Insects, orchids and fire:

The effects of fire on orchid pollinators in eucalypt woodlands of South Australia

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Contents

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Prologue

The initial goal of this study was to explore the effect of prescribed spring and autumn burning on orchid pollinators in eucalypt woodlands. The 3-year study was carried out from 2013 to 2015 and was designed as a "Before - After, Control - Impact" (BACI) experiment. Changes in fire-affected sites were compared to unaffected control sites before fire, and one and two years after fire. The four field locations selected for this study were located in the Mt. Lofty Ranges in South Australia, and two of the four control sites were simultaneously affected by a large summer bushfire in early 2015, just after the second data collection period. The initial shock about the potential "loss of study sites" was soon overcome by the unique opportunity to also investigate the changes in insect communities and their habitat following a wildfire, especially when compared to prescribed burning. Thus, the study investigates the impacts of prescribed spring and autumn burning as well as the impacts of a summer wildfire. Due to the wildfire, two prescribed burn sites could only be investigated over two time periods. In the case of the wildfire, Before-After changes were compared to sites previously affected by prescribed burning, due to the lack of unaffected control sites.

This thesis is presented as a series of manuscripts, which have been prepared for submission as publications.

Following the general introduction discussing fire regimes in South Australia, orchid pollination biology and the potential effect of fire on pollinating insects, Chapter 2 investigates the effects of fire on insect habitat characteristics, which can play an important role in influencing pollinator communities. Chapter 3 focusses on the molecular and morphological identification of orchid pollinator groups, which were used as a foundation for the ecological studies investigating the effect of fire on orchid pollinators. Chapter 4 investigates the effect of fire on hoverflies (Syrphidae) and Chapter 5 discusses the changes in native bee communities following fire. In Chapter 6 the effect of fire on tiphiid wasps (Tiphiidae, pollinators of specialist orchids), is investigated. The final discussion (Chapter 7) connects the findings of the thesis and considers possible implications for terrestrial orchids.

Thank you for reading!

Thesis summary

Populations of orchids are declining throughout Australia, mainly because of habitat destruction and the effects of habitat fragmentation (Coates et al., 2002). In addition the loss of pollinators due to fire events may have an important impact on orchid populations. Given that orchids are frequently pollen limited they may be particularly sensitive and responsive to changes in pollinator communities (Ashman et al. 2004; Burd 1994; Faast et al. 2011). The loss of certain pollinators could have devastating consequences and lead to decline or even extinction for those orchids that are specialists in their pollination strategy and rely on a single species of insect pollinator. This study assessed the response of three families of orchid pollinating insects and their habitat to prescribed burns and wildfires in eucalypt woodlands in southern Australia. Insects and selected habitat characteristics were surveyed over three years during spring at four different locations, and before and after fire events using a "Before-After, Control-Impact" approach.

Fire induced changes of selected insect habitat characteristics including plant composition, floral abundance and nesting resources were investigated. Both prescribed burns and wildfires reduced floral abundance, altered the structure of vegetation and increased the amount of bare ground. Unlike earlier studies (Carrington, 1999; Hubbert et al., 2006), prescribed burning was shown to not reduce litter depth or the abundance of logs and standing litter. An increase in logs after prescribed spring burning, due to limb or tree mortality and fall was also documented. As expected, the impacts of a wildfire were more severe, as it significantly decreased litter depth and the abundance of logs, due to the high intensity of the wildfire resulting in destruction of more biomass.

Potential orchid pollinators were identified using DNA barcoding methods combined with morphological identification. Results show that even just five month after a fire there was no severe effect on the abundance of members of the three most common orchid pollinator families (Apidae s.l., Syrphidae, Tiphiidae). Hoverflies (Syrphidae) were not affected by the fire regimes, but abundance differed among the years of sampling. Native bee (Apidea s.l.) abundance showed only a marginal decline in the second year after prescribed burning. Some bee species, especially ground nesters, showed a positive response to fire, which is most likely associated with fire induced habitat changes such as the increased availability of bare ground. No effects of fire on the abundance of tiphiid wasps could be found. Thiphiid wasps are from great importance for some sexual deceptive orchids, which rely on specific wasp species for pollination.

Although prescribed burning and wildfires affected insect habitat differently, pollinator responses to both fire regimes did not differ. The fire-induced decline in floral resources did not affect pollinator communities in the first year following fire, but the availability of nesting sites seems to influence the responses of certain insect species. The results suggest that pollination success of orchids in South Australia is unlikely to be negatively impacted by both prescribed burns or wildfires, as orchid pollinators were still abundant and diverse in fire prone habitats.

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CHAPTER 1

Introduction

Three different methods of lighting a prescribed burn in South Australian eucalypt forests. Photos by author.

1. Introduction

Orchid populations throughout Australia are declining, predominantly due to habitat destruction and fragmentation (Coates et al., 2002). Over one-third of the approximately 350 orchid species in South Australia are listed as vulnerable, rare, or endangered (North and Barker, 2005, Bates, 2011). Threatening processes can also include the disruption of plant-pollinator dynamics (Lennartsson, 2002). Animal-mediated pollination can be disrupted by a range of environmental changes, such as habitat changes, resource availability, and fire (Potts et al., 2010). Different reports show that the flowering of some orchids is promoted by fire (Coates et al., 2006, Orians and Milewski, 2007), but a higher flowering rate does not necessarily translate into an increased seed output as pollination plays an important role for the increase of progeny. Fire induced changes in pollinator communities can lead to reduced pollination success and, subsequently, to a decline in populations of plants (Cunningham, 2000). This leads to a critical need to investigate the response of insect populations, specifically orchid pollinating insects, to prescribed burns and wildfires. The fire induced changes in pollinator habitat also require investigation, as habitat resources are likely to play a paramount role in shaping pollinator communities.

1.1. Fire regimes in South Australia

It is not without reason that James Cook named Australia the "the continent of smoke" (Martin and Handasyde, 1999). Fire has played a major role in shaping Australian landscapes long before the existence of human beings. Fire has a very complex relationship with the Australian flora and fauna, but in particular wildfire has been a major force in changing the current composition and structure of Australian vegetation (Williams and Gill, 1995, Thomas et al., 2003) and in the overall shaping of plant communities (Ojeda et al., 2010). Mediterranean type vegetation seems to be quite resilient to fire and the reestablishment of post fire communities is rapid (Trabaud, 1994). The relationships between fire, fuel, plants, and animals have various levels of dependency, but current knowledge of interactions among fires and most organisms is very limited. Many Australian plant species have adapted specific traits to help them regenerate after fire, with two key regeneration types in Mediterranean ecosystems: fire persisting and fire recruiting plants (Pausas et al., 2004, Pausas and Keeley, 2014).

Insect pollinators which rely on specific plants as food resource or living environment can be affected by these changes. Fire can furthermore affect soil properties and litter depths, which in turn affect plant growth, vegetation structure, and nesting habitat for insects. The studies conducted on the effects of fire on ecosystems are diverse and responses to fire are varied, highly complex. Variations can occur within and among study sites and also for the same species (Woinarski, 1999, Keith et al., 2002).

Wildfires and prescribed burns

Two types of fire can be distinguished: wildfires (often called bushfires) and prescribed fires, but note that prescribed burning sometimes turns into wildfires. Both influence and change all types of ecosystems (Whelan, 2002). A wildfire is always unplanned, so it can vary in timing, frequency (Banks, 1982, Cary and Banks, 2000), intensity, size (Gill et al., 1981), and heterogeneity (Mackey, 2002). It is hard to predict how a wildfire behaves, as it is influenced by climate (including wind and temperature), the topography, and by the habitat (vegetation type, litter load, moisture and so on) it goes through. All these factors also influences the following successional dynamics. The legacies of a wildfire include recovering vegetation, fallen and standing logs, ash soil, canopy stored seed, and surviving plants/animals.

A prescribed burn is a low to moderate intensity planned and controlled fire (Morley et al., 2004) and can be seen as the ”purposeful application of fire to particular landscapes” (Penman et al., 2011). A prescribed burn regime has four components: fire intensity, between fire interval, and season (Gill et al., 1981, Whelan, 1995, Gill, 1999, Bradstock et al., 2002). Since 2004 the South Australian Government has increased the amount of prescribed burning performed. Prescribed burns are mainly used to manage wildfires by reducing the fuel load, to control weeds (Hodgkinson and Harrington, 1985), and to promote biodiversity. The Department of Environment, Water and Natural Resources of South Australia (DEWNR) (see www.environment.sa.gov.au) states that prescribed burning is used for many ecological reasons, such as: the protection and maintenance of animal habitats, the regeneration of plant species and communities that are reliant on fire, and to improve biodiversity within parks and reserves. However, the impacts on biodiversity are still controversial (Williams and Gill, 1995, York, 1999, Collett, 2003). Prescribed burning can affect plant and animal communities in similar ways to wildfires and can change species compositions (Nieuwenhuis, 1987). Regeneration of burn sites takes place trough survival of species or through recolonisation from unburnt habitat, adjacent to the burnt sites (Noble and Slatyer, 1980). A landscape affected by prescribed burning often shows a higher heterogeneity than after a wildfire, mainly due to the lower intensity.

Many Australian plant and animal species are threatened by inappropriate fires (Bradstock et al., 2002, Brook et al. 2002, Keith et al., 2002a) and have become locally extinct due to fire (Leigh et al., 1984, Gill, 1994). Very little research has been conducted about the differential effects of prescribed fires and wildfires, with only few studies conducted in Australia (McCarthy et al. 2001, Watson 2001). Specifically, studies investigating the response of prescribed burns and wildfire in South Australian eucalypt woodlands are missing. Furthermore, the very valuable approach of BACI ("Before - After, Control - Impact") studies is very rare for wildfires. The BACI design investigates the impacts of a disturbance before and after the event, in comparison to an unaffected control site. However, the BACI approach is nearly impossible to arrange/design for wildfire studies and consequently, information on before wildfire data is missing in most wildfire studies.

Fig. 1-1: Prescribed spring burn (November 2013) in Kersbrook Native Forest Reserve, Adelaide Hills. During the fire (left) and shortly after the fire (right). Photos by author.

