the effects of pollinator loss on plant communities in the field (but see Pauw (2007); Brosi & Briggs (2013); Lundgren et al. (2016)). Although bird pollinators are vital in various parts of the world, to our knowledge no studies have considered the community-wide effects of losing bird pollinators. Two important studies of bird pollinator extinction demonstrated declines of ornithophilous plants in the absence of birds, however, these studies considered only one or two plant species (Mortensen et al. 2008; Anderson et al. 2011). Other community-level work has assessed pollen limitation in hummingbird-pollinated plants, but did not exclude birds from flowers (Wolowski et al. 2013).

In this study, we investigate the effects of bird pollinator loss on the fecundity of communities of bird-visited plants in a part of the Cape Floristic Region. We experimentally excluded birds, but not insects, from entire communities; the first study to do so. This allowed us to assess potential emergent community-level effects that cannot be addressed by typical inflorescence-level or plant-level exclusion studies. A natural fire that occurred during our study provided an opportunity to consider the demographic effects of bird pollinator loss.

We address the following specific questions: 1) How specialised in terms of pollinators are plants with bird-adapted flowers? 2) How does the exclusion of bird pollinators affect the fecundity of bird-visited plants? 3) What are the demographic consequences of bird pollinator loss combined with a short fire return interval for plants? 4) How will plant community composition be affected by bird pollinator extinction?

Methods

Study site and species

We conducted our study in the Jonkershoek Nature Reserve near Stellenbosch. Six study sites were established in 4-year old Boland Granite Fynbos at altitudes of 380 – 467 m (Rebello et al. 2006). Sites number 1, 2 and 3 were on the valley's southwest-facing slopes

with granite-derived soil. Sites number 4, 5 and 6 were on the drier north-facing slopes, where the soil was sandier and the vegetation sparser. Coordinates of study sites: Site 1: 33°59'18.60"S, 18°58'20.87"E; Site 2: 33°59'24.92"S, 18°58'26.97"E; Site 3: 33°59'28.39"S, 18°58'44.04"E; Site 4: 33°59'31.69"S, 18°58'2.97"E; Site 5: 33°59'32.30"S, 18°57'57.48"E; Site 6: 33°59'32.65"S, 18°57'47.74"E. Three different types of plots were established at each site – for further description of the experimental setup, see Chapter 2.

We focused our study on plant species that conformed to the bird pollination syndrome, namely *Protea neriifolia* R. Br., *Protea coronata* Lam., *Protea repens* L., *Protea nitida* Mill., *Mimetes cucullatus* (L.) R. Br. (all members of the family Proteaceae) and *Erica plukenetii* L. (Ericaceae). *Protea neriifolia* was the most dominant species at Sites 1, 2 and 3, where it comprised 27% of total vegetation cover. At these sites *Protea coronata* was also among the dominant species, making up 9% of vegetation cover. *Mimetes cucullatus* occurred only at Site 3, where it ranked 6th in terms of percentage vegetation cover, although comprising only 3% of the total cover. On the other side of the valley, at Sites 4, 5 and 6, *Protea repens* made up 11% of vegetation cover and was the most dominant species at two sites. Here *Protea neriifolia* had cover of 7% and ranked 3rd, 7th and 8th in terms of overall dominance at the respective sites. *Protea nitida* occurred only at Site 5, where it made up 4% of cover, but it was much more dominant in certain other parts of the valley. *Erica plukenetii* occurred at Sites 5 and 6, where it made up 2% of vegetation cover. The vegetation was five years old when we did our vegetation survey, in older fynbos vegetation *Protea* sp. typically become more dominant. Insect-pollinated plant species that flowered at the same time as our focal species included *Diosma hirsuta*, *Oxalis tenuifolia*, *Leucadendron salignum*, *Brunia noduliflora* and a *Thesium* sp.

We selected study plots so that vegetation was as similar as possible among the different plots at a study site. To account for possible differences and to assess density dependent effects, we counted all the *Protea* and *Mimetes* individuals in all study plots.

Protea inflorescences could be divided into two broad morphological classes. *Protea neriifolia* (Fig. 1B) and *P. coronata* inflorescences never open widely and the flowers are covered in hairy fluff. The apple-green involucral bracts that make up the inflorescence of *P. coronata* remain very closed and inflorescences are capped with dense, white fluff. In contrast, the inflorescences of *P. repens* and *P. nitida* open widely and are hairless (Fig. 1C,E). *Mimetes cucullatus* flowerheads are bright red with contrasting white aggregations of flowers. *Erica plukenetii* subsp. *plukenetii* (Oliver & Oliver 2002), hereafter *Erica plukenetii*, has pale yellow-green flowers with long flower tubes and exerted brown anthers (Fig 1F). For Proteaceae nomenclature we follow Rebelo (2001).

Flower visitor observations

To assess the effect of bird exclusion on the pollinator fauna of our focal plant species we observed visits by insects and birds in our study plots. We conducted observations at Sites 1, 2 and 3, where peak *Protea* flowering was earlier, from 25 April to 12 May and at Sites 4, 5 and 6 we made observations from 23 May to 24 June. An inflorescence was considered “open” as soon as some of its flowers were in anthesis, i.e. when pollen presenters separated from the perianths. Flower visitors were observed simultaneously in the different plot types at a study site by three different observers. All flower visitors were recorded, but only visitors touching the reproductive structures of a flower were considered pollinators. Visitation rates were calculated as number of visits / number of inflorescences observed / hours of observation. We did not conduct pollinator observations on rainy or windy days. Birds were observed in the early morning (8:00 to 10:30) and late afternoon (16:30 to 18:00), when they were most active. Insects were observed during 20 minute observation periods in between the two bird watching sessions. Insect flower visitors were caught in vials filled with 70% ethanol for identification. Voucher specimens of Hymenoptera are housed at the Iziko Museum in Cape Town and other insects at the Department of Botany and Zoology, Stellenbosch University. We observed bird flower visitors for a total of 40 hours and insects for a total of 60 hours.

Seed set

We collected *Protea* seed cones after our entire study area burned down on 9 March 2015. The *Protea* species in our study are serotinous, only releasing their seeds after a fire, except for *P. nitida* which releases its seeds 9 to 12 months after flowering. We harvested *Protea* seed cones from all study plots in the days after the fire, before the cones opened to release their seeds. We collected 12 cones of each species at random from each plot, or the maximum number available. In the case of *P. coronata* few cones could be collected. Only cones from the 2014 flowering season were harvested and each cone was sampled from a different plant individual.

In order to test for autonomous selfing and pollen limitation, we had applied three different treatments to *Protea* inflorescences in all enclosure types between April and June 2014: 1) “bag” – exclusion of all pollinators by means of organza mesh bags; 2) “hand pollen addition” – application of outcross pollen to stigmas of all open flowers; and 3) “control” – no manipulation. Since the fire destroyed the tags that marked treated inflorescences, we could not identify treated inflorescences. There was thus a small chance that we harvested cones after the fire that had been bagged or subject to hand pollen addition without knowing. In open plots and bird exclusion plots we had treated ten inflorescences per treatment and in the smaller shade control plots we had treated seven to ten inflorescences per treatment.

For *P. neriifolia* the probability of collecting a bagged or hand pollinated cone was 10%, thus approximately one in 12 of the cones we collected in open and bird enclosure plots may have been contaminated by the inflorescence treatments. In the smaller shade control plots where there were fewer cones, this probability was considerably higher. Bagging of cones would be expected to have lowered the proportional seed set below control levels, while hand pollen addition would likely have increased it above control levels. Outliers in both directions in the proportional seed set data would thus result. We account for this in our statistical analysis. *Protea coronata* and *P. repens* received all three treatments at all sites where the species occurred. *Protea neriifolia* was subjected to all treatments at Sites 4, 5 and 6, but was only bagged and not hand pollinated at Sites 1, 2 and 3. *Protea nitida* did not receive any inflorescence treatments.

We determined the proportional seed set of each *Protea* cone by counting the number of “filled” and “empty” achenes. A “filled achene” contains a fully developed, fertilised ovule and is filled with white endosperm when sectioned; in *P. neriifolia* it feels plump between the fingers. An “empty” achene (i.e. dry sterile floret) has an unfertilised ovule and consists of only woody tissue; in *P. neriifolia* it is smaller, flatter and feels harder than a filled achene. We assessed *P. neriifolia* achenes by feeling, after validating our method by first feeling and then sectioning a subset of achenes. Achenes of the other *Protea* species were sectioned.

Ten *Erica plukenetii* plants were selected in each enclosure treatment at study sites 5 and 6, on 22 November and 5 December 2014. The species did not occur at any other study sites. On each plant individual a branch was marked and the number of flower buds or open flowers on the branch was recorded. The number of flowers per branch ranged from 15 – 90, with a mean of 33 (median 30). Marked branches were collected on 7 January 2015 and fruit set was determined by cross-sectioning the ovaries. An ovary was counted as a fruit when the ovary was clearly thicker than the immature ovaries of open flowers and when partially or fully developed seeds were present. Fruit set was calculated as the number of fruits per branch divided by the number of flowers originally marked on the branch.

We measured the seed set of *Mimetes cucullatus* at Site 3, the only site in our study area where the species occurred. *Mimetes* species carry their flowers in an unusual arrangement. Flowers are aggregated in an inflorescence, technically a “pseudanthium”. Pseudanthia are arranged into a conflorescence, the top part of a single flowering branch. Each flower contains a single ovule. For *M. cucullatus* in our study 3 – 6 (median 4) flowers formed a pseudanthium, with 3 – 22 (median 12) pseudanthia per conflorescence. We marked all the flowering *M. cucullatus* individuals in our plots early in the flowering season, on 2 November 2014. On each plant we selected 1 – 3 conflorescences and recorded the number of

pseudanthia for each confluence. Only pseudanthia that were in bud, open or had flowered in the current season were counted. We collected the marked confluences on 12 December 2014, when seeds were nearly mature, and counted the seeds. Obvious signs of seed predation by insects were recorded.

Post-fire seedling counts

Protea seeds have hairs that enable them to disperse by tumbling along the ground in the wind when they are released after a fire (Bond 1988). After our study area burned down we erected seed dispersal barriers of 30 cm high shade cloth around our study plots. The barriers were intended to contain the seeds of plants that grew in a plot and to prevent seeds from outside entering a plot. We would thus be able to relate seedling recruitment in plots to the exclosure treatments applied to plots before the fire. The barriers at Site 1 were erected three to four days after the fire, those at Sites 2, 4 and 5 after four to five days and those at Sites 3 and 6 after five to six days. We estimate that 70% of cones had released their seeds within 24 hours after the fire and that 90% of cones had opened within a few days. Unlike the *Protea* species, *Mimetes cucullatus* seeds are released after every year of flowering and dispersed by ants to underground nests.

The plastic netting of the bird exclosures and shade control roofs was melted by the fire and formed patchy crusts on top of the soil surface. We removed these plastic crusts in the month after the fire (April 2015) using small spades, taking care to minimise soil disturbance.

We counted the seedlings of the *Protea* sp., *Leucospermum lineare* and *Mimetes cucullatus* between 1 February and 17 April 2016. To ensure comparability among plots at a site we completed all plots at a particular site within a given period of two weeks, before moving on to the next site. Seedlings were counted in subplots of 2.5 x 2.5 m; eight subplots per 20 x 20 m plot and two subplots per 10 x 7 m shade plot. We counted both dead and live seedlings.

Statistical methods

All data analyses were done in R version 3.3.0 (R Core Team 2016). Linear mixed models (LMMs) and generalised linear mixed models (GLMMs) were fit using the functions `lmer` and `glmer` in the package `lme4` (Bates et al. 2015), unless indicated otherwise.

Flower visitors

Data on insect flower visitors to the different *Protea* species were analysed with GLMMs with Gaussian errors and log links, using the function `glmmPQL` in the package `MASS` (Venables & Ripley 2002), with exclosure type as fixed effect and (site/observation period) as random effects. Observation period was added as random effect in order to account for pseudo-replication from multiple observation periods within a plot. Bird visitation rate was

compared between open plots and shade control plots with a Wilcoxon signed-rank test, since observations were paired in time between open and shade control plots and the data were non-normal. Since open plots were always observed by one observer and shade plots always by two observers for bird visits, we summed observations in shade plots for a given observation period and divided by two, to account for the difference in sampling effort.

Seedset

For *Protea neriifolia* seed set data we accounted for the possible presence of hand pollinated and bagged samples among the control inflorescences in two different ways. For the first approach we created box and whisker plots for each site and removed positive outliers at Sites 4 – 6, i.e. points larger than 1.5 times the upper quartile. Only three data points were removed in this way. For the second approach, we removed data points at both the top and the bottom of the distribution of each plot. For open plots and shade control plots, the two largest and two smallest values within a plot were removed. In shade control plots we removed only the largest value and the smallest value, since sample sizes were smaller. At Sites 4 – 6, we removed both the largest and the smallest values in each plot, while at Sites 1 – 3 we removed only the smallest values in a plot, since only the bagging treatment was applied at Sites 1 – 3. We analysed the dataset from each approach in the same way and compared the results. The two approaches lead to the same inferences. Analysis of the full data set (no data points removed) also gave similar results. We report the results for the first approach. Seed set data were overdispersed relative to a binomial distribution, thus we fit a beta-binomial GLMM (Harrison 2015), using the package `glmmADMB` (Skaug et al. 2016), with enclosure type, slope aspect and *Protea* density as fixed effects and site as random effect.

Data for *Protea repens* were analysed in the same manner as for *P. neriifolia*. We present the results of removing three obvious outliers from the dataset and fitting a beta-binomial GLMM using the package `glmmADMB`. We obtained similar results from the alternative approach of removing the two highest and two lowest values (except for the smaller sample sizes of Site 5 Open and Site 6 Shade where only the single highest and lowest values were removed). Analysis of the full data set (no data points removed) also gave similar results. Accounting for overdispersion by adding an observation level random effect to binomial models (Harrison 2015) gave similar results to the beta-binomial models.