1.2. Fire frequency, season and intensity can affect the impact on biodiversity

Fire related variables, such as the different types of fires, the intensities of fires, their seasonality, and frequency have a direct influence on species survival (Gill, 1994).
Fire frequency seems to play a major role in species survival. Frequent fire has been widely considered to have negative effects on biodiversity throughout Australia (Trainor and Woinarski, 1994, Bradstock et al., 1997). Potential effects are the killing of young recruited obligate seeder plants before maturity or by depleting the seedbanks (Keith 1996, Knox and Clarke, 2006). The response to a single fire event can also show a great richness of plant species observed directly after the fire (Gill 1981, Noble and Slayter 1981). In contrast, too low fire frequencies can lead to a lack of recruitment in species dependent on fire for germination (Campbell et al. 2012). Therefore, an appropriate fire frequency is required for the persistence of plant species in these types of ecosystems. This goes in hand with the “Intermediate Disturbance Hypothesis” (IDH), (Fox and Connelll, 1979) which suggests that local species diversity is maximised when ecological disturbance (like fire) is neither too rare nor too frequent. This can also be applied for the response of insects to frequent fires. Although studies on the response of insects to frequent fires are diverse and often report contradicting results. Species richness of insects can be increased by frequent fires (Similä et al., 2002, Moretti et al., 2004) but studies in Mediterranean type ecosystems have also reported a decline in insect taxon richness (York, 1999) or significant changes in species composition (Andrew et al., 2000) after frequent prescribed burning.

Single fire events can also have different effects on insect communities, bee abundance for example, has been reported to decline (Ne’eman, 2000, Moldenke, cited in Ne’eman, 2000), increase (Potts, 2003) or to be not affected (Moretti et al., 2006, Winfree et al., 2009, Rubene et al., 2015) following fire. The effect of fire on insect communities seem to depend on various factors and seem to be closely linked to the fire induced changes in their habitat (Ne'eman et al., 2000, Williams et al., 2010), which are in turn influenced by fire intensity, patchiness, size and season (Gill, 1975, Gill et al., 1981).

The timing of the fire seems to play an important role as well and can have different effects on plant and insect communities, depending on the season in which it takes place. In temperate climates, fires occurring in autumn, winter, or spring, when plants are actively growing, may have a more severe impact on the plant community, depending on life cycles of individual species and their stage of growth. Furthermore, it has been demonstrated that fire season and fire intensity have the strongest influence on seedling emergence (Knox and Clarke, 2004). Additionally, the conditions for seedlings emerged after a fire can also play an important role. Hot conditions in summer can cause mortality to young recruited seedlings, but also a cold and wet winter after an autumn burn can possibly affect the growing success (Knox and Clarke, 2006). Interestingly, most shrub species show greater seedling emergence following burns in spring (Knox and Clarke, 2004, Knox and Clarke, 2006). The fire-altered plant community can subsequently affect animals indirectly. Fires occurring in spring and summer could also affect flying insects within their active flying phase and might cause higher direct mortality in insects than fires occurring during dormancy periods in autumn and winter.

Fire intensity controls the direct mortality of animals and plants (Moreno and Oechel, 1994, Wikars and Schimmel, 2001), and can also stimulate the recruitment of seeds through heat (Tieu et al., 2001). Very intense fires, consistent with most wildfires, lead to high mortality in plants, but can possibly lead to germination of fire dependent plants (Tyler, 1995, Penman et al., 2007). Low to moderate severity fires can additionally promote renovation of dominant vegetation through elimination of undesired plant species (Certini, 2005), but have also been reported to promote weeds in eucalypt forests (Briese, 1996). Other studies have shown that low intensity fires reduce understory vegetation and litter (Ealey, 1984, Abrahamson, 1996, Nobles et al., 2009). Furthermore, burning severity can modulate the recovery of plants (Chafer, 2008). Low severity fires can also lead to a higher heterogeneity of the affected landscape. The severity of low intensity fires is more likely to be influenced by factors such as topography, weather condition and the available fuel load than the severity of high intensity wild fires. One of those factors could temporary stop the advance of the fire, leaving patches of the vegetation unburnt (Bradstock et al., 1996, Thomas et al., 2003). Those unburnt islands could not just provide shelter/refuges for surviving fauna but also allow a quick recolonization of flora into the burnt patches.

The results of studies conducted on burns varying in frequency, size, and season are diverse, often contradictory and ecosystem specific. Research needs to be carried out on specific types of habitats/vegetation to be able to predict fire related responses. Very little is known about the different impact of prescribed burns in different seasons of the year and the possible implications for biodiversity. Research on the response of orchid pollinating insects to prescribed burns and wildfire is sparse in Meditareanen type ecosystems, especially for wasps and syrphid flies (but see Brown et al., 2016). The study presented here is unique, comparing the effects of wildfires and prescribed burns on orchid pollinators and their habitat with a BACI approach.

1.3. Orchids

Orchids are a very large and diverse group including between 25,000 and 35,000 species (Jones, 2006) growing in almost every kind of habitat. Around 1300 species of orchids occur in Australia, with the majority being terrestrial (growing in the ground) and occurring in the southern regions of Australia (Jones, 2006). The detailed status and distribution of orchid populations in South Australia is poorly known (Coates et al., 2002). Over 35 % of the of the approximately 350 orchid species in South Australia are listed as vulnerable, rare, or endangered (North and Barker, 2005, Bates, 2011). Approximately 220 orchid species have been recorded in the Mount Lofty Ranges, South Australia (Bates, 2011).

Pollination strategies

Plants rely on vectors, mainly insects, to transport their pollen to ensure sexual reproduction. The quantity of pollen transferred can be reduced due to fewer insect visits or less pollen delivered per visit (Ashman et al., 2004).

Orchid reproduction is often strongly pollen limited due to inadequate quality or quantity of pollen, which can reduce their reproductive success, as it translates into low quantity and quality of seeds (Ashman et al., 2004). Resource constraints, such as limited water and nutrient availability can affect the severity of pollen limitation.

Most plants and most orchids reward their pollinators with nectar or pollen for their services. In 1793, Sprengel (1793) discovered the absence of nectar in several species of orchids. Many orchids do not produce a floral reward but imitate floral signals to attract their pollinators (Daumann, 1971, Schiestl, 2005).

The most commonly employed deceptive strategy is food deception. Flowers may cheat by not producing nectar, as pollinators cannot detect the presence of nectar before entering a flower (Thakar et al., 2003). While this unrewarding system of deception is widespread among plants (Thakar et al., 2003) it is especially common in orchids. Around one-third of the estimated orchid species worldwide (approximately 28,000) are food deceptive (Dafni, 1984). These orchids look like rewarding flowers, but do not actually provide a reward. The orchid takes advantage of the pollinators, which tend to associate colour, shape or odour with food and therefore still attempt to feed from the flowers (Jersakova et al., 2006). Food deceptive orchids can be pollinated by a variety of insects, especially bees, but also butterflies, moths, flies and even beetles (Adams and Lawson, 1993, Phillips et al., 2009b). Therefore, food deceptive orchids are usually generalists in their pollination strategies.

Another spectacular case of floral mimicry is sexual deception, in which flowers attract pollinators by producing scents that mimic sex pheromones. The orchid flowers imitate the mating signals of certain wasps and are pollinated by males looking for a female. In a process known as "pseudocopulation", the wasp collects and deposits pollen while attempting to mate with the flower (Schiestl, 2005). The flowers are only visited by male wasps, which have a higher motivation to mate instead of searching for food. The floral odour, more precisely the mimicry of the pollinator's sex pheromone and the floral structure imitating the visual cues, are crucial for pollinator attraction (Schiestl and Schlueter, 2009). This phenomenon of sexual deception is up to now, only known in orchids (Anders Nilsson, 1992). In Australia, there are over 100 sexual deceptive orchid species (out of 1300 orchid species), representing 11 sexually deceptive genera (Gaskett, 2011). The common pollinator is a male haploid wasp (Gaskett, 2011). Pollination is often highly species-specific and typically one wasp species is involved (Schiestl et al., 2003). In Australia, several species of male wasps as well as the males of one species of ant and a species of sawfly are sexually exploited by orchids (Peakall and Beattie, 1996). The most common pollinators within sexually deceptive orchids are male thynnine wasps (Family Tiphiidae, Subfamily Thyninnae). Within Australia, more than 100 species of terrestrial orchids are known to have a sexually deceptive relationship with thynnine wasps, exploiting them as pollinators (Peakall, 1990, Peakall and Beattie, 1996, Phillips et al., 2009a, Peakall et al., 2010). Thyninne wasps are parasitoids and often pollinate *Caladenia* species. Fourteen of 40 *Caladenia* species in Western Australia have been observed to be pollinated by thynnine wasps (Stoutamire, 1983, Phillips et al., 2009b). Female wasps are wingless and often ant-like. To attract males, they climb up on to vegetation and release sex pheromones (Salzmann et al., 2006). Male wasps are sexually attracted to the orchid, first by scent, mimicking the female wasp pheromone, and then by visual similarity of the orchid labellum to the female wasps. Sexual deceptive orchids usually do not offer nectar rewards, but thyninne wasps are nectar feeders, which means they still need nectar-producing flowers for survival.

Sexually deceptive specialist orchids are likely to be more susceptible to fire induced changes in pollinator communities than orchids with generalist pollination strategies. The possible loss or decline in abundance of pollinator species such as thyninne wasps, due to inappropriate fire regimes, could have detrimental consequences for the reproduction of certain orchid species and could even lead to their local extinction.

Orchids in the Mount Lofty Ranges and their pollinators

This study will concentrate on the effect of fire on pollinators of spring flowering orchids, occurring in the Mt. Lofty Ranges. Orchids encountered at the designated study sites include *Pheladenia deformis*, *Diuris* spp., *Thelymitra* species, *Caladenia* spp. and *Glossodia major*. These species include both generalists and specialists, and are pollinated by a diverse range of insects (see Table 1-1). According to previous studies from our laboratory and general knowledge about South Australian orchids, the expected pollinators for orchids occurring in the Mt. Lofty Ranges include bees such as honeybees and solitary native bees, hoverflies (Fig. 1-2), bee flies, and certain genera of wasps (Faast et al., 2009, Bates, 2011).

Table 1-1: Orchid genera likely to occur in the Mt. Lofty Ranges, their pollination strategies and their suspected pollinators (for more details and photographs see Appendix 1).

Orchid genus	Pollination strategies	Pollinators
Caladenia	Food deception, Sexual deception, Nectar reward	Bees, wasps, syrphid flies, bee flies
Diuris	Food deception	Bees, wasps
Microtis	Nectar reward, Sexual deception?	Wasps, ants
Thelymitra	Food deception	Bees, syrphid flies, other flies, Sawflies
Pheladenia	Food deception	Bees
Glossodia	?	Syrphid flies, bees

This project will focus on hoverflies, bees, and thynnine wasps. Beetles are unlikely to play an important role in orchid pollination in this region, but examples can be found in some South African orchid species (Johnson and Steiner, 1994, Steiner et al., 1994). Ants will also be left out of our pollination studies as they are uncommon pollinators for South Australian orchids, with only *Microtis parviflora* known to be pollinated by different ant families (Adams and Lawson, 1993).

Fig. 1-2: Left: Syrphid fly on *Caladenia rigida*. Right: Syrphid fly with orchid pollinia attached. Photos by author.