Protea nitida's proportional seed set data were overdispersed relative to a binomial distribution, thus we fit a "quasibinomial" GLM, with enclosure type as fixed effect.

Fruit set data for *Erica plukenetii* were overdispersed relative to a binomial distribution, thus we added "plant individual" as observation level random effect (Harrison 2015) to our GLMM.

The model had enclosure type as fixed effect and plant individual and site as random effects. As alternative approaches we also fit a beta-binomial GLMM (Harrison 2015) using the package `glmmADMB` and a GLM with “site” as fixed effect. Both these alternatives lead to the same inferences as the first model. We report only the first model’s results.

Mimetes cucullatus seed count data were zero-inflated relative to a Poisson distribution, thus we fit a zero-inflated GLMM, using the package `glmmADMB`. We included enclosure type as fixed effect and plant individual as random effect.

Post-fire seedling emergence

For *Protea neriifolia* we fit the number of seedlings per adult in a GLMM with a Gaussian error distribution and a log link function, with enclosure type as fixed effect and site as random effect, but the model failed to converge. We thus dropped site as random effect, fitting a GLM. In order to account for site differences, we also fit a GLMM with the raw seedling counts (not divided by number of adults) as response variable, with adult density and enclosure type as fixed effects and site as random effect, with negative binomial errors and a log link, using the function `glmer.nb` in package `lme4`. We report only the results from this GLMM, since the GLM on seedlings per adult gave the same inferences.

For *Protea coronata* and *Protea repens* the number of seedlings per adult was fit in a GLMM with a Gaussian error distribution and a log link function, with enclosure type as fixed effect and site as random effect.

Results

Flower visitors

Protea neriifolia was visited mainly by Cape Sugarbirds that mostly probed inflorescences from above (78% of all Cape Sugarbird visits, $n = 54$), thus acting as pollinators (79% of all pollinating visits by birds, $n = 53$). Orange-breasted sunbirds visited *Protea neriifolia* less often, typically thieving nectar from the side of inflorescences (74% of all Orange-breasted Sunbird visits, $n = 39$), but sometimes also pollinating by probing from above (19% of all pollinating visits by birds, $n = 53$). Malachite Sunbirds were present in our study area, but we recorded only a single Malachite Sunbird visit to *P. neriifolia*. A number of different insect pollinator species visited *P. neriifolia* (Fig. S3.1). The only insect pollinating visitor that was common was a beetle in the genus *Chirodica* (Chrysomelidae: Alticidae) (Fig. 3.1D). Seven other Coleoptera species visited *P. neriifolia* in low numbers, with the Protea Beetle, *Tricostetha fascicularis*, being observed only once. Occasional flower visitors included four non-ant Hymenoptera, three Diptera, two Hemiptera species and ants. We recorded

significantly more insect pollinator visits per inflorescence per hour to *P. neriifolia* in open plots than in bird enclosure and shade control plots (Fig. 3.2B)(Table S3.1).

Protea repens was pollinated by Cape Sugarbirds, Orange-breasted Sunbirds and insects. Flocks of Red-winged Starlings, *Onychognathus morio*, also visited inflorescences on two occasions, collecting pollen on their heads. The most prominent insect pollinators visiting *Protea repens* were the *Chirodica* beetle that also visited *P. neriifolia*, as well as honey bees (*Apis mellifera capensis*). Honey bees did not always act as pollinators as they also drank nectar from inflorescences without contacting the pollen presenters. The *Chirodica* beetles climbed up styles to feed on pollen during hand pollen supplementation, indicating that these beetles contact pollen presenters and may thus act as pollinators; although pollen feeding can also be detrimental to pollination. Inflorescences were also visited by six other Coleoptera species and three other non-ant Hymenoptera species, as well as a Diptera species and ants. There were no differences in insect pollinator visits per inflorescence per hour to *P. repens* among different enclosure types (Fig. 3.2B)(Table S3.2).

Protea repens received fewer bird visits than *Protea neriifolia* (Fig. 3.2A) and there was little overlap between *P. repens* and *P. neriifolia* in terms of insect visitors (Fig. S3.1).

Protea coronata was pollinated by Cape Sugarbirds and occasionally by Orange-breasted sunbirds, but we saw few birds visiting *P. coronata*. The *Chirodica* beetle also found on the other *Protea* was the only common insect visitor. Four other Coleoptera species also visited *P. coronata*. There were no differences in insect pollinator visits per inflorescence per hour to *P. coronata* among different enclosure types (Table S3.3).

Bird visitors to *Protea nitida* were Cape Sugarbirds, Malachite Sunbirds, Orange-breasted Sunbirds and twice flocks of Red-winged Starlings, but we did not conduct intensive observations on *P. nitida*. Honey bee visits were common and three Coleoptera, three Diptera and a Hemiptera also paid visits.

Erica plukenetii was pollinated by Orange-Breasted Sunbirds and some flowers had pierced corollas due to insect robbing. *Mimetes cucullatus* received pollinating visits from Cape Sugarbirds and Orange-breasted Sunbirds, but we saw few bird visitors. Ants were the most common insect visitors to *M. cucullatus*, thieving floral nectar and visiting extra-floral nectaries. Few other insects visited *M. cucullatus*.

Bird pollinators moved freely underneath shade control roofs. There was no difference between bird visitation rate under shade control roofs (mean 0.18 ± 0.51 (SD) visits per

flower per hour, $n = 115$) and open plots (mean 0.4 ± 1 (SD) visits per flower per hour, $n = 112$) (Wilcoxon signed rank test, $V = 410$, $p = 0.23$).

Seedset

In *Protea neriifolia* average proportional seed set in the bird exclusions was reduced by 46% relative to open controls. The proportional seed set of *P. neriifolia* was significantly lower in bird exclusion plots (mean = 0.020 ± 0.042 (SD), $n = 67$) than in open plots (0.037 ± 0.042 (SD), $n = 67$) and shade control plots (mean = 0.057 ± 0.086 (SD), $n = 48$) (Fig. 3.3A) (Table 3.1). Slope aspect was influential (Table 3.1) and proportional seed set was significantly higher at south-facing sites (mean = 0.052 ± 0.073 (SD), $n = 96$) than at north-facing sites (mean = 0.018 ± 0.027 (SD), $n = 86$).

Protea repens did not suffer any reduction in proportional seed set in the absence of birds, with similar values in bird exclusion plots (mean = 0.14 ± 0.10 (SD), $n = 35$) as in open plots (mean = 0.16 ± 0.08 (SD), $n = 33$) and shade control plots (mean = 0.15 ± 0.10 (SD), $n = 31$) (Fig. 3.3B) (Table 3.2).

In *Protea coronata* mean proportional seed set was comparable among bird exclusion plots (0.023 ± 0.031 (SD), $n = 10$), open plots (0.024 ± 0.028 (SD), $n = 12$) and shade control plots (0.042 ± 0.022 (SD), $n = 5$) (Fig. 3.3C). However, due to the small sample sizes and unbalanced sampling we did not do statistical analysis. No data could be collected at Site 1 and at Site 2 no data were available for the shade control plots.

For *Protea nitida* mean proportional seed set was similar in the bird exclusion (0.082 ± 0.076 (SD), $n = 8$), open (0.047 ± 0.038 (SD), $n = 10$) and shade control plots (0.079 ± 0.060 (SD), $n = 5$) at Site 5 (Fig. 3.3D) (Table 3.3).

In addition to *P. neriifolia*, two other plant species experienced significant reductions in reproductive output when birds were excluded. *Erica plukenetii* saw its proportional fruit set being reduced by 31% on average relative to open controls. Proportional fruit set was significantly lower in bird exclusion plots (mean = 0.46 ± 0.19 (SD), $n = 19$) than in open plots (mean = 0.67 ± 0.18 (SD), $n = 19$) or shade control plots (mean = 0.65 ± 0.14 (SD), $n = 16$) (Fig. 3.3E) (Table 3.4). In *Mimetes cucullatus*, exclusion of birds lowered seed set by 92% on average relative to open controls and by 54% relative to shade controls. Seed set was significantly higher for conflorescences in open plots (mean = 1.81 ± 2.46 (SD) seeds, $n = 22$) and shade control plots (mean = 0.83 ± 2.12 (SD) seeds, $n = 12$) than in bird exclusion plots (mean = 0.14 ± 0.36 (SD) seeds, $n = 21$) (Fig. 3.3F) (Table 3.5).

Post-fire seedlings

Seedling emergence

Protea neriifolia seedling emergence did not differ significantly among exclosure types across all sites (Fig. 3.4A) (Table 3.6). Adult plant density did not have a significant effect on seedling emergence (Table 3.6). The mean number of *P. neriifolia* seedlings emerging per adult were 3.5 ± 3.7 (SD) in bird exclosure plots, 4.4 ± 5.1 (SD) in open control plots and 3.2 ± 1.7 (SD) in shade control plots. The number of *Protea repens* seedlings emerging per adult plant was similar among exclosure types across all sites (Figure 3.4B) (Table 3.7). For *Protea coronata* there was also no difference in the number of seedlings emerging per adult plant among the different exclosure types across sites (Figure 3.4C) (Table 3.8). In *Mimetes cucullatus* seedling emergence per adult was lower in the bird exclosure (median = 0, IQR = 0.8, n = 8) than in the open plot (median = 1.1, IQR = 4.8, n = 8) and shade plots (median = 7.6, IQR = 8.6, n = 4), however, due to the small sample size and zero-inflation we did not conduct a statistical test (Fig. 3.4D). We encountered only two *P. nitida* seedlings, in the shade control and bird exclosure plots at Site 5. We searched for *Erica plukenetii* seedlings, but at the time of data collection they had either not yet emerged or were indistinguishable.

Seedling mortality

We found no evidence for density dependent mortality of *Protea* seedlings at the 2.5 x 2.5 m scale. In *P. neriifolia* the density of conspecific seedlings did not explain proportion seedling mortality (Figure S3.2), nor did the combined seedling densities of *P. neriifolia*, *P. coronata*, *P. repens*, *L. lineare* and *Mimetes cucullatus* (Figure S3.3). The proportion seedling mortality of *Protea neriifolia* did not differ among plot types (Figure S3.4). For *P. repens* proportion seedling mortality was also not explained by conspecific seedling density (linear regression, $r^2 = 0.03$, $p = 0.16$), nor by the combined seedling densities of *P. neriifolia* and *P. repens* (linear regression, $r^2 = 0.03$, $p = 0.17$). In *P. coronata* we observed the same pattern: no relationship between seedling mortality and conspecific seedling density (linear regression, $r^2 = 0.02$, $p = 0.3$) or the combined seedling densities of *P. neriifolia*, *P. coronata*, *L. lineare* and *Mimetes cucullatus* (linear regression, $r^2 = 0.0009$, $p = 0.8$).

Discussion

Loss of bird pollinators reduced the fecundity of some bird-visited plant species in fynbos communities: the seed set of *Protea neriifolia*, *Mimetes cucullatus* and *Erica plukenetii* declined significantly in the absence of birds. Other bird-visited plant species were not affected by the loss of bird pollinators: *Protea repens* and *Protea nitida* maintained seed set without birds, likely thanks to insect pollinators. This is the first study to exclude birds from entire communities and thus the first to document the community-level effects of bird pollinator extinction.

Cape Sugarbirds and Orange-breasted sunbirds were the main bird pollinators of all our study species. Cape Sugarbirds were the most important bird pollinators for *P. neriifolia* and *P. coronata*. Orange-breasted Sunbirds were the main pollinator of *Erica plukenetii*, were among the pollinators of *P. repens* and *P. nitida* and mostly thieved nectar from *P. neriifolia*. Malachite Sunbirds occurred but were uncommon and inconsistently present, in accordance with their itinerant nature (Fraser 1997). Our occasional observations of Red-winged Starlings opportunistically visiting *P. repens* and *P. nitida* agree with evidence that generalist birds can also sometimes act as pollinators (Johnson & Nicolson 2008).

In addition to birds, various insect pollinators visited the flowers of *P. repens*, *P. nitida* and *P. neriifolia*. Other bird-pollinated plant species like *P. coronata* and *M. cucullatus* received few insect pollinator visits. Honey bees, *Apis mellifera capensis*, were important for *P. repens* and *P. nitida*, but did not visit *P. neriifolia* and *P. coronata*. This pattern may be due to the different inflorescence morphologies: *P. repens* and *P. nitida* have wide open inflorescences with hairless flowers, while *P. neriifolia* and *P. coronata* inflorescences are more closed and their flowers are covered with fluffy hair that may impede access to pollen and the nectar at the bottom of inflorescences (Fig. 3.1). Honey bees collecting pollen are likely to be good pollinators, but at high densities they may display interference competition with Cape Sugarbirds (Geerts & Pauw 2011). In contrast to honey bees, various beetle species visited both types of *Protea* inflorescences (Fig. S3.1). A beetle species in the genus *Chirodica* (Chrysomelidae) (Fig. 3.1D) was by far the most numerous insect flower visitor to *P. neriifolia*, *P. repens* and *P. coronata*. They often moved between inflorescences and among plants and likely contributed to pollination. Previous work also found *Chirodica* beetles to be the most abundant insects in *P. repens* inflorescences and showed that *Protea* pollen adheres to insects (Coetzee & Giliomee 1985). Besides the *Chirodica* beetles, a number of other small beetle species visited *P. repens* and *P. neriifolia*, but each plant species had its own collection of beetle visitors (Fig. S3.1). Small beetles in general are thought to be pollinators of *P. repens* and many kinds have been collected in *P. neriifolia* inflorescences (Coetzee & Giliomee 1985; Coetzee 1989). Similar results have been found outside the CFR: Various bird-pollinated plants in southwest Australia also frequently receive insect visits and some ornithophilous species are pollinated primarily by insects (Phillips et al. 2010).

Although insect visitation rates were much higher than bird visitation rates, bird pollinators are important, since their large bodies transfer much greater quantities of pollen and they most likely contact pollen presenters more consistently than insects. The daily nectar requirements of bird pollinators also mean that they must visit many inflorescences (Collins

& Rebelo 1987), transferring more pollen than less mobile insects (excluding active honey bees).

We observed that flying insects moved freely through the 2 x 2 cm mesh of the bird enclosure netting. There were no differences in insect visitation rates to *P. repens* and *P. coronata* among open, shade control and bird enclosure plots (Tables S3.2, S3.3). However, in *P. neriifolia* insect visitation rate was significantly lower in bird enclosures and shade control plots than in open plots (Table S3.1) (Fig 3.2B). A similar trend was seen in *P. repens*, but differences were not significant (Table S3.2) (Fig 3.2B). These results suggest that some effect of the bird enclosures and shade controls reduced insect activity directly, or indirectly by somehow changing the attractiveness of inflorescences. If nectar (and possibly pollen) accumulated in inflorescences in the absence of birds removing it, as in *Leucospermum lineare* (Chapter 2), then one would expect elevated insect visitation in bird enclosures. Mesopredator release may also explain lower insect visitation in bird enclosures, spiders that prey on insects may increase in the absence of birds (Rogers et al. 2012).

Our findings confirm that *Protea* species visited by bird pollinators rely on birds to varying degrees (Coetzee & Giliomee 1985; Wright et al. 1991; Wright 1994; Schmid et al. 2015). In *Protea neriifolia* proportional seed set declined by half in the absence of birds and in general proportional seed set of this species was low (Fig. 3.3A)(Table 3.1). This corresponds to previous work that also found a significant reduction of 27% in *P. neriifolia* seed set when excluding birds from single inflorescences with wire cages, as well as low seed set in general (Horn 1962; Wright et al. 1991). For *Protea coronata* we do not have enough seed set data to determine the role of birds (Fig. 3.3C), but its “closed” bearded inflorescence morphology and few insect visitors suggest that *P. coronata* is bird pollinated. The low seed set of *P. coronata* in our study is similar to older findings of 3.5% proportional seed set in this species (Horn 1962). In *Protea repens* seed set did not decline with bird exclusion and mean proportional seed set was much higher than the other *Protea* species (Fig. 3.3B). Previous studies that excluded birds from single inflorescences also concluded that *P. repens* does not need bird pollinators to set seed and is adequately pollinated by insects (Coetzee & Giliomee, 1985; Schmid et al., 2015). Insect pollinators are crucial for *P. repens*, since it produces little seed by autonomous selfing (Schmid et al. 2015). For *Protea nitida* we also found no reduction in seed set in the absence of birds (Fig. 3.3D), in accordance with previous work (Wright et al. 1991).

It is possible that our *Protea* seed set data from cones harvested after the fire included some inflorescences that had been hand pollen supplemented or bagged, but it is highly unlikely

that this influenced our findings, since the likelihood of harvesting a treated cone was small and our statistical analyses accounted for the possible outliers resulting from the treatments.

Taken together, our pollinator observations and seed set results indicate that *Protea neriifolia* requires bird pollinators, while *P. repens* and *P. nitida* can maintain seed set with only insect pollination, (possible also autonomous selfing in the case of *P. nitida*). Lower bird visitation rate to *P. repens* than to *P. neriifolia* (Fig. 3.2A) possibly contributed to the lack of effect of bird exclusion on *P. repens*, but the maintenance of seed set in *P. repens* was most likely due to insect pollination, also considering previous work on the species (Schmid et al. 2015). Birds may have preferred *P. neriifolia* over *P. repens* during our observations, but in general *P. repens* is known to receive many bird visits (e.g. (Geerts & Pauw 2011). Although insect visitation to *P. neriifolia* was lower in bird exclosures than in open plots, reduced seed set is explained by the lack of birds and not by lower insect visitation, since seed set was not reduced in shade control plots where insect visitation frequency was just as low as in bird exclosure plots (Fig. 3.2B, Fig. 3.3A).

Our findings and those of previous studies suggest a pattern of bird pollinator dependence in *Protea*. Species that show seed set declines without birds are either in the section *Speciosae* (“Bearded Sugarbushes”), with evidence for *P. neriifolia*, *P. laurifolia* and *P. magnifica*, or in the section *Ligulatae* (“Spoon-bract Sugarbushes”), with evidence for *P. compacta*, *P. eximia* and the summer rainfall region species *P. roupelliae* (Wright et al. 1991; Wright 1994; Hargreaves et al. 2004; Schmid et al. 2015). In contrast, bird-visited *Protea* species that maintain seed set without bird pollinators are in various sections of the genus, with evidence for *P. repens*, *P. nitida*, *P. cynaroides* and *P. longifolia* (Wright et al. 1991; Schmid et al. 2015). However, *P. longifolia* is also in the section *Ligulatae*, thus this section contains species that respond in both ways to bird loss. In an assessment of these and some other *Protea* species, inflorescence morphology traits did not predict bird dependence (Schmid et al. 2015). Further work informed by phylogeny is required to determine the evolutionary patterns of *Protea* pollination modes.

Mimetes cucullatus produced almost no seeds in the absence of avian pollinators (Fig. 3.3F). This is the first study to experimentally determine the importance of bird pollinators for this species, the most widespread of *Mimetes*. Previous work on *M. hirtus* found a similar result when excluding birds from inflorescences with wire cages (Collins 1983). The mean natural proportional seed set of *M. cucullatus* (17%) is lower than that of *M. hirtus* (35%), which is expected, since the former is a resprouter while the latter is a reseeder (Collins 1983). Although a resprouting species like *M. cucullatus* is less reliant on seeds and could

persist by resprouting for many years, it would become the “living dead” without its pollinators and eventually go extinct (Bond 1994).

Erica plukenetii had significantly lower seed set when excluded from its bird pollinator, the Orange-breasted Sunbird (Fig. 3.3E). Our findings prove the expectation of bird pollination based on the flower morphology of long corollas and brush-like, protruding anthers (Rebello et al. 1985) and on observations of the relationship between floral morphology and flower visitors in the various subspecies of *Erica plukenetii* (Van der Niet et al. 2014).

Besides reduction in the quantity of seeds, loss of bird pollinators may also reduce the quality of seeds if inbreeding occurs (Forrest et al. 2011). This can occur if a plant relies on autonomous selfing in the absence of birds, as shown in *Leucospermum lineare* (Chapter 2), as well as in plants normally pollinated by both insects and birds, such as *P. repens* and *P. nitida*. Birds may be better outcrossing pollinators than insects, since they carry pollen over longer distances and have shorter within-plant foraging bouts (Collins & Rebello 1987; Llorens et al. 2012), although in the beetle-pollinated, bird-visited species *Protea caffra* outcrossing rates are similar with and without birds (Steenhuisen et al. 2012). In many bird-pollinated *Protea* seed quality as indicated by germination does not seem to be lower when birds are excluded (Schmid et al. 2015). The seed quality of the *Protea* species in our study has not been assessed under different pollination regimes and future studies should investigate this. Reduced seed quality in the absence of birds may compound the demographic effects of lower seed set and can also have significant consequences by itself (Chapter 2).

Protea fecundity can vary substantially with the density of adult plants, with lower seed set above certain adult densities resulting in lower seedling to parent ratios (Bond et al. 1995). In our study the combined adult density of *Protea* species in a community did not have an influence on the seed set of any of the *Protea* species (Tables 3.1, 3.2, 3.3). This may be because density dependent effects on fecundity had not yet manifested in *Protea* stands that were only six years old. Slope aspect was important for *Protea neriifolia*: we found higher seed set on the cooler, south-facing slopes than on the hotter north-facing slopes (Table 3.1). Soil differences may also have caused this pattern, the south-facing side of the valley had finer clay soils which likely contained more nutrients than the more sandy soil of the north-facing side. *Protea neriifolia* was the only *Protea* that occurred on both sides of the valley.

Lower seed set or reduced seed viability due to pollinator loss may result in reduced seedling recruitment (Anderson et al. 2011; Chapter 2). Since *P. neriifolia* seed set was significantly lower in bird exclosures, we expected reduced seedling recruitment in bird

exclosures after fire, but there were no differences among plot types (Fig. 3.4A) (Table 3.6). This lack of difference was likely due to the movement of the *Protea* tumble seeds by wind prior to the erection of seed dispersal barriers. Most seed cones had released their seeds within the first day after the fire, but the barriers could only be erected a few days after the fire. Thus seeds released by plants in a particular plot did not stay in that plot and seeds also moved into study plots from outside. This unfortunately precluded any inferences about the effects of bird exclusion on seedling recruitment. We also found no differences in *P. repens* and *P. coronata* seedling recruitment among plot types (Fig. 3.4B, 3.4C) (Tables 3.7 and 3.8). If the seeds had not moved, the post-fire seedling recruitment of *Protea repens* in bird exclosure plots might have been reduced if seed viability was lower without bird pollinators.

Mimetes cucullatus seedling recruitment did not appear to differ among treatments (Fig. 3.4D). However, this may be for experimental rather than biological reasons. We excluded birds from this species for two flowering seasons, but during the second flowering season (2014) we harvested many seeds to assess seed set. Furthermore, much of the soil seed bank may have originated from flowering in the years before the bird exclosures were erected. As a resprouter *Mimetes cucullatus* flowers in the first year after fire and profusely in the second year (pers. obs.). Bird exclosures were erected three flowering seasons after the fire of 2009.

Seedling mortality of *Protea* species was not dependent on the density of conspecific seedlings nor on the density of all Proteaceae seedlings (Fig S3.2, S3.3), thus strong intra- or interspecific competition in our *Protea* species does not manifest in the first year post-fire. This contrasts with (Maze & Bond 1996), who found significant first-year density dependent seedling mortality in *P. neriifolia*, at densities comparable to our study. Our findings are also contrary to high first-year density-dependent seedling mortality in Australian *Banksia* (Lamont et al. 1993).

If fire return intervals are too short for plants to produce enough seed for replacement, reseeders species, such as many *Protea*, may become locally extinct (Van Wilgen 1981). Our study area burned when the vegetation was six years old, a short interval for the region (Kraaij & Van Wilgen 2014). Overall, *Protea* seedling densities one year after the fire were high enough to replace adults, with mean seedling to adult ratios of 3.4 ± 3.5 (SD) for *P. neriifolia*, 4 ± 4.6 (SD) for *P. coronata* and 8.7 ± 6.8 (SD) for *P. repens*. However, mortality in the following years will still reduce plant numbers and since *Protea* seedlings quite often emerged in dense clumps (tumble seeds accumulate in depressions or against obstacles) this may be substantial. Wind-dispersed *Protea* seedlings were much less frequently clumped than seedlings of ant-dispersed species like *Leucospermum lineare* (Chapter 2).

Extensive previous work on *Protea* demography indicates that the seedling to parent ratios we observed will be sufficient to maintain adult populations (Bond et al. 1995; Kraaij et al. 2013; Kraaij & Van Wilgen 2014; Treurnicht et al. 2016). Earlier work in our study area showed that a four year fire return interval leads to local extinction of *Protea* and other reseeders (Van Wilgen 1981).

We show that loss of bird pollinators can cause important changes in plant communities. The most dominant species in many of our study communities, *Protea neriifolia*, will likely be lost without its bird partners. Two less abundant species, *Mimetes cucullatus* and *Erica plukenetii* will likely also disappear from the communities if their bird pollinators go extinct, thus reducing community diversity. Collectively, the plant species that rely on birds, including *Leucospermum lineare* (Chapter 2), make up an average of 19% (range 8 – 42%) of the vegetation cover in our study communities.

Protea repens, often dominant where it occurs, and *P. nitida* will persist thanks to insect pollination. Will these persisting *Protea* species be able to fill the gap left by the loss of *P. neriifolia*? *Protea repens* was completely absent from our study sites on the south-facing side of the valley, where *P. neriifolia* and *P. coronata* dominated. This may be due to the superior competitive ability of *P. neriifolia* and *P. coronata* at cooler, wetter sites with richer soil. The fact that *P. repens* occurs in a very wide range of habitats across its distribution (Rebello 2001) suggests that may not be fundamentally excluded from the parts of our study area where it did not occur.

Protea neriifolia is one of the most widespread *Protea* species and is often dominant where it occurs. The loss of dominant overstory *Protea* species like *P. neriifolia* will have far-reaching effects on fynbos ecosystems. Besides *P. neriifolia*, a number of other bird-obligate *Protea* species have been specifically identified in the literature (Wright et al. 1991; Schmid et al. 2015) and considering knowledge of flower visitors many other *Protea* likely also depend on bird pollinators. Overstory *Protea* species fulfil a variety of ecological roles, one example is that they may maintain understory species diversity (Vlok & Yeaton 1999) and can facilitate shallow-rooted sub-shrub and grass plants by bringing water and nutrients from deeper underground closer to surface (Hawkins et al. 2009).

Species that are not dominant in communities can still play important functional roles. *Erica plukenetii* and *Mimetes cucullatus* are the most important nectar resources for birds in November and December in our study area, since few other bird pollinated plants flower at that time (Rebello et al. 1984). Likewise, *Leucospermum lineare* is an important nectar resource when it flowers in October and November. Specialised nectar-feeding birds require nectar throughout the year. If these plant species decline with low abundance of bird

pollinators there may thus be feedback effects leading to further declines in bird pollinator numbers. Ultimately the populations of all the many plant species that rely on bird pollinators could also collapse.