Caladenia contains 376 species and subspecies in six subgenera with the greatest diversity in temperate southern Australia (Phillips et al., 2009a). The majority of species are endemic to Australia, with 64 of these recorded in South Australia (Barker et al., 2005) and 25 species occurring in the Mt. Lofty Ranges. The genus *Caladenia* is currently controversially discussed and the taxonomic status remains contentious (Hopper and Brown, 2004, Jones and Clements, 2005). In this thesis, *Caladenia* sensu lato will be considered as one single genus (Hopper and Brown, 2004). The genus is unusual in comprising species that have evolved two different pollination syndromes, both food deception and sexual deception (Salzmann et al., 2006). Food-deceptive species attract male and female nectar and pollen seeking insects (Stoutamire, 1983), while sexually-deceptive *Caladenia* species, such as *Caladenia tentaculata* (Fig.1-3, left) attract male thyninne) wasps. As such *Caladenia* is a perfect model as it contains both generalist and specialist pollination strategies. Several *Caladenia* species occur in the project’s designated study field sites. *Caladenia behrii* is an endangered species endemic to the

Mt. Lofty Ranges. *Caladenia behrii* is thought to use sexual deception as a pollination strategy, and is exclusively pollinated by a species of thynnine wasp (Bates, 2011). *Caladenia tentaculata* is a common and widespread species which is also sexually deceptive using a different species of thynnine wasp for pollination (Peakall and Beattie, 1996, Phillips et al., 2009b). *Caladenia rigida* is in contrast a food advertising orchid producing small amounts of nectar and using a broad range of bee species as pollen vectors, such as *Exoneura* spp., *Homalictus* spp. or *Lasioglossum* spp. as well as species of Diptera, such as syrphid flies and bee flies (Faast et al., 2009, Phillips et al., 2009b).

Fig. 1-3: Left: *Caladenia tentaculata*, which has a sexually deceptive pollination system.
Right: *Caladenia rigida*, a food advertising orchid. Photos by author.

Glossodia major is a species in a genus closely related to *Caladenia*, belonging to the subtribe Caladininae. It is a widespread and common orchid, but its pollinators are unknown. Possible pollinators include hoverflies and native bees, which have been observed to visit the brightly coloured flowers (personal observation). Bates (2005) mentions that *G. major* might be a generalist in its pollination strategy. It is unknown whether this species produces nectar, and therefore its status as a rewarding or deceptive species remains to be determined. Flowering occurs from August to October.

No research has been carried out to investigate species composition and abundance of orchid pollinator guilds in Southern Australia. The recent implementation of prescribed fires in the region creates the need to understand the potential consequences this management can have on both orchid and pollinator communities.

1.4. Potential effects of fire on orchid pollinators

Animal-mediated pollination can be profoundly disrupted by a range of environmental changes (Potts et al., 2010), such as habitat changes, resource availability and fire. There are also several factors that can influence pollinator abundance and community composition. Fire may affect orchid pollinators such as bees, wasps and flies, either directly by fire-related mortality or indirectly by changing plant communities, limiting food resources (Hermann et al., 1998), and availability of nesting sites (Potts et al., 2005). The direct survival of insects after fire is related to the fire regime itself, the location of the individuals at the time of the fire (e.g. above or below ground), their mobility, and their stage of development (egg, larva, pupa) (Swengel, 2001). To date, few studies on the effects of fire on pollinator activity have been published (but see Ne'eman et al., 2000, Potts et al., 2003a). Fire might have negative short term effects on pollinator communities, but Pryke and Samways (2012) suggested that burning and long-term recovery is important for overall pollinator diversity. It has been reported that fire induced changes in insect habitat can play an important role in shaping insect communities. For example the loss of plant diversity can cause higher insect abundances and lower insect species richness (Siemann et al., 1998, Knops et al., 1999), whereas higher plant diversity leads to higher insect diversity (Haddad et al., 2001, Knox and Clarke, 2006). Both plant species richness and plant functional group richness have significant positive effects on the total insect species richness (Haddad et al., 2001). Therefore, the diversity of insects is related to the diversity of their resources (Siemann et al., 1998, Knops et al., 1999).

Floral composition can also be altered by fire and may also have a large influence on pollinator diversity, abundance, and community structures (Potts et al., 2003a). Flowers are an essential food resource for pollinator communities and have been shown to predict pollinator diversity (Roulston and Goodell, 2011). Bee diversity for example is closely linked to floral diversity, nectar resource diversity, and time since age (Potts et al., 2003b, Nyoka, 2010). Also, the availably of bare ground, the depths of litter, and the abundance of logs and dead trees, which be altered by fire, are likely to influence nesting behaviour of insects and are therefore very important factors for determining the structure of pollinator communities (Potts et al., 2005).

Although fire is an important ecological factor affecting South Australian ecosystems, most of its effects on biodiversity are still unclear and fire induced changes in ecological interactions, such as pollination, are understudied. No research has been carried out to investigate how fire changes orchid pollinator populations and their habitat, which is likely to play a paramount role in shaping pollinator communities. There is also a need for more field based empirical work on the impacts of fire in Australia and there is a critical need for more monitoring on impacts of different fire regimes in different vegetation types (Gill, 1999, Keith et al., 2002, Whelan, 2002, Cary et al., 2003). To investigate the effect of fire on pollinator communities, an identification of insect is necessary. Identifying pollinators in South Australia is difficult, as they are generally understudied.

1.5. Identification of Australian orchid pollinators

There are few field guides for Australian native bees, wasps, and flies available (but see Stevens et al., 2007). Identification of native bees and thynnine wasps requires a high level of taxonomic expertise (Sheffield et al., 2009). Identification-keys often rely on quantitative characters difficult to ascertain, or do not exist for many species; more than 57 % of Australian bees are not covered by existing keys (Batley and Hogendoorn, 2009). DNA barcoding can be used to assign unknown specimens to species that have previously been described and it can facilitate the discovery of new species (Meyer and Paulay, 2005, Whitworth et al., 2007). While previous studies have shown that DNA barcoding can provide a reliable, cost-effective, and accessible method for species identification (Hebert et al., 2003), there has been very little implementation of DNA barcoding on Australian insects. It is important to explore if DNA barcoding can be used as an effective and reliable tool to identify Australian orchid pollinator species and it would be of great benefit to build a DNA reference library for South Australian orchid pollinators.

1.6. Research aims

The overarching aim of this research was to assess the effect of fire on selected orchid pollinator communities and their habitat. The project was undertaken in the Mt. Lofty Ranges (near Adelaide, South Australia) on sites where prescribed burns were conducted and in nearby control (unburnt) sites. In the final year of the project, a wildfire affected two control sites, which were additionally used to study effects of wildfires. Specific research questions for this research are:

1. How does orchid pollinator habitat change following prescribed burns and wildfire? (Chapter 2)
2. Can insect pollinators be reliably identified using DNA barcoding methods (combined with morphological identification)? (Chapter 3)
3. How do selected orchid pollinator communities change following prescribed burns and wildfire?
    3.1 Effects of fire on Syrphidae (hoverflies) (Chapter 4)
    3.2 Effects of fire on native bee communities (Apidae s.l.) (Chapter 5)
    3.3 Effects of fire on Thynninae (thynnine wasps) (Chapter 6)

The results will give important information about the community composition of potential orchid pollinators (on field sites before and after prescribed burns as well as in wildfire affected sites) and will offer valuable clues on how pollinator communities respond to fire. Declines or changes in pollinator communities could lead to reduced pollination success and consequently declines in orchid pollination. This study will also assess the impacts of prescribed burns and wildfires on important orchid pollinator habitat characteristics which can play a major role in altering pollinator abundance and diversity (Potts, 2005). Rapid molecular techniques for the identification of insect pollinators were developed. This work will also help to establish a DNA reference library of pollinating insects of South Australia. This means, that once a species has been identified taxonomically, other specimens can be rapidly identified to the species level, using DNA barcoding and an established reference library. This will benefit the field of Southern Australian insect biology. This research will play an important future role for the optimal management of selected orchid and pollinator species under fire-managed regimes in the Mount Lofty region of South Australia, as well as more generally in south-eastern

Australia. It will furthermore provide insight into fire induced changes in the vegetation of eucalypt woodlands.

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CHAPTER 2

Impact of prescribed burns and wildfires on pollinator habitat characteristics

Freshly burnt habitat after a prescribed autumn burn in South Para (April 2014). Photo by author.

Statement of Authorship

Title of Paper	'Impacts of prescribed burns and wildfires on pollinator habitat characteristics	
Publication Status	☐ Published ☐ Submitted for Publication	☐ Accepted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted all field work, conducted all statistical analyses and wrote the manuscript	

Principal Author

Name of Principal Author (Candidate)	Anita Marquart		
Contribution to the Paper	Conducted all field and lab work, conducted all statistical analyses and wrote the manuscript		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	29/9/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Renate Faast		
Contribution to the Paper	Advice on experimental design, supervised development of work, helped with field work and with data interpretation		
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Contribution to the Paper	Advice on experimental design, supervised development of work, helped in drafting process		
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2. Impacts of prescribed burns and wildfires on pollinator habitat characteristics

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2.1. Abstract

Fire is known to cause profound changes in Australian ecosystems and can produce changes to habitat characteristic that can be important for pollinating insects. These changes can include vegetation composition and structure (Williams and Gill, 1995, Thomas et al., 2003) flower resources (Hermann et al., 1998) and availability of nesting sites (Potts et al., 2005). The temporal and spatial effects of fire on pollinator habitats can often lead to changes in pollinator abundance and diversity. This study investigated the short-term effects of both prescribed burns and wildfire on selected insect habitat characteristics. The results of this study show that fire causes profound changes in the structure of South Australian eucalypt woodlands. Both prescribed burns and wildfires impacted floral abundances and reduced vegetation density. Of particular importance is the observed decrease in floral abundance, which may negatively impact foraging success of pollinating insects (Ne'eman et al., 2000). Prescribed burning did not reduce litter depth or the abundance of logs and standing litter. The seasonality of prescribed burning did not have an important influence on the impact of fire on insect habitat, whereas the intensity of the fire seems to be a driving factor. A high intensity wildfire had a more severe impact on habitat characteristics and was found to reduce litter depths as well as the abundance of logs and standing litter. Wildfire also altered the composition of the ground cover, which is likely to influence the availability of nesting resources for pollinating insects. This study also reported an increased availability of bare ground after both prescribed burns and wildfires which might benefit many Australian ground nesting bee species.