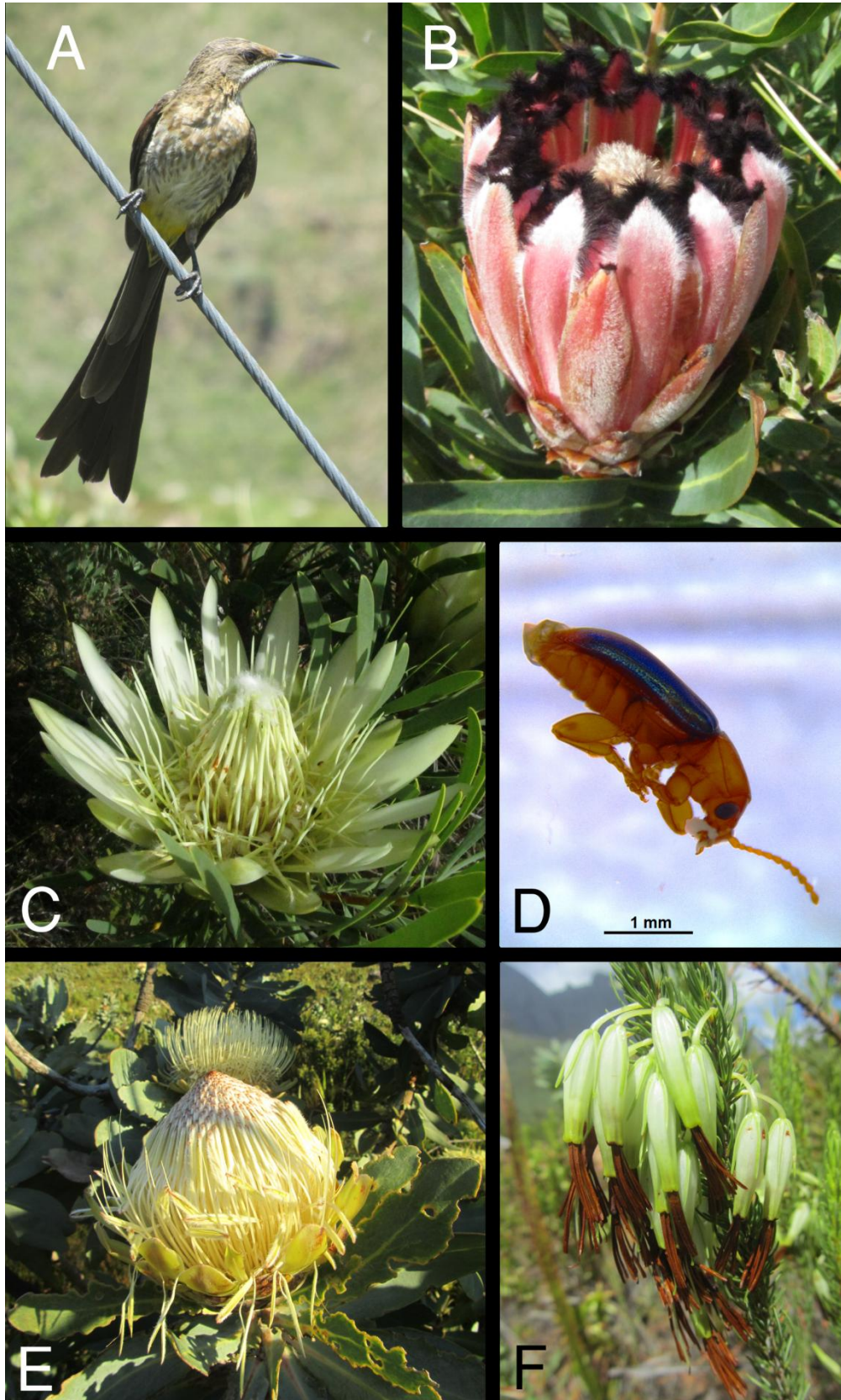


Figure 3.1. A) Cape Sugarbird; B) *Protea neriifolia*; C) *Protea repens*; D) *Chirodica* sp. (Coleoptera: Chrysomelidae); E) *Protea nitida*; F) *Erica plukenetii*.

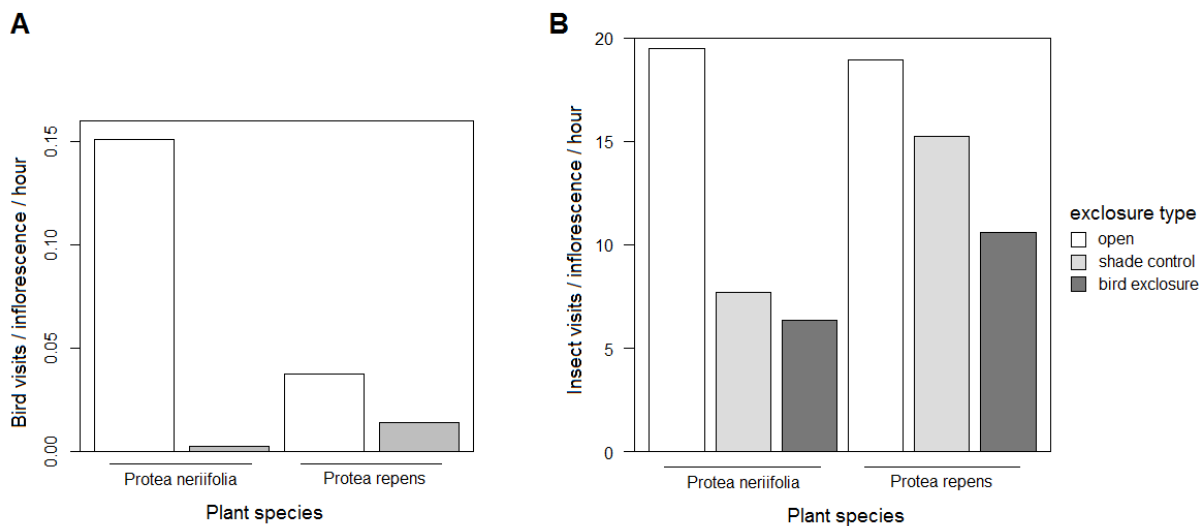


Figure 3.2. A) Sum of bird visits per inflorescences observed per hours of observation to *Protea neriifolia* and *Protea repens* in open plots and shade control plots. B) Sum of insect visits per inflorescences observed per hours of observation to *Protea neriifolia* and *Protea repens* in different enclosure types. Data for both species and both graphs from Sites 4, 5 and 6, thus excluding Sites 1, 2 and 3 where *P. repens* did not occur.

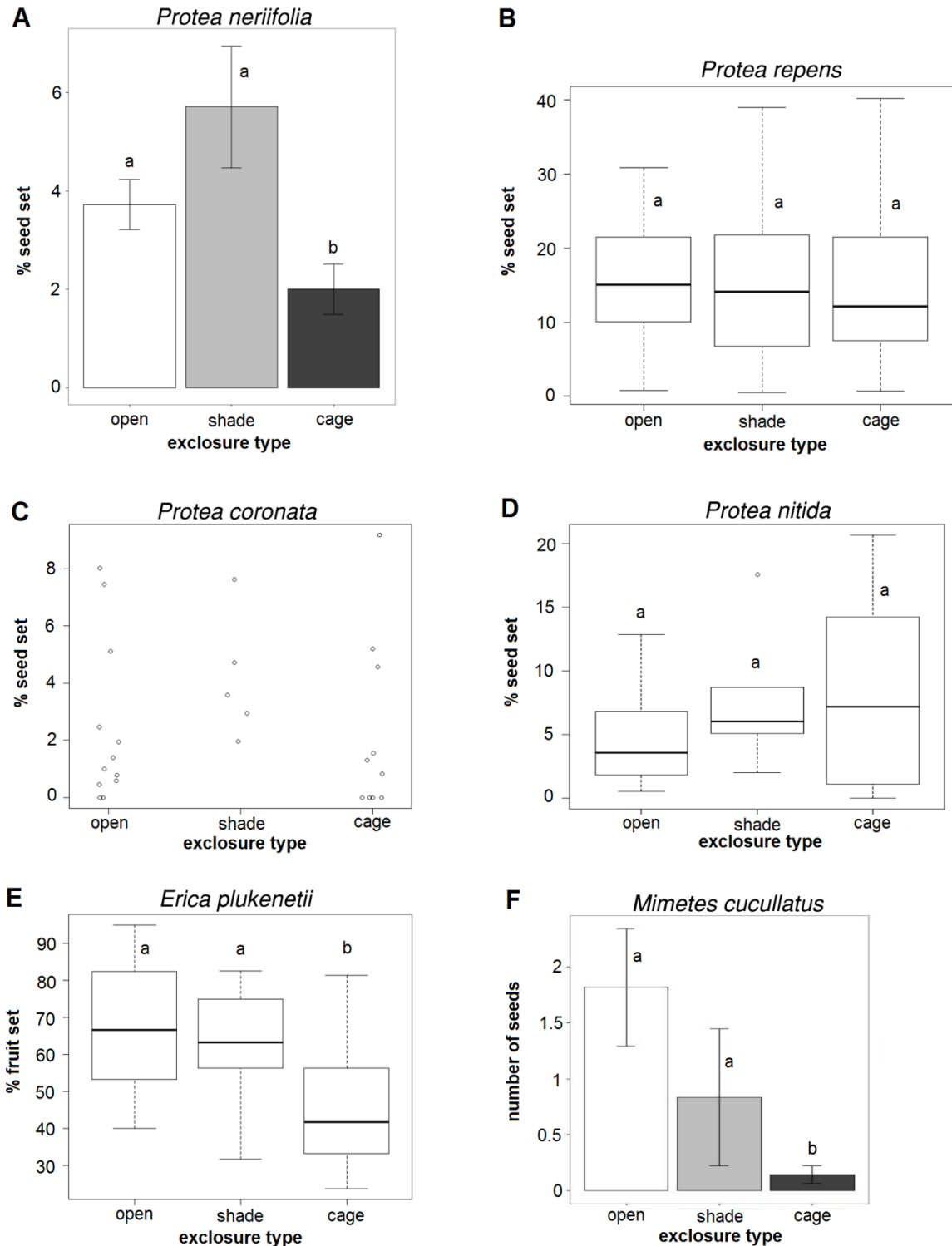


Figure 3.3. Fecundity of bird-pollinated species in different enclosure types. Enclosure type “shade” = shade control and “cage” = bird enclosure. Different letters indicate significant differences (GLMMs in Tables 3.1, 3.2, 3.3, 3.4, 3.5). In panels A) and F) the values are means and bars are standard errors. In panels B), D) and E) bold lines are medians, bars show upper and lower quartiles, whiskers indicate ranges and dots are outliers.

Table 3.1. Results of GLMM comparing the proportional seed set of *Protea neriifolia* among different exclosure types. Estimate and SE values are on the logit scale. Beta-binomial dispersion parameter: 18.135 ± 2.7828 (SE).

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	-4.19	0.24	-17.34	p < 0.001	182
Open	0.77	0.19	4.05	p < 0.001	
Shade control	0.81	0.22	3.67	0.00024	
Intercept (Open)	-3.42	0.23	-15.11	p < 0.001	182
Bird exclusion	-0.77	0.19	-4.05	p < 0.001	
Shade control	0.032	0.21	0.16	0.88	
Aspect (North facing)					
South-facing	0.97	0.29	3.34	0.00084	
Protea density	-0.082	0.048	-1.72	0.086	
	Variance	SD			
<u>Random effects</u>					
Site	0.053	0.23			6

Table 3.2. Results of GLMM comparing the proportional seed set of *Protea repens* among different exclosure types. Estimate and SE values are on the logit scale. Beta-binomial dispersion parameter: 13.552 ± 2.266 (SE).

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	-1.87	0.30	-6.25	p < 0.001	99
Open	0.12	0.19	0.63	0.53	
Shade control	-0.025	0.19	-0.13	0.89	
Intercept (Open)	-1.74	0.37	-4.69	p < 0.001	99
Bird exclusion	-0.12	0.19	-0.63	0.53	
Shade control	-0.15	0.20	-0.72	0.47	
<i>Protea</i> density	0.13	0.40	0.32	0.75	
	Variance	SD			
<u>Random effects</u>					
Site	0.00046	0.022			3

Table 3.3. Results of GLM comparing seed set of *Protea nitida* among different exclosure types at Site 5. Estimate and SE values are on the logit scale. Dispersion parameter for quasibinomial model = 9.43.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	-1.58	2.01	-0.78	0.44	23
Open	-0.56	0.56	-1.00	0.33	
Shade control	-0.34	0.55	-0.62	0.54	
Intercept (Open)	-2.13	2.38	-0.90	0.38	23
Bird exclusion	0.56	0.56	1.00	0.33	
Shade control	0.22	0.82	0.27	0.79	
<i>Protea</i> density	-0.93	2.37	-0.39	0.70	

Table 3.4. Results of GLMM comparing proportion fruit set of *Erica plukenetii* among different exclosure types. Estimate and SE values are on the logit scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	-0.18	0.18	-0.99	0.32	54
Open	0.98	0.24	4.09	p < 0.001	
Shade control	0.80	0.25	3.14	0.0017	
Intercept (Open)	0.80	0.18	4.51	p < 0.001	54
Bird exclusion	-0.98	0.24	-4.09	p < 0.001	
Shade control	-0.18	0.25	-0.72	0.47	
	Variance	SD			
<u>Random effects</u>					
Site	0.0051	0.072			2
Plant individual	0.38	0.62			54

Table 3.5. Results of GLMM comparing seed set of *Mimetes cucullatus* among different enclosure types. Estimate and SE values are on the log scale. Zero-inflation: 0.491 ± 0.104 (SE).