Keywords: prescribed burning, wildfire, fire, eucalypt woodland, insect habitat, floral abundance

2.2. Introduction

Fire is an important ecological factor that alters the species composition and abundance of Australian biota (Noble and Slatyer, 1980, Coates et al., 2006, Spehn et al., 2006) and controls the structure of Australian vegetation (Williams and Gill, 1995, Thomas et al., 2003). Many of the effects of fire on biodiversity and ecological interactions are still unclear, especially the effects on insect pollinators. Given the recent worldwide decline of pollinators (Allen-Wardell et al., 1998, Kearns et al., 1998, Potts et al., 2010, Vanbergen, 2013), the possibility that fire negatively affects pollination is a concern. Animal-mediated pollination can be disrupted by a range of environmental changes (Potts et al., 2010), such as habitat changes and resource availability which can be influenced by fire. Fire may affect insect pollinators, either by directly fire-related mortality or indirectly by altering their habitat. In addition to investigating pollinator communities we need to document the changes that fire causes in their habitat to fully understand the effects of fire on pollinator communities. The fire induced changes commonly reported for plant communities (Ojeda et al., 2010), vegetation composition and structure (Williams and Gill, 1995, Thomas et al., 2003) as well as changes in flower resources (Hermann et al., 1998) and abundance of nesting sites (Potts et al., 2005), are likely to have an important impact on insect pollinator abundance and diversity. There is general consensus about the need for field based empirical work on the impacts of fire in Australia and there is a critical need for more monitoring of the impacts of different fire regimes in different vegetation types (Gill, 1999, Keith et al., 2002, Whelan, 2002, Cary et al., 2003).

2.2.1. Effects of fire on plant communities and consequences for insects

The response of plant communities to fire plays a paramount role in shaping the habitat for insects. Fire can influence plant communities on different temporal and spatial scales, as it can affect plants during the fire through heat and removal of biomass but also throughout their recovery process and may also affect plant communities many years after the actual fire event. Differential responses to fire of various species creates a dynamic process of changes in community structure that can last for decades. Fire can immediately eliminate certain plant species from local areas (Williams and Gill, 1995) but it can also benefit species that may require fire for seed recruitment (Keeley and Fotheringham, 2000). Many Australian plant species have adapted specific traits to help them regenerate after a fire. There are two main plant regeneration types in Mediterranean type ecosystems: fire persisting and fire recruiting plants (Keeley, 1986, 1995). The plants either survive the fire and are able to quickly resprout after the disturbance (fire persisting plants/resprouter), whereas others are killed by fire but new individuals germinate from large seedbanks (fire recruiting plants/obligate seeders).

The intensity of fire plays a key role influencing a plant community. Fire intensity controls the direct mortality of plants (Tieu et al., 2001) and very intense fires, such as most of the wildfires can lead to high mortality. The higher soil temperatures of wildfires can also lead to a better recruitment of seeds that rely on fire for germination (Penman et al., 2007) and better establishment of seedlings because of less competition directly after the fire (Tyler, 1995, Tyler and D'Antonio, 1995, Tyler, 1996).

Low fire intensity, such as a prescribed burn, can lead to more direct survival of plants after a fire, but may not allow fire dependent recruiters to establish seedlings. Furthermore, burning severity can modulate the recovery of plants (Chafer, 2008). The effect of fire on plants can also depend on the respective life stages, for example old eucalyptus trees hold bark that is thicker and more resistant to fire compared with young seedlings. In contrast, large and already decayed trees are more flammable (Gibbons and Lindenmayer, 2002).

Post-fire recovery is generally expected to be a dynamic progress, depending not only on the intensity of the fire but also on the season of the burn (Knox and Clarke, 2004) and the pre-fire plant community (Purdie and Slatyer, 1976). A habitat with low intensity competition directly after a fire could also turn into a habitat with strong competition dominated by dense fire promoted r-strategist. However, fire response can also vary within and among sites even for the same species (Woinarski, 1999, Keith et al., 2002a). Little is known about the specific responses of plants to single fire events in Australian eucalypt woodlands (Bradstock et al., 2012) and there is a general need for more knowledge on the impacts of prescribed burning and wildfires on plant species abundance and diversity in meditareanean climates such as in South Australia.

Fire has been reported to change characters of plant communities that determine properties of insect habitats. Especially changes in vegetation structure and plant species composition and abundance can affect insect pollinators. Changes in the structure of vegetation, for example a reduced density after recent fire (Christensen, 1994) can have important implications for insects. Campbell (2007) investigated the effects of prescribed burns on pollinators in North Carolina and showed that total insect abundance and the abundance of most orders and families was correlated with reduced tree basal areas and an increase of herbaceous plant cover, caused by fire.Heliophilous insects like hoverflies might thrive in a fire affected landscape with more open vegetation, as they are attracted to open spaces (Gittings et al., 2006) and their feeding from flowers occurs significantly more in the sun than in the shade (Gilbert, 1985).

It has been shown that the loss of plant diversity can result in higher insect abundances and lower insect species richness (Siemann et al., 1998, Knops et al., 1999), whereas higher plant diversity leads to higher insect diversity (Haddad et al., 2001, Knox and Clarke, 2006). Both plant species richness and plant functional group richness have significant, positive effects on total insect species richness (Haddad et al., 2001). Therefore, the diversity of pollinating insects is related to the diversity and availability of their resources (Siemann et al., 1998, Knops et al., 1999). Vice versa, declines in pollinators can also lead to declines of the plants they pollinate (Biesmeijer et al., 2006). No research has been carried out to investigate insect pollinator responses to fire affected eucalypt forests.

2.2.2. Effect of fire on floral abundance and diversity and implications for pollinating insects

So far, very little attention has been paid on the effect of fire on floral abundance and diversity. Potts et al. (2003a) found that species richness of flowering plants is the highest immediately after fire but then declined sharply in the following decade. A wide range of Australian plant species show fire stimulated flowering (Lamont and Downes, 2011), which could lead to a high floral diversity directly after the fire. Fires are also known to increase the flowering rates of some plants in the next season (Gill and Ingwersen, 1976, Gill et al., 1981) and abundant flowering provides the opportunity to increase sexual reproduction and therefore the potential for a population increase and genetic recombination. Fire stimulated flowering is recorded in 34 families, headed by terrestrial orchids with almost half of the species (Lamont and Downes, 2011). For resprouting species, fire stimulated flowering plays an important role, as they are taking advantage of optimal resources and a reduced competition for growth and reproduction after fires (Lamont and Downes, 2011). According to Lamont (2011), peak flowering can be observed 5 - 18 months following fire in Mediterranean-type ecosystems. In some Mediterranean-type systems, floral abundance was relatively low in the first year after fire before reaching a maximum the following year, but then declined successively (Potts et al., 2003b). However, there is a general lack of knowledge about the response of specific flowering plants in Australian eucalypt woodlands to fire regimes, especially to prescribed burning.

The floral community composition can have a large influence on pollinator diversity, abundance and community structures (Potts et al., 2003a). Flowers are an essential food resource for pollinator communities and have been shown to be a robust predictor of pollinator diversity (Roulston and Goodell, 2011). Bee diversity and species richness is closely linked to floral diversity, nectar resource diversity and time since last fire (Potts et al., 2003b, Nyoka, 2010). Potts et al. (2003b) showed that "absolute bee species richness and absolute floral species richness were highly significantly correlated". Diet specialists such as oligolectic bees (which are specialized in their pollen preference, often for a single genus) might be more vulnerable to changes in the floral community structure than diet generalists (Grundel et al., 2011). Nyoka (2010) reported a significantly greater flower cover and a greater bee abundance and diversity in burned treatments in pinyon-juniper woodland, in Arizona. Potts (2003a) also showed that nectar availability and quality are greatest immediately after fire, followed by a steady decrease as regeneration proceeded. Also Roulston and Goodell (2011) demonstrated strong evidence for food resource availability regulating bee populations, but found little clear evidence that other direct factors may be limiting. A study in the east Mediterranean scrublands of Israel surveyed post-fire areas and found bumble bees and a few honeybees, while solitary bees were almost absent from fire affected areas (Ne'eman et al., 2000). Reasons for their absence could either be direct mortality or indirect effects due to limited nectar resources (Ne'eman et al., 2000). So far, there have been few reports regarding the response of flower visiting flies (Diptera: Syrphidae, Conopidae and Bombyliidae) to fire. Many flies are nectar feeders, with for example syrphid flies feeding on both nectar and pollen (Holloway, 1976) and as such they are strongly dependent on flowering plants as food sources. As fire affects plant communities, it can be assumed that flies will also be affected by fire in some way.

2.2.3. Effect of fire on pollinator nesting sites (Ground cover, litter depth, standing litter and logs) and possible consequences for insects

Further habitat characteristics such as ground litter and dead wood in the form of standing litter (dead trees and shrubs) as well as logs on the ground often serve as nesting sites for insect pollinators. Fire can affect these nesting habitats in different ways, but mainly by consuming biomass and reducing organic material (Certini, 2005). It can also affect the ground cover, since a ground previously covered by litter and moss may turn into bare ground or might get covered in ash after a fire. The severity of the fire induced changes is once again determined by the intensity of the fire: a hot wildfire will consume more biomass than a prescribed burn with lower intensity. Nesting sites are very important resource for determining the composition of insect pollinator communities (Potts et al., 2005) as shown by reported positive correlations between bee richness and the abundance of potential nesting resources (Grundel et al., 2010).

Fire induced reduction of litter cover and litter depths can have important influences on community organisation and dynamics not just for plants (Facelli and Pickett, 1991), but also for ground nesting insects. A decrease in litter depths, as reported for prescribed burns in Florida (Carrington, 1999) and California (Hubbert et al., 2006), can lead to reduced diversity and density of ground dwelling species (Springett, 1976). In contrast, a high number of bee species, most often solitary bees, are ground-nesting species and may not be directly affected by the loss of nesting opportunities due to fire, but might actually gain resources due to an increased amount of bare ground after a fire. It has been shown that the availability of bare ground and the occurrence of pre-existing burrows are important factors for bee communities, guild composition and species abundance (Potts et al., 2005).

Nesting resources for stem or log-nesting insects could be drastically reduced by fires due to the loss of potential nesting habitats in the form of standing litter, logs or a general loss of vegetation (e.g. a less dense vegetation structure). During fire, stem-nesting insects and those with burrows in shallow soil are at risk to have the highest mortality rates (Potts et al., 2003a). However, it has also been shown that fire can enhance the abundance of nesting sites for the stem nesting bee *Exoneura nigrescens*, which nests in *Xanthorrhoea* stems (Stow et al., 2007). As previous studies report contradictory effects of fire on stem-nesting pollinators, it can be assumed that the effects of fire on stem nesters can be species-specific or may depend on the fire itself and the habitat it goes through. Further research is needed for a better understanding of the response of insect nesting resources like ground litter, standing litter, dead logs and the general ground cover to prescribed burns and wildfires.