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Enclosure type					
Intercept (Bird excl.)	-1.46	0.723	-2.02	0.044	55
Open	2.37	0.738	3.21	0.0013	
Shade control	2.60	0.84	3.08	0.0021	
Intercept (Open)	0.91	0.30	3.05	0.0023	55
Bird exclusion	-2.37	0.74	-3.21	0.0013	
Shade control	0.22	0.59	0.38	0.71	
	Variance	SD			
<u>Random effects</u>					
Plant individual	0.36	0.60			27

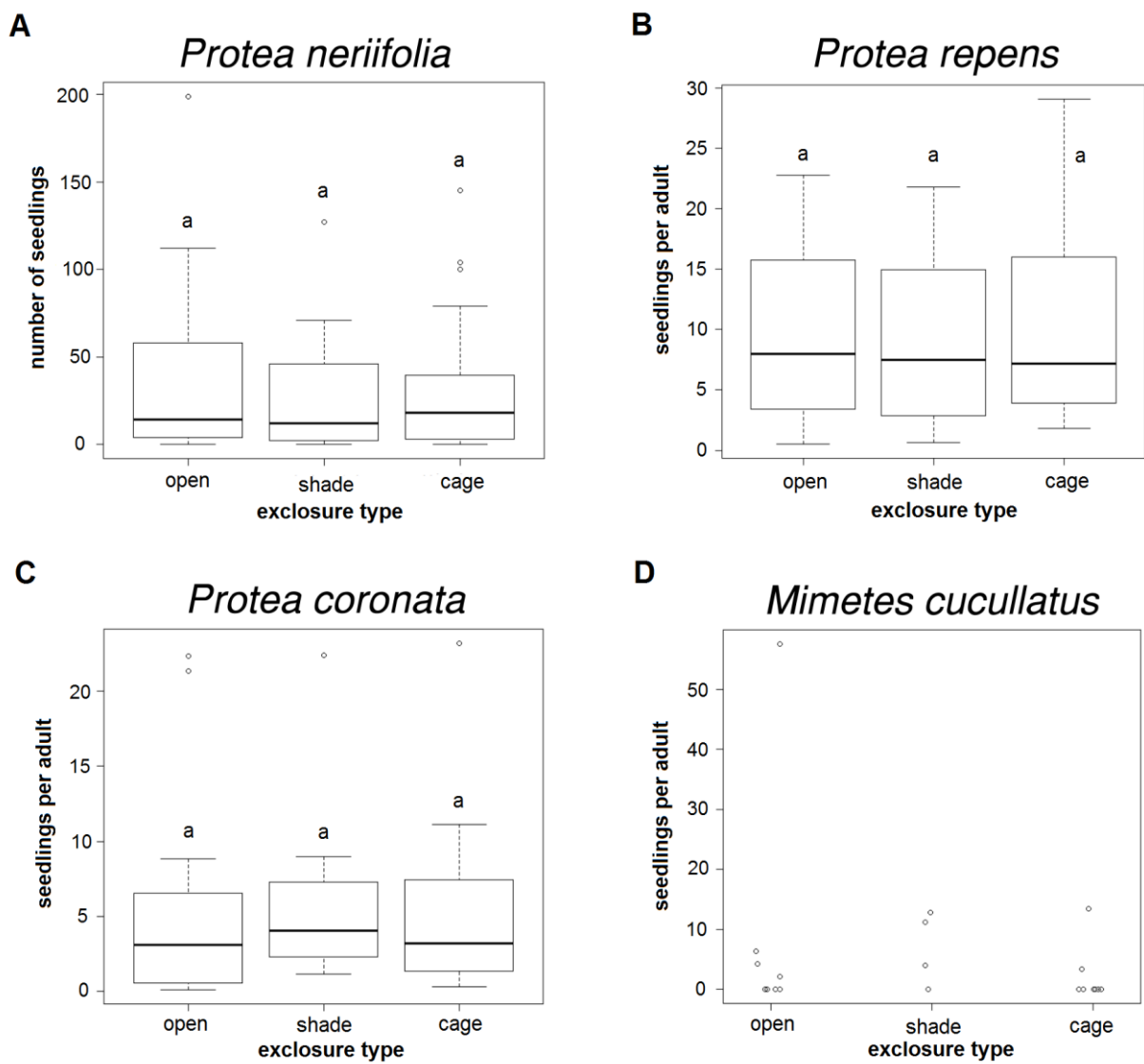


Figure 3.4. Post-fire seedling emergence of bird-pollinated plant species. Different letters indicate significant differences (GLMMs: Tables 3.6, 3.7, 3.8).

Table 3.6. Results of GLMM comparing post-fire seedling emergence of *Protea neriifolia* among different exclosure types. Estimate and SE values are on the log scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	2.39	0.45	5.27	p < 0.001	120
Open	0.11	0.18	0.58	0.56	
Shade control	-0.093	0.22	-0.42	0.68	
Intercept (Open)	2.49	0.47	5.34	p < 0.001	120
Bird exclusion	-0.11	0.18	-0.58	0.56	
Shade control	-0.20	0.23	-0.85	0.40	
<i>P. neriifolia</i> adult density	0.24	0.15	1.6	0.11	
	Variance	SD			
<u>Random effects</u>					
Site	0.80	0.89			6

Table 3.7. Results of GLMM comparing post-fire seedling emergence per adult of *Protea repens* among different exclosure types. Estimate and SE values are on the log scale.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	2.27	0.27	8.57	p < 0.001	60
Open	-0.14	0.15	-0.94	0.35	
Shade control	-0.19	0.19	-0.99	0.32	
Intercept (Open)	2.13	0.27	7.87	p < 0.001	60
Bird exclusion	0.14	0.15	0.94	0.35	
Shade control	-0.050	0.20	-0.26	0.80	
	Variance	SD			
<u>Random effects</u>					
Site	6.02	2.45			3

Table 3.8. Results of GLMM comparing post-fire seedling emergence per adult of *Protea coronata* among different exclosure types. Estimate and SE values are on the log scale.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird excl.)	1.55	0.28	5.51	p < 0.001	60
Open	-0.02	0.30	-0.066	0.95	
Shade control	0.12	0.34	0.34	0.74	
Intercept (Open)	1.53	0.27	5.62	p < 0.001	60
Bird exclusion	0.02	0.30	0.066	0.95	
Shade control	0.14	0.35	0.39	0.70	
	Variance	SD			
<u>Random effects</u>					
Site	4.01	2.00			3

Chapter 4: Rodents and baboons reduce seed cone production of *Protea neriifolia*

This chapter has been published in the *South African Journal of Botany*.

Abstract

Rodents are important pollinators and seed predators of fynbos Proteaceae, but their role as florivores has received little attention. Chacma baboons (*Papio hamadryas ursinus*) are known to feed on Proteaceae inflorescences, however, their effect on plant reproduction has not been quantified. We recorded the extent of damage by rodents and baboons to inflorescences of the dominant shrub, *Protea neriifolia*, in 20 x 20 m plots in the Jonkershoek Nature Reserve near Stellenbosch. Damage was distributed patchily across the landscape. Rodents damaged up to 23% of the inflorescences per plot by feeding on styles and nectar. We observed the striped mouse (*Rhabdomys pumilio*) climbing up plants to feed on inflorescences. Up to 14% of inflorescences in some plots were destroyed by rodents gnawing through inflorescence stems. Baboons damaged or destroyed 12% to 29% of inflorescences at three study sites, but did not forage at all in the three remaining study sites. Among plant individuals affected by baboons or rodents, a mean proportion of 0.49 ± 0.31 (SD) of individuals' inflorescences were damaged or destroyed. Florivory by rodents and baboons can thus reduce the fecundity of *P. neriifolia* significantly. *Protea* inflorescences may be an important seasonal food source for certain rodents. In an evolutionary context, rodent florivory may have been an important selective force that caused certain Proteaceae species to shift to rodent pollination. Further work is needed to determine which rodent species feed on *Protea* inflorescences and whether rodent florivory is widespread in fynbos.

Highlights

- Rodents and baboons consume many *Protea neriifolia* inflorescences
- Striped mice (*Rhabdomys pumilio*) climb up to 1.6 m high in *Protea* plants to feed
- Rodent gnawing severs stems carrying *P. neriifolia* inflorescences

1. Introduction

Florivory, the consumption of flowers prior to seed coat formation, is an important biotic interaction, but it has received relatively little attention in its own right (McCall and Irwin, 2006). Florivory should be distinguished from leaf and shoot herbivory, since the effects on plants can differ (McCall and Irwin, 2006). Plant fitness may be reduced by florivory in a number of ways. Flower damage or loss can directly affect plant fecundity by reducing both

seed set (female fitness) and pollen export (male fitness). Flower loss also represents a loss of invested resources, which is especially costly for plants that reabsorb nutrients from old flowers in resource-limited environments (Ashman, 1994). Florivory can indirectly reduce plant fitness too, by altering floral display or reward, thus decreasing the attractiveness of plants to pollinators (Krupnick et al., 1999). Florivory can act as selective force on flower traits, e.g. plants may evolve less conspicuous floral displays to reduce florivory (De Waal et al., 2012).

Herbivory is often considered to be less prevalent in the fynbos vegetation of the Cape Floristic Region than in other biomes and is thus not well studied. Fynbos typically occurs on nutrient poor soils; hence fynbos foliage is usually low in nutrients. The leaves of many fynbos plants are also well-defended against herbivory by sclerophylly, pubescence and/or high phenolic content (Anderson et al., 2014). It has been suggested that plant parts such as flowers and seeds, which are potentially less defended and more nutritious, will support a greater abundance and diversity of insect herbivores than leaves in fynbos (Cottrell, 1985). Not much is known about insect herbivory in fynbos in general, hence there is insufficient evidence to test Cottrell's hypothesis. Inflorescences and infructescences of *Protea* (Proteaceae) are attacked by a diversity of insect borers (Roets et al., 2006) and high levels of leaf herbivory by chewing insects has also been recorded in certain *Protea* species (Wright and Giliomee, 1992). The abundance and diversity of herbivorous mammals in fynbos are relatively low, likely as a result of the low forage quality (Pauw and Johnson, 1999). However, even small amounts of herbivore damage may have significant effects on fynbos plants, since plants are slow growing and nutrients are not easily replaced in a low-nutrient system (Anderson et al., 2014).

The Proteaceae family is a characteristic and often dominant component of fynbos vegetation (Rebelo, 2001). Fynbos Proteaceae are ecologically and commercially important, well-studied and considered to be a model for biodiversity research and conservation (Schurr et al., 2012). Proteaceae interact with various animals, including rodents and chacma baboons (*Papio hamadryas ursinus*).

Rodents play a number of important ecological roles in fynbos. Most species in fynbos are wholly or partially herbivorous (Skinner and Chimimba, 2005). Rodents have strong interactions with Proteaceae in particular, acting as seed predators (Bond and Breytenbach, 1985), but also dispersing the seeds of certain *Leucadendron* species (Midgley and Anderson, 2005). Moreover, rodents pollinate several *Protea* species (Biccard and Midgley, 2009; Melidonis and Peter, 2015; Wiens et al., 1983; Zoeller et al., 2016) and three *Leucospermum* species (Johnson and Pauw, 2014; Johnson, 2015). As many as 35 species

of *Protea* and *Leucospermum* are thought to be rodent-pollinated (Rebello and Breytenbach, 1987). Inflorescences of rodent-pollinated plants are typically robust and present nectar rewards in an accessible manner to prevent damage by rodents (e.g. Johnson and Pauw, 2014; Wester et al., 2009). Inflorescences of some rodent-pollinated *Protea* are also robust enough to withstand foraging by larger mammals such as mongooses (Steenhuisen et al., 2015). Rodent-pollinated *Protea* may often be gnawed by rodents, but usually inflorescences are not destroyed, however, *Protea nana* (nomenclature of Proteaceae species in this article follows Rebello (2001)), loses many inflorescences to rodent gnawing (Biccard and Midgley, 2009). The styles and fleshy involucral bracts of geoflorous rodent-pollinated species such as *P. subulifolia*, *P. amplexicaulis* and *P. decurrens* are also sometimes consumed by rodents (Wiens et al., 1983; Zoeller et al., 2016). Rodents are not known to feed on the inflorescences of bird-pollinated *Protea*. The only literature records we know of are by Wiens et al. (1983) who noted occasional light chewing of *Protea laurifolia* bracts by rodents and Rebello and Breytenbach (1987) who mention the presence of rodent faeces in *Protea repens* inflorescences.

Chacma baboons are opportunistic omnivores (Skinner and Chimimba, 2005) that include the flowers of at least 36 fynbos species in their diet, notably from the Amaryllidaceae, Ericaceae and Proteaceae families (Davidge, 1978; Hall, 1962). Baboons on the Cape Peninsula may spend as much as half of their time in summer feeding on Proteaceae inflorescences, especially *Leucospermum conocarpodendron*, as well as *L. hypophyllocarpodendron* subsp. *hypophyllocarpodendron* and *Mimetes fimbriifolius* (Davidge, 1978), while *Protea lepidocarpodendron* is a main food item from April to June (Hall, 1962). Inflorescences of *Protea scolymocephala* (Hall, 1962), *P. humiflora* and *P. repens* (Wiens et al., 1983) are also consumed by baboons. Entire inflorescences are broken from *Protea* and *Leucospermum* plants, and sometimes baboons tear open inflorescences and lick out the insides, most likely feeding on the nectar (Hall, 1962). Baboons also destroy the inflorescence buds of cultivated *Protea* when searching for insect borers (Malan, 2012).

Despite the potential importance of florivory by rodents and baboons, their effect on plant reproduction has not been quantified. In this study we aimed to determine the extent of damage by rodents and baboons to the inflorescences of *Protea neriifolia* R.Br., a widespread bird-pollinated species.

2. Materials and Methods

2.1 Study area

We conducted our study in the Jonkershoek Nature Reserve near Stellenbosch. Study plots were established at six different sites in 5-year old Boland granite fynbos (Rebello et al., 2006), at altitudes of 380 – 467 m. Sites number 1, 2 and 3 were on the valley's southwest-facing slopes, where the granite-derived soil supported dense stands of *Protea neriifolia* and *Protea coronata*. Sites number 4, 5 and 6 were on the drier north-facing slopes, where the soil was sandier and the sparser vegetation was dominated by *Protea neriifolia* and *Protea repens*. At each study site, two 20 x 20 m plots were established, as part of a larger study on bird pollination. Birds and baboons, but not rodents, were excluded from one of the plots at each site by a 20 x 20 x 2.2 m cage of plastic netting (mesh-size 2 x 2 cm), while the other plot consisted of unmanipulated open vegetation. Rodents moved freely into bird enclosure plots, evidenced by numerous rodent runways underneath the netting. Coordinates of study sites: Site 1: 33°59'18.60"S, 18°58'20.87"E; Site 2: 33°59'24.92"S, 18°58'26.97"E; Site 3: 33°59'28.39"S, 18°58'44.04"E; Site 4: 33°59'31.69"S, 18°58'2.97"E; Site 5: 33°59'32.30"S, 18°57'57.48"E; Site 6: 33°59'32.65"S, 18°57'47.74"E.

2.2 Baboon damage to *Protea* inflorescences

We assessed all *Protea neriifolia* inflorescences in the open plots at Sites 4, 5 and 6 for baboon damage on 24 and 28 July 2014, after baboons passed through the area on 19 July 2014. *Protea* inflorescences are hermaphrodite. In addition, styles first serve a male function by acting as pollen presenters and thereafter serve the female function when stigmas become receptive. Inflorescences were scored as “damaged by baboons” if part of the inflorescence had been removed by the feeding baboons, but the remaining part of the inflorescence was intact and considered potentially capable of pollination and seed production. If all the flowers had been removed from an inflorescence or the inflorescence stem had been snapped by the baboons, the inflorescence was scored as “destroyed by baboons”. Inflorescences that were open and producing nectar at the time were scored “open”, while inflorescences that had finished flowering were scored “closed”. Inflorescences that were not yet open and flowering were scored as “bud”. We examined a random sample of 25% of *P. neriifolia* individuals (range 70 to 600) for baboon damage at Sites 1, 2 and 3 in October 2014. We also checked all six study sites regularly from March to October 2014 for indications of baboon activity such as digging, faeces, plant damage and overturned rocks.

2.3 Rodent damage to *Protea* inflorescences

We recorded rodent damage to *Protea neriifolia* inflorescences on 8 and 9 August 2014, at Sites 1, 4 and 5. Rodent gnawing at Site 2 was recorded in February 2015. All *P. neriifolia* individuals at Sites 4 and 5 were assessed (including those previously affected by baboons),

and a random representative sample of individuals at the other sites (Site 1 Cage: $n = 61$, Site 2 Open: $n = 123$). All inflorescences on an individual were assessed. An inflorescence was scored as “damaged by rodents” when the central flowers of the inflorescence had been consumed by rodents. Rodent feeding of this kind hollowed out the centre of an inflorescence, with most styles gnawed off close to the base, and it left loose styles lying around the rim of the inflorescence and sticking out beyond the top of the involucre bracts (Fig. 4.1A,B). When the stems below inflorescences were completely gnawed off by rodents, with tell-tale gnawing marks, we scored it as “gnawed off by rodents” (Fig. 4.1C,D). Inflorescences that were in “bud”, “open” and “closed”, were scored as described above. Rodent damage could be distinguished from baboon damage, since rodents gnawed on inflorescences and stems while baboons plucked flowers and snapped stems. In addition, baboons could not enter caged plots. We recorded cones produced before 2014 at Sites 4 and 5 to determine the lifetime reproductive output of individuals.

3. Results

3.1 Baboon damage to *Protea neriifolia*

Chacma baboons damaged and destroyed substantial proportions of the total *Protea neriifolia* inflorescences on one side of the valley (Site 4: 12%, $n = 150$; Site 5: 28.6%, $n = 189$; Site 6: 18.4%, $n = 103$; sample sizes are inflorescence counts), but did not forage on the other side of the valley (Site 1: 0%, $n = 91$; Site 2: 0%, $n = 223$; Site 3: 0%, $n = 314$), as also determined by the lack of signs of baboon activity (described below). Baboons plucked flowers, i.e. styles and ovules, clean off the base of inflorescences, mostly leaving the involucre bracts. A number of inflorescences were partially damaged in this way (Site 4: 6.7%; Site 5: 9%; Site 6: 7.8%; sample sizes as above), but had some potentially functional flowers remaining (Table 4.1). Many inflorescences were completely destroyed, with all flowers lost or inflorescence stems snapped (Site 4: 5.3%; Site 5: 19.6%; Site 6: 10.7%; sample sizes as above) (Table 4.1). The way inflorescences were damaged suggests that baboons fed on nectar rather than flower parts, as also described by Hall (1962). Baboons damaged or destroyed the majority of open, nectar-producing inflorescences available to them at the time of foraging (Site 4: 56.3%, $n = 32$; Site 5: 71.1%, $n = 76$; Site 6: 86.4%, $n = 22$), i.e. inflorescences that had already closed after flowering and inflorescence buds were not targeted by baboons. Within sites that were visited by baboons, substantial proportions of flowering *P. neriifolia* individuals were affected by baboon feeding (Site 4: 22.5%, $n = 40$; Site 5: 36.7%, $n = 60$) (Table 4.1). Among affected *P. neriifolia* plants at Sites 4 and 5, large proportions of individuals' inflorescences were damaged or destroyed (median = 0.43, interquartile range = 0.25); individual level data for Site 6 not available. Baboons damaged plants in general by snapping branches, in a few cases snapping main stems of plants.

Some plants with snapped main stems continued growing, others died. Many *P. neriifolia* plants in the areas surrounding study sites were also affected by baboon feeding. Although we never directly observed baboons feeding on inflorescences, baboon calls were often heard in the area and many tell-tale signs indicated baboon activity at Sites 4, 5 and 6, e.g. overturned rocks, plant bulbs dug up and baboon faeces. There were no signs of baboon activity at Sites 1, 2 and 3.

3.2 Rodent damage to *Protea*

Rodent feeding damaged a substantial proportion of *Protea neriifolia* inflorescences (mean 9.1%, range 0 to 22.5%) in five plots across Sites 1, 4 and 5 (Table 4.1). A larger proportion of inflorescences were damaged in caged plots (Site 4 Cage: 22.5%, Site 5 Cage: 16.6%) than in open plots (Site 4 Open: 2.2%, Site 5 Open: 0%). A small number of *Protea repens* inflorescences were damaged, but few *P. repens* plants were still flowering at the time of data collection. We observed the striped mouse (*Rhabdomys pumilio*) climbing up *P. neriifolia* plants and into open inflorescences up to 1.5 m above the ground to consume flowers on four different occasions on two separate days at Site 4, damaging inflorescences as in Fig. 4.1A,B. Rodent faeces were found in some damaged inflorescences of *P. neriifolia* and *P. repens*.

In addition, many *P. neriifolia* inflorescences were severed from plants by rodents gnawing through stems (Site 1 Cage: 13.7%, n = 117 inflorescences, Site 2 Open: 9.5%, n = 153) (Table 4.1); this was observed across all plots at Sites 1, 2 and 3, but not at Sites 4, 5 and 6. *Protea coronata* inflorescences were also gnawed off by rodents. Severed *P. neriifolia* inflorescences on the ground below plants had rodent feeding marks and contained rodent droppings (Fig. 4.1E). Among *P. neriifolia* plants affected by rodent damage and gnawing at Sites 1, 4 and 5, considerable proportions of individuals' inflorescences were affected (median = 0.37, interquartile range = 0.71). At Site 2, 15 out of 16 plants affected by rodent gnawing lost their sole inflorescence.

3.3 Life-time inflorescence production

At Site 4 and Site 5, 56% (n = 245) of the reproductive *P. neriifolia* plants flowered for the first time in 2014 (a further 71 plants had not yet produced any inflorescences). Inflorescences from 2014 made up 80% (n = 978) of the life-time inflorescence production at Sites 4 and 5. Baboons damaged or destroyed 13% (n = 381) of the life-time inflorescence production in open plots at Sites 4 and 5. Rodents damaged or destroyed 15% (n = 597) of the life-time inflorescence production in caged plots at Sites 4 and 5. These numbers do not include inflorescences that might have been lost due to baboons or rodents before 2014.

Discussion

Destructive feeding on inflorescences by rodents and baboons substantially reduced the fecundity of *Protea neriifolia* in three out of six stands in Jonkershoek. Baboons affected up to a third of *P. neriifolia* plants where they foraged, while rodent feeding affected one in five plants on average. Individual plants in their second year of flowering lost substantial proportions of their inflorescences. This is the first study of the effect of mammal florivory on the fecundity of a *Protea* species and the first record of extensive rodent feeding on a bird-pollinated *Protea*.

Rodent and baboon florivory may affect *Protea* plants in a number of ways. Most significantly, the loss of inflorescences will reduce the reproductive output, and thus fitness, of plants. Substantial inflorescence loss due to rodent and baboon activity has been recorded previously for rodent-pollinated *Protea*. In *Protea nana*, rodents gnawing through stems reduced the average number of inflorescences per plant by 20% over a two-month period (Biccard and Midgley, 2009), while baboons removed an average of eight inflorescences per *Protea humiflora* individual (Wiens et al., 1983).

Feeding by rodents and baboons did not always sever or completely destroy *Protea neriifolia* inflorescences. In many cases inflorescences were only partially damaged and it is possible that the remaining flowers on such inflorescences could still set seed. When rodents damaged inflorescences, they gnawed off the styles close to the base, but did not typically damage the ovules, and also left some flowers intact. Baboons plucked entire flowers, including the ovules, from inflorescences, but did not always remove all the flowers from an inflorescence. In response to baboon damage, an inflorescence's involucre bracts, mostly still intact, closed in the same way as in an inflorescence that had finished flowering. This might serve to protect the remaining ovules. However, inflorescences that lost some of their styles may still not be able to set seed, if the normally tightly packed dry styles of *Protea neriifolia* cones serve to protect the developing seeds from water, fungi and insects.

The effect of inflorescence loss on plant fecundity may be mitigated by resource reallocation. Plants that have lost inflorescences may have more resources available per remaining inflorescence and thus the seed set or seed quality of the undamaged inflorescences may increase, provided that seed set is resource-limited (McCall and Irwin, 2006). Resource reallocation is not evident among *Protea* inflorescences exposed to different pollination treatments (Schmid et al., 2015a), although the effect of a bagged inflorescence on a plant's resource allocation to its other inflorescences will not be as great as completely losing one or more inflorescences. If nutrients are allocated to seeds after pollination, a plant will only lose the nutrients invested in producing the lost inflorescence.

When an inflorescence is lost, a plant not only loses ovules, the pollen that would have been exported by the inflorescence is also lost, thus possibly reducing the plant's male fitness. (*Protea* inflorescences are hermaphrodite). A plant carrying fewer inflorescences may also be less attractive to pollinators and will be disadvantaged if it thus receives fewer pollinator visits per inflorescence. Bird-pollinated *Protea* plants offering small rewards (few inflorescences and/or less sugar per inflorescence) relative to their neighbours receive fewer pollinator visits per plant (Schmid et al., 2015b), but whether this in fact involves fewer visits per inflorescence was not shown. In the present study, baboons destroyed a significant proportion of inflorescences over an area of at least ten hectares in relatively young vegetation and may thus have reduced the overall attractiveness of the area to bird pollinators. However, at the high *Protea* inflorescence densities of mature vegetation, a reduction of density may also reduce competition for bird pollinators, thus increasing per-plant visitation rates (Schmid et al. 2015b).

Lastly, the stem wounds caused by rodents and baboons removing inflorescences could make plants more susceptible to infection by diseases, as is the case for pruning wounds in cultivated proteas (Malan, 2012).

We provide the first description and quantification of baboons feeding on *Protea neriifolia* inflorescences. It should be noted that possibly only one baboon troop was involved in our study, although more than one troop occurred in the greater area and in general baboon troop home ranges are known to overlap. The nature of the damage to *P. neriifolia* inflorescences in our study is consistent with observations of baboon feeding behaviour on other Proteaceae (Hall, 1962). In addition to records of baboon florivory on Proteaceae in the literature (Davidge, 1978; Hall, 1962; Wiens et al., 1983), various other observations indicate that baboons feed on the inflorescences of many *Protea* species, namely *P. burchellii*, *P. cynaroides*, *P. laurifolia*, *P. longifolia*, *P. lorifolia*, *P. pendula*, *P. repens* (Protea Atlas Project), and *P. canaliculata* and *P. recondita* (S.L. Steenhuisen, pers.comm.).

We observed the striped mouse (*Rhabdomys pumilio*) climbing up *P. neriifolia* plants to feed inside inflorescences up to 1.5 m above the ground. Hitherto, this species was not known to feed on flowers of bird-pollinated *Protea*, except for a YouTube video record on *Protea obtusifolia*. Previous studies have found that *R. pumilio* pollinates various geoflorous *Protea* species (Melidonis and Peter, 2015; Wiens et al., 1983; Zoeller et al., 2016) and climbs up to a metre high to forage on (and pollinate) *Protea nana* (Biccard and Midgley, 2009). However, *R. pumilio* also forages very destructively on the rodent-pollinated legume *Liparia parva* (Letten and Midgley, 2009). We did not observe *R. pumilio* gnawing through inflorescence stems, but the species has been recorded gnawing off canopy-borne inflorescences of

Protea nana, a species with relatively thin stems, in captivity in order to consume them on the ground and the result of such feeding was observed in the field (Biccard and Midgley, 2009). A number of other rodent species may have fed on the *Protea* inflorescences in our study. Besides *R. pumilio*, six other rodent species that occur around the study area are wholly or partially herbivorous (Skinner and Chimimba, 2005; Van Hensbergen et al., 1992).