2.2.4. Research approach, expectations and key research questions

The main aim of this study was to document the impacts of fire on important pollinator habitat characteristics in eucalypt woodlands of the Mt. Lofty Ranges in South Australia. The study was set up to investigate the impacts of prescribed burning in different seasons (spring or autumn), using a "Before - After, Control - Impact" (BACI) approach (Smith, 2002). The BACI design is used here to investigate field sites before and after fire and to compare the control sites to the fire affected impact sites. Unexpectedly, a wildfire affected two of four control sites and, while it caused problems with the experimental design, it also gave the opportunity to explore the effects of wildfire on pollinator habitats and to compare the impacts of both fire regimes.

For prescribed burn sites, minimal changes in insect habitat are expected, such as the creation of more open space due to a fire caused lower vegetation density, especially in the year directly after burning. Drastic changes in the plant community composition are not expected, as fire intensity is likely to be too low to eliminate species and not high enough to trigger germination of fire dependent species (Keeley and Fotheringham, 2000). Changes in species abundance and diversity as well as in vegetation structure are difficult to predict, as fire responses can vary greatly within vegetation types and even within species (Gill et al., 1981, Cowling and Lamont, 1985, Vivian et al., 2010) and we lack comprehensive studies in South Australian ecosystems. Studies documenting the reduction of potential nesting grounds like litter and dead logs are missing, although the reduction of these "fuel loads" is one of the main drivers for conducting prescribed burning in South Australia.

More drastic changes are expected in wildfire affected sites as those can be much hotter than prescribed burns, although they can vary greatly in intensity (McCarthy et al., 1999). The plant community is expected to change as wildfires have been reported to alter species compositions (Nieuwenhuis, 1987), and it has been shown that wildfires can stimulate the growth of "fire-dependent" species by breaking dormancy of seeds (Keeley and Fotheringham, 2000). Changes in potential insect nesting resources, such as of litter and logs are also expected, due to the high intensity of the fire. Especially a decrease in litter following wildfire has been previously reported in several other studies (Carrington, 1999, Hubbert et al., 2006) for Mediterranean type ecosystems. Studies including ‘before’ wildfire data (BACI approach) are generally rare, as they are difficult to effectuate.

This study aims to answer several research questions on how selected habitat characteristics, which were shown to be from importance to pollinating insects, change after prescribed burning and wildfires.

The overarching research question is:
How does prescribed burning and wildfire affect potential habitat and resources availability for pollinating insects?

It was specifically focussed on the impacts of fire on plant diversity and species composition, vegetation structure and floral abundance, as well as on potential insect nesting resources (ground cover, litter depth, abundance of logs and standing litter).

2.3. Methods

2.3.1. Study sites

The study was conducted in the Mt. Lofty Ranges, a range of mountains to the east of Adelaide, South Australia. The area has a Mediterranean-type climate, with moderate rainfall (600 - 1000 mm/year mostly in winter), hot summers and mild winters. Forests in the Mt. Lofty ranges are often dominated by *Eucalyptus* species such i.e. *Eucalyptus goniocalyx*, *E. obliqua*, *E. leucoxylon*, and *E. fasciculosa*, mixed with golden wattle trees (*Acacia pycnantha*) on the lower slopes. The understory is frequently dominated by *Pultenea* spp., *Hibbertia* spp., *Gonacarpus* spp., *Lepidosperma* spp., *Arthropodium* spp. and *Xanthorrhoea* spp.

Four locations were selected (Fig. 2-1) to assess insect pollinator abundances and pollinator habitat characteristics and their respective response to fire. Two prescribed autumn burns and two spring burns were chosen, which were conducted by the South Australian government. Study locations were at Parra Wirra Recreation Park (PW), in the

South Parra Reservoir (SP), in the Millbrook Reservoir (M) and in the Kersbrook Native Forest Reserve (K).

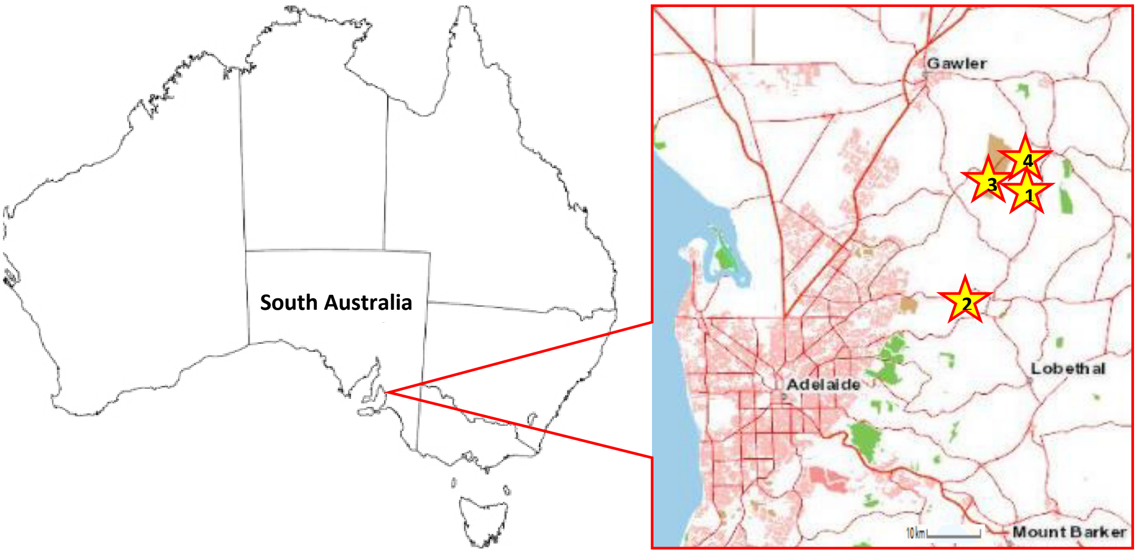


Fig. 2-1: Map of sites. Stars represent the four different field locations: 1 Kersbrook, 2 Millbrook, 3 Para Wirra, 4 South Para.

Each location contained one burn ('pburn') and one adjacent control ('control') site (Table 2-1) with similar habitat attributes, such as vegetation density and plant species present. Control and burn sites were adjacent, but separated by wide fire tracks. Surveys were carried out over three seasons, spring 2013, 2014 and in spring 2015 at the four locations. The "Sampson Flat Bushfire" was a series of wildfires from 2nd to 9th January 2015 that affected the Mt. Lofty Ranges and burnt two control sites before the third data collection was undertaken. The wildfire affected sites were still sampled and were analysed separately with the previous prescribed burn site serving as control site.

Table 2-1: Field sites in the Mount Lofty Ranges with respective coordinates, field site size, burn time and patchiness of the prescribed burn.

	Field site	Coordinates (approx. centre of field site)	Size	Prescrib. burn	Patchiness	Bushfire
1 - burn	Kersbrook Native Forest Reserve	138°50'16.412"E 34°43'11.971"S	Ca 28 Ha	Spring 2013	Medium patchy (ca 70 % burnt)	
1 - control	Kersbrook Native Forest Reserve	138°50'11.227"E 34°43'36.655"S	Ca 30 Ha			January 2015
2 - burn	Millbrook Reservoir	138°49'39.874"E 34°48'48.801"S	Ca 19 Ha	Autumn 2013	Well burnt (85%)	
2 - control	Millbrook Reservoir	138°49'44.153"E 34°48'29.622"S	Ca 15 Ha			January 2015
3 - burn	Para Wirra Recreation Park	138°49'10.545"E 34°41'45.284"S	Ca 35 Ha	Spring 2013	Very patchy (50% burnt)	
3 - control	Para Wirra Recreation Park	138°49'29.626"E 34°41'48.45"S	Ca 35 Ha			
4 - burn	South Para Reservoir	138°52'31.472"E 34°40'35.612"S	Ca 27 Ha	Autumn 2014	Very patchy (ca 60% burnt)	
4 - control	South Para Reservoir	138°52'9.396"E 34°40'25.268"S	Ca 35 Ha			

2.4. Study design, data collection and statistical methods

The impact of fire on pollinator habitat was assessed by estimating changes at unaffected control sites and compared to fire affected sites, using a BACI study design (Stewart-Oaten et al., 1986, 1992). Data were collected at four combinations of sites: affected and unaffected sites, each sampled before and after the occurrence of the fires. Two prescribed burn locations were sampled for six combinations, affected and unaffected sites, each sampled before, after and two years after the fire.

Data collection periods were categorised into: before fire ('before'), one year post fire ('post1') and two years post fire ('post2'), as indicated in Table 2-2. A special case is the field site in Millbrook Reservoir, where a prescribed burn happened before this study was undertaken. Data from all field sites were analysed separately to account for season and site effects. Sites in South Para and Parra Wirra were analysed for three time periods. Sites in Kersbrook and Millbrook were analysed for two periods only, as the third data collection period was affected by the Sampson Flat Bushfire. Data collected in Millbrook were used to compare the one year post burning period with two years post burning period analysis, as prescribed burning in this site was conducted before this study was undertaken.

Table 2-2: List of study location, sites and their respective fire treatments and the monitored time periods per location**.**

Location	Treatment sites	Fire event	Monitored time periods		
Para Wirra	pburn control	prescribed spring burn	before	post1	post2
Kersbrook (PB)	pburn control	prescribed spring burn	before	post1	
South Para	pburn control	prescribed autumn burn	before	post1	post2
Millbrook (PB)	pburn control	prescribed autumn burn		post1	post2
Millbrook (WF)	pburn WF	prescribed autumn burn (post1) summer wildfire	before	post1	
Kersbrook (WF)	pburn WF	prescribed spring burn (post1) summer wildfire	before	post1	

Six permanent belt transects (Fig. 2.2) were set up in each location, always with three transects located in the control site and the other three transects in the burn site, to collect habitat characteristics. Transects were placed at least 200 m away from the edge of the site and at least 400 m away from the respective treatment site. It was ensured that transect in burn treatment sites got burnt by using hand flame throwers when they were not ignited by the original fire treatment on the same day.

Locations were chosen to ensure good easy access to the transects, and to represent the general habitat and ecological characteristics across each site. Transects were 50 m long and 10 m wide and were spaced at least 300 m apart.