The fact that rodents feed on a widespread bird-pollinated *Protea* species like *P. neriifolia* in addition to the rodent-pollinated *Protea* species, lends further support to the idea that *Protea* inflorescences may be an important seasonal food source for rodents. Although *P. neriifolia* started flowering at the end of April, we did not observe rodent feeding on inflorescences before the last week of July 2015. This might be explained by increased energy requirements of rodents breeding in late winter. Nectar of the rodent-pollinated *P. humiflora* is a valuable food source for rodents and flowering coincides with late winter rodent breeding (Fleming and Nicolson, 2002). Rodent pollinators in the Cape, including *R. pumilio*, are also able to digest and gain meaningful amounts of protein from *Protea* pollen (Van Tets and Nicolson, 2001). Furthermore, consuming styles and bracts will also provide sustenance to rodents. Damage to *P. neriifolia* inflorescences in our study (Fig. 4.1), suggests that rodents fed on nectar, pollen and inflorescence parts. Nectar sugar concentration is typically higher in rodent-pollinated than bird-pollinated *Protea* (Van Tets and Nicolson, 2001). However, the mean sugar concentration of *P. neriifolia* nectar in our study area was 29.8% (SD = 4.4, n = 12 inflorescences) (P.W. Botha, unpublished data), which is comparable to the average sugar concentration of 36.1% of four rodent-pollinated *Protea* species (Van Tets and Nicolson, 2001), as well as that of rodent-pollinated species in the Cape in general (Turner et al., 2011).

The cost of flower destruction by rodents may be a selective force that contributes to the evolution of rodent pollination. The only available phylogeny of *Protea* suggests that rodent pollination has evolved independently a few times, once most likely from bird-pollinated ancestors (Valente et al., 2009).

Many *Protea* species in the Cape, including *Protea neriifolia*, are serotinous, only releasing their seed from cones after fire, and do not resprout after burning. Populations thus depend entirely on seeds for recruitment after fire. Can mammal florivory have significant effects on the demography of *Protea neriifolia*? Maze and Bond (1996) found that a nine year old stand of *Protea neriifolia* was not seed limited and was able to replace itself with up to 80% of infructescences removed. However, mammal florivory may have meaningful effects in combination with short fire return interval (less than 7 years), since younger *Protea* plants with relatively few inflorescences may lose significant proportions of their inflorescences and

the inflorescences on smaller plants are more accessible to rodents. Our study area burned in March 2015 when the vegetation was six years old, thus *P. neriifolia* plants lost 13 – 15% of their lifetime inflorescence production to mammal florivory (Section 3.3). By reducing the density of a dominant shrub, rodents and baboons may be enhancing local plant diversity.

Further studies are needed to determine how widespread mammal florivory is in fynbos, particularly rodent florivory, which Proteaceae species are affected, which rodent species are involved and how loss of inflorescences affects the seed set of inflorescences that remain on plants.

Acknowledgements

The authors thank CapeNature for access to the Jonkershoek Nature Reserve and acknowledge the National Research Foundation and Stellenbosch University for funding.



Figure 4.1. *Protea neriifolia* inflorescences damaged by rodent feeding. A), B) Rodents climbed up plants and into inflorescences to consume flowers. C), D) Inflorescences were also severed by rodents gnawing through stems, and consumed by rodents on the ground (E).

Table 4.1: Proportions of *Protea neriifolia* plants affected and inflorescences damaged or destroyed by rodents and baboons in Jonkershoek near Stellenbosch in 2014. See Methods and Materials for definitions of “damaged”, “destroyed” and “gnawed off”. Superscripts denote sampling differences: a) all study sites (n = 6); b) study sites with data available (n = 5); c) study sites with data available (n = 4); d) study plots with data available (n=5); e) study plots with data available (n = 6)

Measure	Mean (%)	Range (%)	n	# study sites
Inflorescences damaged by rodents ^d	9.1	0 – 22.5	869	3
Inflorescences gnawed off by rodents ^c	5.8	0 – 13.7	1022	4
Flowering individuals affected by rodents ^e	21.4	0 – 45.8	413	4
Inflorescences damaged by baboons ^a	3.9	0 – 9	1070	6
Inflorescences destroyed by baboons ^a	5.9	0 – 19.6	1070	6
Flowering individuals affected by baboons ^b	11.8	0 – 36.7	1135	5

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Chapter 5: General Conclusions

The loss of bird pollinators from fynbos communities has varying consequences for the reproduction of different plant species. Various species that are specialised for bird-pollination suffer sharp declines in fecundity without birds, either due to reduced seed set or as a result of lower seed viability and seedling recruitment. Other bird-pollinated species are able to maintain their seed set in the absence of birds. In sum, bird pollinator extinction may ultimately reduce the biomass and diversity of fynbos plant communities.

The exclusion of avian pollinators affects the floral reward, flower visitors and fecundity of *Leucospermum lineare*, a typical bird-pollinated member of the Cape Proteaceae (Chapter 2). *Leucospermum lineare* is specialised for bird-pollination and its nectar volume and concentration fall in the range of ornithophilous plants in general. The Cape Sugarbird, *Promerops cafer*, is the main pollinator, while Orange-breasted Sunbirds, *Anthobaphes violacea*, visit occasionally, mostly thieving nectar but sometimes pollinating. Insect visitors are rare, with only pollen-collecting bees possibly sometimes facilitating pollination. Without birds consuming it, nectar accumulates in flowers. The higher volume and concentration appears to increase ant visitation to flowers. However, ants do not contribute to pollination and only steal nectar. Thus, contrary to certain theoretical predictions, in this case lost pollinator function is not replaced by rewiring of pollinator interactions, perhaps due to the specialised morphology of *L. lineare*'s bird-adapted inflorescences. Instead the surfeit of nectar only attracts more thieves.

Although *Leucospermum lineare* is specialised for bird pollination, its seed set is not reduced when birds are excluded, thanks to autonomous selfing. In this regard *L. lineare* fits the general pattern of its genus, in which selfing is associated with bird pollination, putatively as a means of reproductive assurance in unpredictable environments. But selfing comes at a cost: Seed viability and post-fire seedling recruitment in *L. lineare* are substantially lower without bird pollinators. This could ultimately cause populations to decline. I thus demonstrate the demographic consequences of bird pollinator extinction for *L. lineare*. My findings show that pollinator loss can have insidious effects on fecundity, via inbreeding that manifests after seed set. This suggests that studies assessing only seed set are stopping short and may underestimate the effects of pollinator loss.

In addition to *Leucospermum lineare*, a number of other bird-pollinated species showed reduced reproduction without birds. The loss of bird pollinators could thus cause important changes in plant communities (Chapter 3).

The most dominant shrub in my study communities, *Protea neriifolia*, is pollinated by Cape Sugarbirds while Orange-breasted Sunbirds typically thieve its nectar. Some small beetle species visit *P. neriifolia* inflorescences, possibly contributing to pollination. However, when birds are excluded the seed set of *P. neriifolia* declines by half. In contrast, the seed set of *Protea repens*, also a dominant plant in some of the communities, is not lowered by the absence of birds. Similarly, *Protea nitida* maintains its seed set without avian pollinators. These two species are pollinated by both Cape Sugarbirds and Orange-breasted Sunbirds and receive various insect visitors. Insect pollinator visitation rates are similar for *P. repens* and *P. neriifolia*, but mostly from different insect species. The ability of *P. repens* and *P. nitida* to maintain seed set by insect pollination (and perhaps selfing), as opposed to *P. neriifolia*, may be explained by differences in accessibility to insects due to inflorescence morphology: *P. neriifolia* inflorescences do not open widely and flowers are covered in fluffy hair, while *P. repens* and *P. nitida* inflorescences open completely and flowers are naked. Based on this morphological hypothesis, I predict that another important bird-pollinated community member, *Protea coronata*, is also bird-dependent since it has very closed and fluffy inflorescences and is not visited by many insects.

Protea fecundity is often affected by the density of adults in a stand. However, in this study *Protea* seed set was not influenced by the combined adult density of *Protea* species in the communities, possibly because the stands were too young for density dependent effects to realise. The seedling mortality of *Protea* species in the first year after fire can also be density dependent, but here the first-year seedling mortality of *Protea* species did not depend on the density of conspecific seedlings or on the combined density of all Proteaceae seedlings. Thus strong intra- or interspecific competition among seedlings does not manifest in our *Protea* species in the first year post-fire.

When fynbos vegetation burns too frequently, many plant species that rely on seed for regeneration may become locally extinct. My study site went up in flames when the vegetation was six years old. In this case the fire return interval appears to have been long enough for *Protea* species to regenerate adequately: The densities of *Protea neriifolia*, *Protea coronata* and *Protea repens* seedlings one year post-fire were high enough to replace the adults. This result does not include the effects of bird exclusion, since seed dispersal barriers could not be erected fast enough after the fire to contain the *Protea* tumble seeds in experimental plots.

In addition to *Protea neriifolia* and *Leucospermum lineare*, two other plant species in our communities rely on bird pollinators. The seed set of *Mimetes cucullatus* (Proteaceae) collapses when birds are excluded. Although this species is a resprouter, in the long term it will most likely go extinct without its bird partners. Considering that most *Mimetes* species are bird-adapted, the loss of bird pollinators would likely be a serious threat to this charismatic Cape genus. The fruit set of *Erica plukenetii* also declines significantly without its pollinator, the Orange-breasted Sunbird. If this response is representative of ornithophilous *Erica* in general, decline of bird pollinators would have far-reaching consequences: approximately 15% of the genus is bird-pollinated.

Although *Erica plukenetii* and *Mimetes cucullatus* are not dominant community members, they may still play an important functional role as bridging nectar resources for birds during lean times of the year. Along with *Leucospermum lineare*, these species flower from October to December, when very few other bird-pollinated plants in the area flower. If these species decline with low abundance of bird pollinators there may thus be feedback effects leading to further declines in bird pollinator numbers.

The plant species in this study that rely on birds, including *Leucospermum lineare*, are also important in terms of vegetation cover, collectively making up an average of 19% (range 8 – 42%) of the vegetation cover in the study communities. In general many other bird-pollinated Proteaceae, that are often dominant where they occur, are also likely to depend on bird pollinators. Overall my results suggest that bird pollinator extinction will reduce biomass and diversity of plant communities in the Cape Floristic Region. Avian pollinators are thus keystone mutualists in the region. In a global context, this study adds to our understanding of the demographic and community consequences of pollinator loss. I also demonstrate the feasibility and utility of experimentally excluding birds from entire plant communities.

Lack of bird pollination is not the only factor that may reduce the fecundity of ornithophilous *Protea* species, florivory by mammals can also play a part (Chapter 4). Baboons and rodents that feed on inflorescences reduce the fecundity of six year old *Protea neriifolia* plants significantly. The striped mouse, *Rhabdomys pumilio*, climbs up to 1.6m high into plants to consume the styles and nectar of *P. neriifolia* inflorescences. Rodents also gnaw through inflorescence-bearing stems in order to eat inflorescences on the ground. This is the first study to quantify the effects of mammal florivory in fynbos and it contributes to our general understanding of the oft-neglected phenomenon that is florivory.

Fire frequency in the CFR is set to increase as much of the region becomes hotter and drier due to climate change and the numbers of fire-starting humans increase. A shorter fire cycle may interact with low bird pollinator abundance to reduce plant fecundity. Nectar-feeding

birds are sensitive to fire, and their abundance is much lower in the first few years after a burn due to lack of nectar resources. If a large proportion of the landscape is recently-burnt it might not support enough nectar feeding birds to provide adequate pollination for plants, potentially also in patches of older vegetation, resulting in lower plant fecundity. Plants that rely on seeds for regeneration may thus need more time to produce sufficient seed to replace populations killed by fire, thus becoming more vulnerable to increased fire frequency. A positive feedback may thus result, potentially leading to the extinction of both plants and bird-pollinators.

Unexpectedly a burning ember flitted across the green and white slope and landed on a pink protea. The Orange-breasted Sunbird seemed unfazed by the snow. May the twangy calls of its kind keep ringing across the Cape mountains in the hotter times ahead.

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Supplementary information

Table S2.1. Results of GLMM comparing proportional seed set of *Leucospermum lineare* among different pollinator enclosure treatments in 2014. Estimate and SE values are on the logit scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
Enclosure type					
Intercept (Bird excl.)	-2.11	0.059	-36.2	< 0.001	77
Open	-0.015	0.082	-0.18	0.86	
Shade control	-0.22	0.097	-2.24	0.025	
Intercept (Open)	-2.13	0.058	-36.7	< 0.001	77
Bird exclusion	0.015	0.082	0.18	0.86	
Shade control	-0.20	0.096	-2.09	0.037	
<u>Random effects</u>	Variance	SD			
Site	2.7e ⁻¹⁸	1.6e ⁻⁰⁹			

Table S2.2. Results of GLMM comparing proportion seedling mortality of *Leucospermum lineare* among different enclosure types. Estimate and SE values are on the logit scale.

Effects	Estimate	SE	z	p	n
<u>Fixed effects</u>					
<i>L. lineare</i> seedling density	0.07	0.03	2.4	0.018	
Enclosure type					
Intercept (Bird exclusion)	-0.93	0.14	-6.7	< 0.001	95
Open	0.44	0.16	2.8	0.006	
Shade control	-0.13	0.16	-0.82	0.41	
Intercept (Open)	-0.49	0.13	-3.7	< 0.001	95
Bird exclusion	-0.44	0.16	-2.8	0.006	
Shade control	-0.57	0.13	-4.3	< 0.001	
<u>Random effects</u>	Variance	SD			
Site	0.006	0.08			3

Table S2.3. Insect visitors to the flowers of *Leucospermum lineare*, *Diosma hirsuta* and *Mimetes cucullatus*.