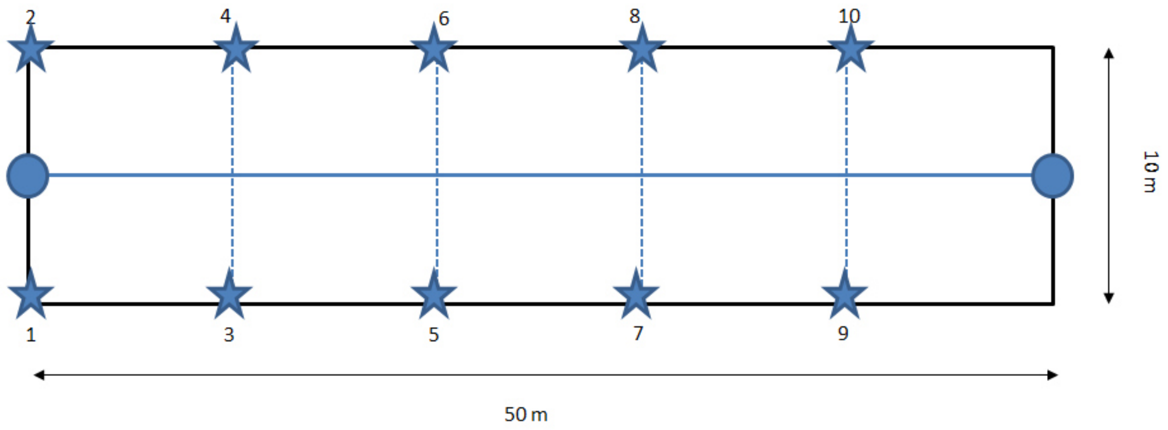


Fig. 2-2: Diagram of the transect lay out. Circles represent star droppers at the end points, while stars represent pegs and data collection points for litter depth. Transect were further divided into 5 quadrats for data recording.

Several habitat characteristics were recorded at each transect (see Table 2-3). Data for every field site were analysed for the data collected at the three transects per treatment over either two or three periods. Data at two transects in 2013 was not collected, due to technical problems. All data analyses were performed in R studio (R© 3.4.1) and results graphed in GraphPad Prism 7.02.

Table 2-3: Habitat characteristics assessed at each transect.

Habitat characteristic	Method of collection along each transect
Plant community	
Plant species composition	Abundance of species present, recorded in Braun-Blanquet cover
Species richness	Count of different plant species present
Vegetation structure	Vegetation touches in different strata, measured along the centre line
Floral diversity and abundance	
Floral abundance	Count of all flowers present
Floral diversity	Count of all flowers per flowering species
Nesting opportunities	
Ground cover	Cover of the ground at every 50cm along the centre line
Litter depth	Measured in mm on points 1 to 10 (see fig.2)
Fallen logs/branches	Count of logs present (in 3 diameter groups)
Standing litter	Count of standing litter (shrubs and trees)

2.4.1. Plant community data

Species composition

The plants present at each transect were either identified to species or allocated to species groups. All grasses were aggregated to one group due to identification issues and considering that grasses might not be as important for orchid pollinators. Some species could be only identified to genus level and multiple species of the same genus were pooled to species pluralis (spp.).

Species richness was defined as the number of species, species pluralis plus the group of grasses. Negative binominal generalised linear mixed models (GLMM's, package glmmADMB) (Fournier et al., 2012) were used to examine differences in the counts of different plant species present between 'control'and 'burn' treatment sites, and how these changed after burning (i.e. pre-burning ('before'), and at two times post-burning ('post1', 'post2') in a BACI design). No data normalisation was applied. Species richness was used as response variable, burn treatment and time period were treated as fixed factors, and individual transects, which were revisited at each time point, were treated as a random effect in the analysis. Plant species counts were modelled using a log link function and an interaction model (species ~ treatment : period). Then, likelihood ratio tests (Chi) were performed to test for significant interactions, using a $p = 0.05$ cut off.

Species composition data were recorded by estimating the cover for each species/spp./group present within each transect using Braun-Blanquet scale of cover (Braun-Blanquet, 1951) with the cover classes 0.1: < 10 plants; 0.5: < 1 %; 1: 1 - 5 %; 2: 5 - 25 %; 3: 25 - 50 %; 4: 50 - 75 %; 5: > 75 %. Data collected as Braun-Blanquet cover was later converted into numerical data in form of percentage cover groups for analysis (0.1; 1; 5; 15; 37.5; 62.5; 87.5).

Plant species composition between 'control' and 'burn' treatment sites, and how these changed after burning, was analysed with a PERMANOVA (Anderson, 2001), based on dissimilarity matrices with Bray-Curtis distances calling the function adonis in the R package Vegan (Oksanen et al., 2013). Contrasts were investigated by fitting subsets of the original datasets and correcting the p - values from multiple testing using Holm correction (Holm, 1979). Dissimilarities between burn and control treatments were illustrated by plotting ordination scores from a nonmetric multidimensional scaling (nMDS) ordination (metaMDS function of the R package multcomp) based on a Bray-Curtis distance matrix (Bray and Curtis, 1957). Species with significant contribution to dissimilarities were investigated with the function envfit (Oksanen et al., 2013). Then, indicator species for treatments and period were detected using multi-level pattern analysis (Dufrene and Legendre, 1997, De Cáceres et al., 2010) from the indicspecies package, and cut offs of $p = 0.05$ were used.

Vegetation structure

The structure and density of the vegetation was sampled by pole point-survey every 50 cm along a 50 m central line of each transect (see Fig. 2-2). We used a 1 m levy pole (1 cm diameter) and recorded the number of live touches by each species touching the pole at four different vegetation strata. The different strata were defined as class1: 0 - 0.25 m, class2: 0.25 - 0.50 m, class3: 0.5 - 1 m, class4: 1 - 2 m to represent four lower heights classes of vegetation likely to be important for insect pollinators.

The effect of fire on the structure of vegetation and the density in the different height classes was assessed by analysing the number of touches of live vegetation in each strata, independently of plant species identity, using a PERMANOVA (response variables: vegetation touches in four different height classes; fixed factors: treatment, period; random effect: transect). An nMDS with a Bray-Curtis distance matrix (Bray & Curtis, 1957) for each field site (see species composition analysis) was performed to illustrate results. The significance of variables (different vegetation classes) was tested using the envfit (Vegan package) function with a permutation test (Oksanen et al., 2013). GLMM's were performed to test for the effect of fire on vegetation density. Analysed was the overall number of touches per transect, disregarding differences in touches per strata. (Response variable: overall vegetation touches; fixed factors: treatment, period; random effect: transect). Likelihood ratio tests (Chi) were performed to test for significant interactions.

2.4.2. Floral abundance and community composition

Transects were divided into 10 quadrats (see Fig. 2-2) per transect to record floral abundance and the floral community composition. Floral data was collected by counting all individual flowers per species in each quadrat. As far as possible, all individual open flowers were counted. The number of individual flowers within large groups of flowers was estimated by counting the individual flowers within the size of one hand and then extrapolated by estimating the number of hands needed to cover the large group of flowers.

The effect of fire on floral abundance was analyzed, using the overall number of flowers present at each transect (all quadrats summed up) and by generating GLMM's (response variable: floral abundance; fixed factors: treatment, period; random effect: transect). Likelihood ratio tests were performed to test for significant interactions. Paired contrast analysis was conducted for prescribed burn sites containing three periods, to look at the effects between the different periods separately.

Another permutational analysis of variance (PERMANOVA, as in previous analysis) was conducted to test for the effect of fire on the floral community composition (response variables: floral abundance per flowering species; fixed factors: treatment, period; random effect: transect). Contrasts were investigated by fitting subsets of the original datasets and correcting the *p* - values from multiple testing using Holm correction (Holm, 1979).

2.4.3. Data on nesting opportunities

Ground cover

Point surveys were conducted to assess the ground cover. Touches were recorded every 50 cm along a 50 m central transect and the presence of litter, bare ground (soil), rock, ash, log, tree or moss was recorded at ground level. The presence of the different ground covers along the 100 measure points of each transect was transformed into relative abundance of each ground condition per transect. A PERMANOVA (like in previous analysis) was performed to test for an effect of fire on the composition of the ground cover. Contrasts were investigated by fitting subsets of the data and then correcting p-values from multiple tests using Holm correction. Results were illustrated using an nMDS (same procedure like in previous analyses). The significance of variables (different ground covers) was tested using the envfit (Vegan package) function with a permutation test (Oksanen et al., 2013).

Litter depth

Litter depth was recorded using calipers, taking 10 measurements along the 10 outer points of each transect (Fig. 2.2). We analysed the average of the 100 measurements per transect for three transects per treatment over either two or three periods. Linear mixed effects models (lme function from the lme4 package) were generated to test for the effect of fire on litter depth, as they allow for non-integer data with random effects. No data normalisation was applied and the model was analysed for residuals. Fixed factors were litter depths (numeric, in centimetres), treatment and period, whereas transect was used as random effect. Data for one transect in the 'before' period in Para Wirra is missing.

Logs

Fallen branches and logs over 1 m length and 2 cm in diameter, were counted within each of the five quadrats per transect, classified into different size diameters groups (2 - 10 cm, 10 - 30 cm and > 30 cm). All fallen logs and branches over one meter in lengths were analysed for the overall number per transect, disregarding the different diameter groups. The effect of fire on the abundance of dead logs present in field sites was analysed using GLMM's like in previous analyses (response variable: log abundance; fixed factors: treatment, period; random effect: transect). The GLMM model was used to generate estimates and 95 % confident intervals of the mean number logs recorded per treatment and period along the sites for graphing. Likelihood ratio tests were performed to test for significant interactions. Data for one transect in the 'before' period in Para Wirra is missing.

Standing litter

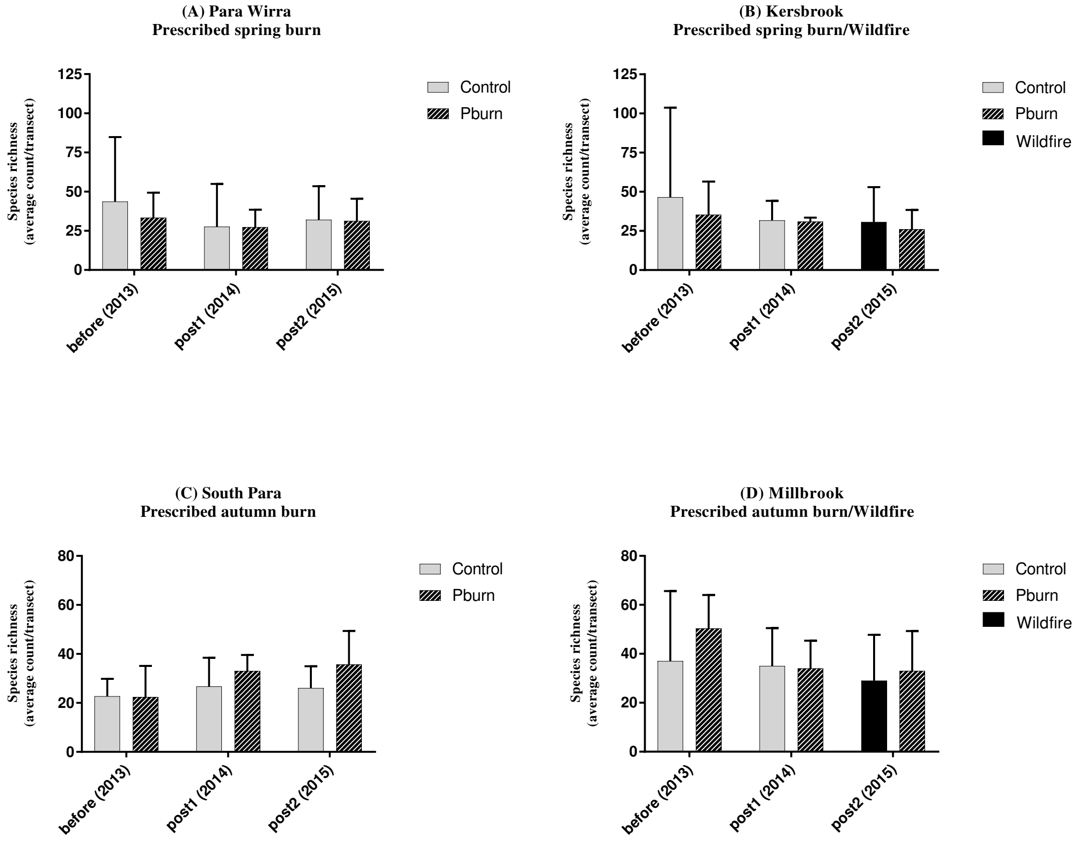
Standing litter was estimated visually, recording the type, the average height (between 0.5 m and 6 m), the width and the number of dead trees and shrubs within each transect. The effects of fire on the abundance of dead trees and dead shrubs were analyzed in two separate analysis. Data were analysed using GLMM's analyses (response variable: dead tree or shrub abundance; fixed factors: treatment, period; random effect: transect). The GLMM model was also used to generate estimates and 95 % confidence intervals of the mean number of standing litter (trees) recorded per treatment and period along the sites for graphing. Missing data: Data for one transect in the before period at the control site in Para Wirra is missing.

2.5. Results

2.5.1. Effects of fire on the plant community

Effects of fire on species richness and composition

The number of different plant species recorded per transect (species richness, Fig. 2-3) was not influenced by any fire events. In contrast, species richness differed among the years of sampling (factor: period), although this observation was only made in field sites affected by prescribed burning (Table 2-4). Wildfire affected sites were analysed independently to prescribed burns. A list of species recorded can be found in Appendix 2.


(A) Para Wirra
Prescribed spring burn

(B) Kersbrook
Prescribed spring burn/Wildfire

(C) South Para
Prescribed autumn burn

(D) Millbrook
Prescribed autumn burn/Wildfire

Fig. 2-3: The effect of fire on species richness (raw numbers, average species count per site) (A), (B) show locations affected by prescribed spring burning. (C), (D) show field locations affected by prescribed autumn burning. (B), (D) were affected by a wildfire in the post2 period.

The plant species composition (defined as the species present and their respective abundances collected as Braun-Blanquet cover) was not related to the occurrence of prescribed burns in all four different field sites.

Table 2-4: Effect of fire on species richness. Likelihood ratio tests (displayed as table of deviance for every field location) for individual levels of factors in the generalised linear mixed model (species richness ~ treatment : period, random effect of transect).

Prescr. spring burns		**Para Wirra**			**Kersbrook (PB)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	10.407	**0.005**	1	5.6855	**0.017**
treatment	1	0.347	0.556	1	1.989	0.158
Period:treatment	2	2.021	0.364	1	1.421	0.233

Prescr. autumn burns		**South Para**			**Millbrook (PB)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	8.114	0.017	1	5.693	**0.017**
treatment	1	3.891	0.049	1	1.949	0.163
Period:treatment	2	2.030	0.326	1	2.971	0.085

Wildfire		**Kersbrook (WF)**			**Millbrook (WF)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	1	0.844	0.358	1	1.037	0.309
treatment	1	0.634	0.426	1	0.163	0.687
Period:treatment	1	0.428	0.513	1	0.566	0.452

Although prescribed spring burning did not affect species composition significantly (adonis interaction (treatment : period): $F = 0.789$, $p = 0.653$) at the location Para Wirra, the period (year) of sampling altered the composition of species significantly ($F = 0.789$, $p = 0.023$). The indicator species analysis did not show an association of specific species with fire. But interestingly, *Caesia calliantha* appeared significantly more in the 'post1' period.

At the other spring burn in Kersbrook, no effect of prescribed spring burning nor period of sampling (adonis level period: $F = 0.755$, $p = 0.313$) on species composition was observed. Indicator species analysis showed an increased appearance *of Stackhousia* sp. one year post fire in the burn site ($p = 0.003$). Only 'before' and one year post fire ('post1') data were used for the analysis, as this site was affected by a wildfire in the third data collection period.

The analysis of the effect of prescribed autumn burning on species composition showed significant differences among the years of sampling (adonis period level; $F = 3.636$, $p < 0.001$) for the location South Para and also a trend towards changes in species composition (adonis interaction level, $F = 1.904$, $p = 0.045$). Indicator species analysis revealed that *Cheilanthes austrotenuifolia* appeared associated with the combination 'burn.post'1 and 'burn.post2' ($p = 0.03$) in post prescribed burn sites.

The effect of wildfire on species composition was tested before and one year after fire. Treatment sites were compared to sites previously affected by prescribed burning. The site in Millbrook showed no significant effect of fire on species composition (adonis interaction, $F = 1.316, p = 0.344$). Indicator species analysis showed that no plants species were significantly associated with wildfire. At Kersbrook, no effect of wildfire on species composition (adonis interaction level, $F = 1.308, p = 0.188$) was detected. An effect of the year of sampling (adonis period level, $F = 1.308$, $p = 0.031$) could be observed, showing that plant species composition differed between the years of sampling. Indicator species analysis showed a trend towards an increased appearance of the weed *Senecio pterophorus* one year following wildfire ($p = 0.04$). *Kennedia prostrata* could only be observed in the 'post1' period (in 2015), but in both control and burn treatment sites.

Table 2-5: Summary of fire induced effects on the plant community. Fire induced changes in plant species richness, plant composition, vegetation structure and the composition of the floral community were assessed using permutation tests. The effect of fire on vegetation density and floral abundance were analysed using GLMM's.

Fire regime	**Time of burn**	**Field site**	**Periods incl in the analysis**	**Effects of fire on potential insect nesting resources, table shows *p* - values and indicates an (+) increase or (-) decline for significant results (cut off: $p < 0.05$)**					
				Plant species richness	Plant species comp.	Veg. structure	Veg. density	Floral abund.	Floral comp
Prescrib. burn	Spring	PW	before, post1, post2	0.364	0.652	**0.004**	**<0.001 (-)**	**<0.001 (-)**	0.083
	Spring	K (PB)	before, post1	0.233	0.313	0.188	0.057 (+)	**<0.001 (-)**	0.375
	Autumn	SP	before, post1, post2	0.362	**0.045**	0.570	0.329	**0.013 (-)**	0.343
	Autumn	M (PB)	post1, post2	0.085	0.156	1.000	0.245	**0.017** (+)	0.063
Wildfire	Summer	K(WF)	before, post1	0.513	0.188	0.156	**0.001 (-)**	**<0.001 (-)**	0.375
	Summer	M (WF)	before, post1	0.451	0.344	**0.0313**	**0.001 (-)**	**<0.001 (-)**	0.094

Effects of fire on vegetation structure and density

The vegetation structure was only affected by a prescribed spring burn in Para Wirra (adonis interaction level, $F = 5.826$, $p = 0.004$). None of the other sites affected by prescribed burning showed changes in vegetation structure caused by fire events. The effect of fire on density changes in the different height classes (strata) showed differences between field sites, indicating the spatial variation. However, vegetation density in height class 3 (0.5 m - 1 m) was reduced by fire in every field location.

The two locations affected by the wildfire showed inconsistent results. Vegetation structure at the location in Kersbrook was not altered by wildfire (adonis interaction, $F = 1.799$, $p = 0.156$), which was not reflected by the site in Millbrook (adonis interaction, $F = 5.68$, $p = 0.03$). Interestingly, both wildfire affected sites showed significant changes in vegetation density in height class 4 (1 - 2 m), but not in height class 3 as it was observed for prescribed burn sites (envfit, MDS Fig. 2-4).

Overall vegetation density (density.e., disregarding height classes) was significantly reduced by prescribed spring burns and following wildfire, but not affected by prescribed autumn burning (Table 2-5). Vegetation density in Para Wirra recovered in the second year following the spring burn (paired contrast ‘before’ vs ‘post*2*’; $p = 0.618$, se = 0.114).

Table 2-6: Effect of fire on vegetation density. Likelihood ratio tests (displayed as table of deviance for every field location) for individual levels of factors in the generalised linear mixed model (vegetation density ~ treatment : period, random effect of transect).

Prescr. spring burns		**Para Wirra**			**Kersbrook (PB)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	3.161	0.206	1	3.752	0.053
treatment	1	0.436	0.509	1	2.837	0.092
Period:treatment	2	59.070	**<0.001**	1	5.179	**0.023**
Prescr. autumn burns		**South Para**			**Millbrook (PB)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	0.120	0.942	1	22.739	**<0.001**
treatment	1	0.239	0.624	1	1.576	0.209
Period:treatment	2	2.23	0.329	1	1.353	0.244
Wildfire		**Kersbrook (WF)**			**Millbrook (WF)**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	1	13.030	**<0.001**	1	3.705	0.054
treatment	1	1.662	0.197	1	10.776	0.001
Period:treatment	1	11.004	**<0.001**	1	32.519	**<0.001**