Visitors				Flower species		
Order	Family	Genus	Species	<i>Diosma hirsuta</i>	<i>Leucospermum lineare</i>	<i>Mimetes cucullatus</i>
Coleoptera	Chrysomelidae		<i>Chrysomelidae sp5</i>	x		
	Cleridae		<i>Cleridae sp2</i>	x		
	Mordellidae	<i>Anaspis</i>	<i>Mordellidae sp1</i>	x	x	
			<i>Mordellidae sp2</i>	x	x	
	Nitidulidae	<i>Pria</i>	<i>Pria cinerascens</i>	x		
Scarabaeidae	<i>Trichostetha</i>	<i>Trichostetha capensis</i>	x	x		
Diptera	Empididae		<i>Empididae sp3</i>			x
	Scatophagidae	<i>Scatophaga</i>	<i>Scatophaga stercoraria</i>	x	x	
Hemiptera					x	
Hymenoptera	Apidae	<i>Apis</i>	<i>Apis mellifera capensis</i>	x	x	
	Colletidae	<i>Hyaleus</i>	<i>Hyaleus sp1</i>		x	
	Formicidae	<i>Anoplolepis</i>	<i>Anoplolepis custodiens</i>			x
		<i>Camponotus</i>	<i>Camponotus niveosetosus</i>	x	x	x
	Halictidae	<i>Lasioglossum</i>	<i>Lasioglossum sp1</i>		x	x
	Ichneumonidae	<i>Lissonota</i>	<i>Lissonota sp</i>			x

Table S3.1. Results of GLMM comparing insect visitation frequency to *Protea neriifolia* inflorescences in different exclosure types.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	2.22	0.21	10.58	0	158
Open	0.61	0.21	2.99	0.0036	
Shade control	-0.57	0.35	-1.65	0.10	
Intercept (Open)	2.83	0.16	17.23	0	158
Bird exclusion	-0.61	0.21	-2.99	0.0036	
Shade control	-1.19	0.32	-3.72	0.0004	
		SD			
<u>Random effects</u>					
Site		0.22			6
Observation period		0.56			69

Table S3.2. Results of GLMM comparing insect visitation frequency to *Protea repens* inflorescences in different exclosure types.

Effects	Estimate	SE	t	p	n
<u>Fixed effects</u>					
Exclosure type					
Intercept (Bird exclusion)	1.19	0.33	3.60	0.0006	83
Open	0.59	0.38	1.58	0.12	
Shade control	0.51	0.39	1.31	0.19	
Intercept (Open)	1.78	0.18	9.80	0	83
Bird exclusion	-0.59	0.38	-1.58	0.2	
Shade control	-0.08	0.28	-0.29	0.78	
		SD			
<u>Random effects</u>					
Site		3.2e-05			3
Observation period		3.6e-05			14

Table S3.3. Results of GLMM comparing insect visitation frequency to *Protea coronata* inflorescences in different exclosure types.

Effects	Estimate	SE	t	p	n
Fixed effects					
Exclosure type					
Intercept (Bird exclusion)	2.40	0.31	7.78	0	45
Open	0.35	0.32	1.08	0.29	
Shade control	-0.44	0.43	-1.02	0.32	
SD					
Intercept (Open)	2.75	0.27	10.35	0	45
Bird exclusion	-0.35	0.32	-1.08	0.2881	
Shade control	-0.79	0.39	-2.04	0.05	
Random effects					
Site		4.5e-05			3
Observation period		0.63			17

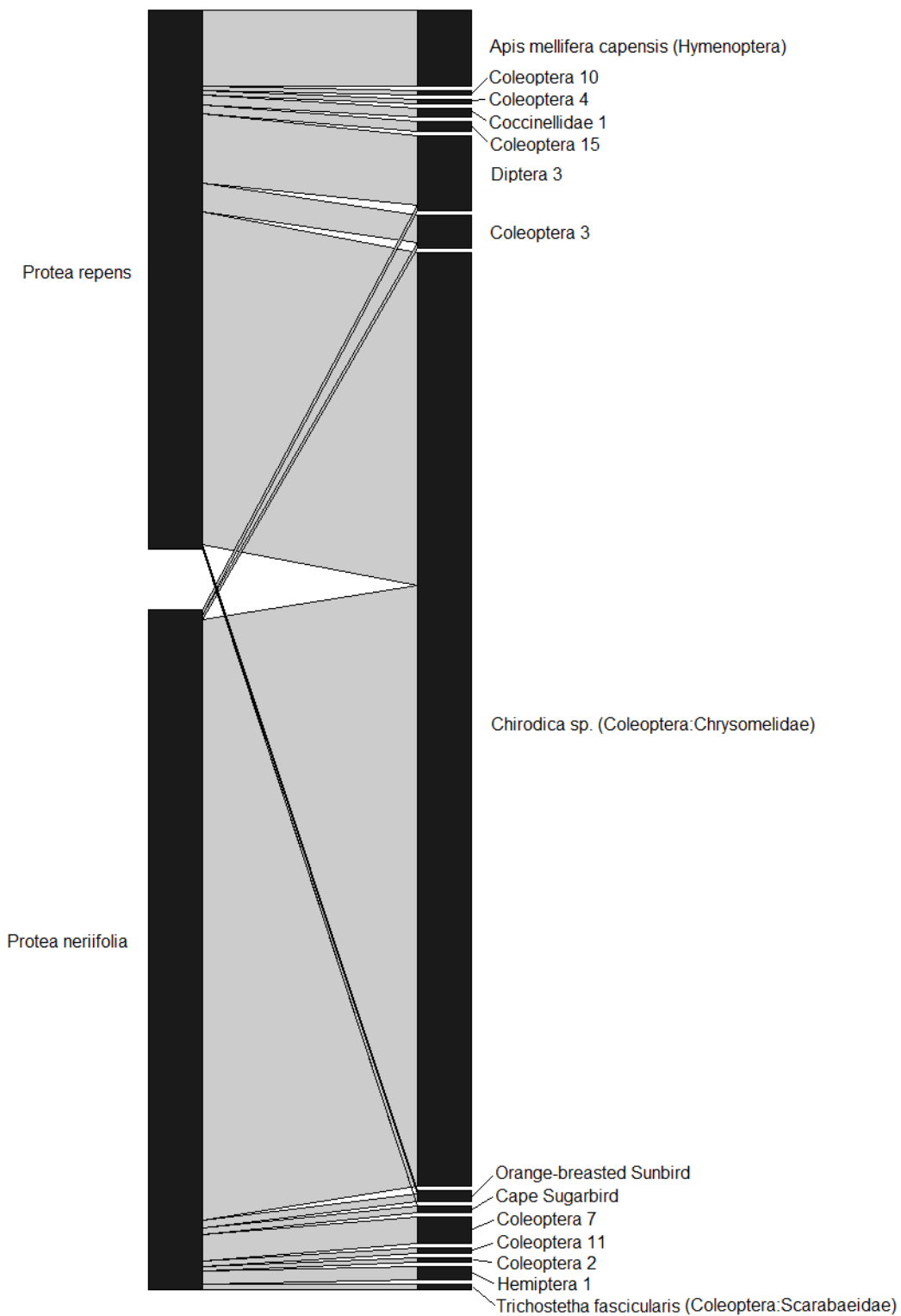


Figure S3.1. Bird and insect pollinators visiting *Protea repens* and *Protea neriifolia* in open plots at Sites 4, 5 and 6. Thickness of links are proportionate to total number of visits per inflorescence per hour observation. *Protea repens* values are standardized to account for difference in sampling effort relative to *P. neriifolia*.

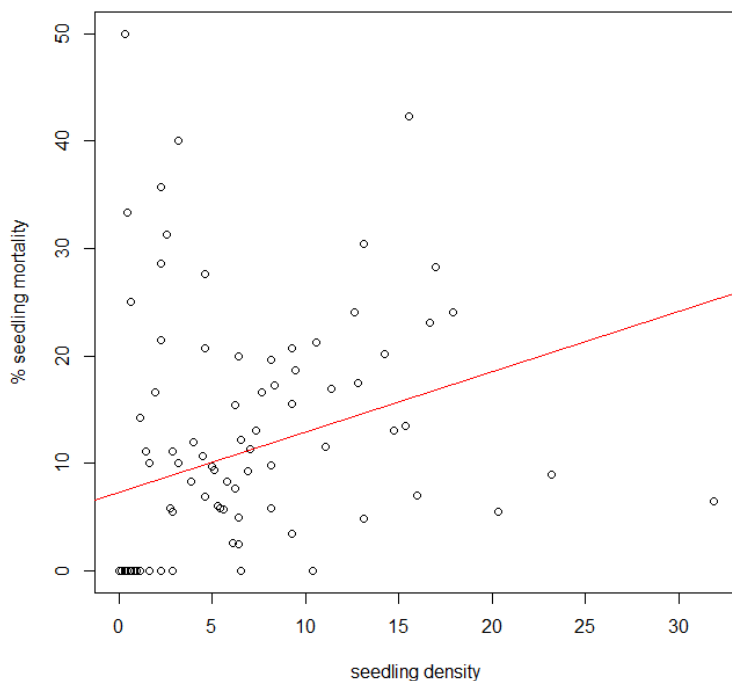


Figure S3.2. Proportion mortality of *Protea neriifolia* seedlings plotted against the seedling density per m² of *P. neriifolia*. Linear regression line fitted ($r^2 = 0.076$, $p = 0.0024$).

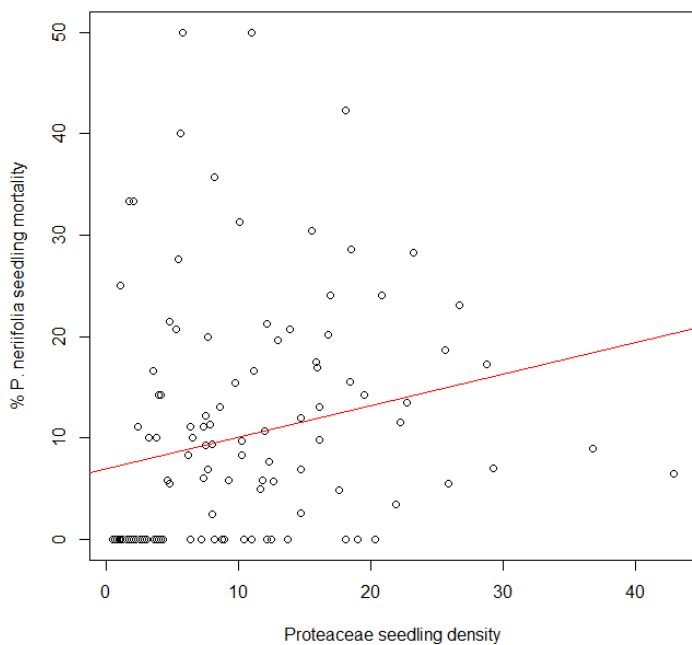


Figure S3.3. Proportion mortality of *Protea neriifolia* seedlings plotted against the combined seedling density per m² of *P. neriifolia*, *P. coronata*, *P. repens*, *Leucospermum lineare* and *Mimetes cucculatus*. Linear regression line fitted ($r^2 = 0.046$, $p = 0.01892$).

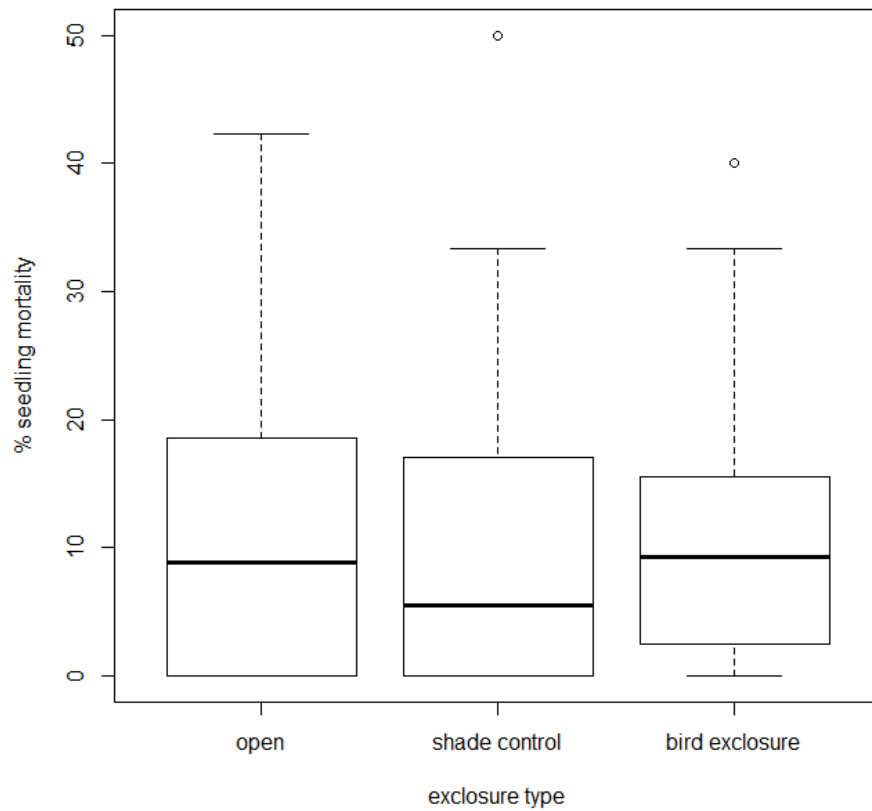


Figure S3.4 Proportion mortality of *Protea neriifolia* seedlings in different exclosure types approximately one year post-fire.