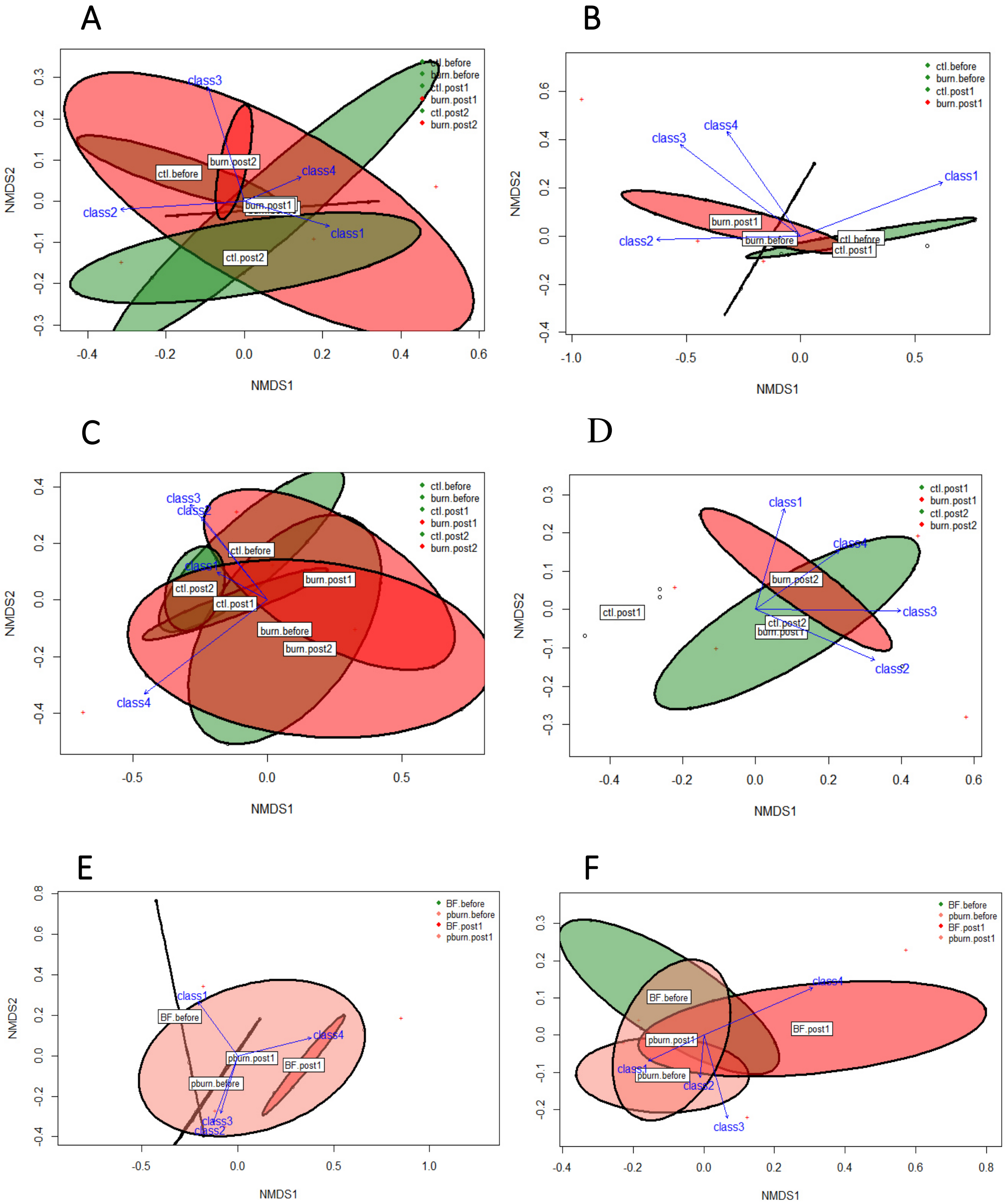


Fig. 2-4: Meta MDS of the number of touches of vegetation in different height classes (class1: 0 - 25 cm, class2: 25 - 50 cm, class3: 50 cm - 1 m, class4:1 m - 2 m) before and after prescribed spring burning for locations A: Para Wirra (stress: 0.089), B: Kersbrook (stress: 0.005). Locations affected by prescribed autumn burning are C: South Para (stress: 0.085), D: Millbrook (stress: 0.024). Locations affected by wildfire: E: Millbrook (stress: 0.047), F: Kersbrook (stress: 0.01). Fire affected sites are red shaded. Long vector lengths indicate stronger effects.

2.5.2. Effects of fire on the floral community

The floral composition was not altered by prescribed burning (adonis; PW: $p = 0.083$, $F = 1.42$; SP: $p = 0.34$, $F = 1.08$; M: $p = 0.063$, $F = 4.01$; K: $p = 0.375$, $F = 1.16$) or wildfire (adonis; M: $p = 0.094$, $F = 4.57$; K: $p = 0.38$, $F = 1.16$). In contrast, floral abundance decreased significantly after all fire events in all field sites (Table 2-7).

Table 2-7: The fire on floral abundance. Analysis of deviance table for a GLMM (abundance ~ period : treatment). Shown are degrees of freedom (*Df*), Chisquare calculations, p - values (Pr(> Chisq) for significance of factors.

Prescr. spring burns	**Para Wirra**			**Kersbrook (PB)**		
	Df	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	2	2.667	0.263	1	83.403	**<0.001**
treatment	1	0.148	0.700	1	0.469	0.493
Period:treatment	2	18.081	**<0.001**	1	13.161	**<0.001**
Prescr. autumn burns	**South Para**			**Millbrook (PB)**		
	Df	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	2	1.137	0.266	1	5.441	**0.019**
treatment	1	0.002	0.276	1	30.747	**<0.001**
Period:treatment	2	8.622	**0.013**	1	5.727	**0.0167**
Wildfire	**Kersbrook (WF)**			**Millbrook (WF)**		
	Df	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	1	18.126	**<0.001**	1	20.677	**<0.001**
treatment	1	0.394	0.530	1	0.191	0.662
Period:treatment	1	51.141	**<0.001**	1	15.871	**<0.001**

Multiple comparison analysis was used to detect fire effects on floral abundance between the different time periods separately (Table 2-8). Results were various across the sites, however, floral abundance in the second year following fire showed always a significant reduction of flowers when compared to the initial abundance before fire in all sites monitored over three time periods. Multiple comparison between floral abundance one year and two years following autumn burning in Millbrook revealed an increase in flowers in the fire affected site, indicating the recovery from fire ($p = 0.0167$). However, this effect was not observed in other sites.

Table 2-8: Paired contrast (p-values) of the effect of fire on floral abundances between the respective years of sampling.

Prescribed spring burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Para Wirra	0.117	**0.008** (-)	**0.001** (-)
Kersbrook	**<0.001**	N/A	N/A
Prescribed autumn burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
South Para	**0.0157** (-)	0.675	**0.005** (-)
Millbrook	NA	**0.0167** (+)	N/A
Wildfire	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Millbrook	**<0.001** (-)	N/A	N/A
Kersbrook	**<0.001** (-)	N/A	N/A

2.5.3. Effect of fire on potential insect nesting resources

The effect of fire on potential insect nesting resources, such as the composition of the ground cover, the abundance of logs and standing litter, or the depth of litter differed, depending on the nature of the fire (prescribed burn or wildfire), the season of the prescribed burn (spring or autumn) and sometimes by the field location itself (results for respective sites below). Nevertheless, overall wildfire had a more severe effect on insect habitat, especially by reducing litter depths, log abundance and by changing the composition of ground covers. A summary of the effect of fire on the observed habitat characteristics can be found below (Table 2-9).

Table 2-9: The effect of fire on: Ground cover composition, analysed using PERMANOVA; Bare ground associated with fire, using MDS vector lengths; Litter depth (lme); Log abundance and the abundance of standing litter (GLMM's).

Fire regime	**Time of burn**	**Field site**	**Periods incl in the analysis**	**Effects of fire on potential insect nesting resources, results in table show *p*-values and indicate (+) increase or (-) decline for significant results**				
				Ground cover	Bare ground associated with fire	Litter depth	Log abundance	Stand. litter (trees)
Prescrib. burn	Spring	PW	before, post1, post2	**0.025**	**0.001** (+)	0.491	0.609	0.794
	Spring	K	before, post1	0.531	**0.002** (+)	0.191	**0.002** (+)	0.065 (+)
	Autumn	SP	before, post1, post2	0.111	0.093	**0.033** (-)	0.959	0.544
	Autumn	M	post1, post2	0.25	**0.001** (+)	0.915	**0.025** (+)	0.431
Wildfire	Summer	K (WF)	before, post1	**0.031**	0.203	**0.066** (-)	**0.004** (-)	0.625
	Summer	M (WF)	before, post1	**0.062**	**0.004** (+)	**0.010** (-)	**0.035** (-)	**0.014** (+)

2.5.4. Effect of fire on the composition of the ground cover

The composition of the ground cover was significantly affected by a prescribed spring burn in Para Wirra (adonis interaction: $F = 4.12$, $p = 0.022$). Changes were driven by the reduction of litter and due to an increase of cover of moss and rocks in burnt sites (Fig. 2-5, A). Testing for contrasts revealed no significant effect of fire on the ground cover composition between the different time periods in Para Wirra, indicating that changes occurred slowly over the three years of the study.

The other spring burn in Kersbrook, which was only monitored before and one year after burning, showed similar results, and no significant changes of the ground cover one year post burning were observed. However, some components of the ground cover were associated with the fire affected site, such as ash (envfit vectors in MDS ordination K: $p = 0.027$) and bare ground (BG, K: $p = 0.019$).

NMDS ordination (Fig. 2-5, C and D) showed similar results for prescribed autumn burning, indicating an increase in ash (A, $p = 0.001$), bare ground (BG, SP: $p= 0.093$, M: $p = 0.012$) and logs (Log, $p = 0.007$) in burnt habitats, whereas moss (M, SP: $p = 0.002$), rocks (Rock, SP: $p = 0.012$) and litter (Litter, SP: $p = 0.004$, M: $p = 0.026$) were significantly associated with unburnt sites. Although some ground cover components were altered by fire, fire was not found to significantly change the relative composition of the ground cover.

In contrast, ground cover composition following wildfire was significantly (adonis: K: $p = 0.031$, $F = 34.42$, M: $p = 0.06$, $F = 11.41$), driven by a reduction of logs and trees on the ground and by an increased amount of bare ground, especially in Millbrook (envfit, BG: $p = 0.004$).

2.5.5. Effects of fire on litter depths, log abundance and standing litter abundance

Changes in litter depths (Fig. 2-6) were generally of small magnitude and the differences were often just a few millimetres. Litter depths was not significantly affected by spring burning, whereas the autumn burn in South Para showed a decrease in litter in burn sites compared to steadily increasing litter in control sites (lme analysis, SP: $p = 0.0328$, sd = 0.259), especially when comparing the before period to the second year after fire (‘post2’). In contrast, wildfire affected sites show mostly a decline in litter depths (K: $p = 0.010$, $sd = 0.25$; M: $p = 0.066$, $sd = 0.00$), although only data for one year post fire was analysed and compared with adjacent prescribed burn sites.

Prescribed spring burning never reduced the mean number of logs (Fig. 2-7). Some sites showed an increase in logs among the years of sampling in both control and treatment site (Para Wirra, Kersbrook), whereas others showed an increase in the fire affected sites (Kersbrook, Millbrook). Wildfires significantly reduced the overall number of logs present at fire affected sites, whereas the number of logs in the control sites increased from one year to the next.

Standing litter, in the form of dead trees and trunks, increased in all field sites over the three periods of sampling. No significant effects of fire on the abundance of dead trees (Fig. 2-8) was detected in the majority of the field sites. Nevertheless, one site (Fig. 2-8, E, Millbrook) showed an increase in standing litter after a wildfire ($p = 0.0143$, $sd = 0.5213$), compared to the prescribed burn site.

The numbers of dead shrubs recorded were very low. The very small variation between sites prevented meaningful tests for these data.

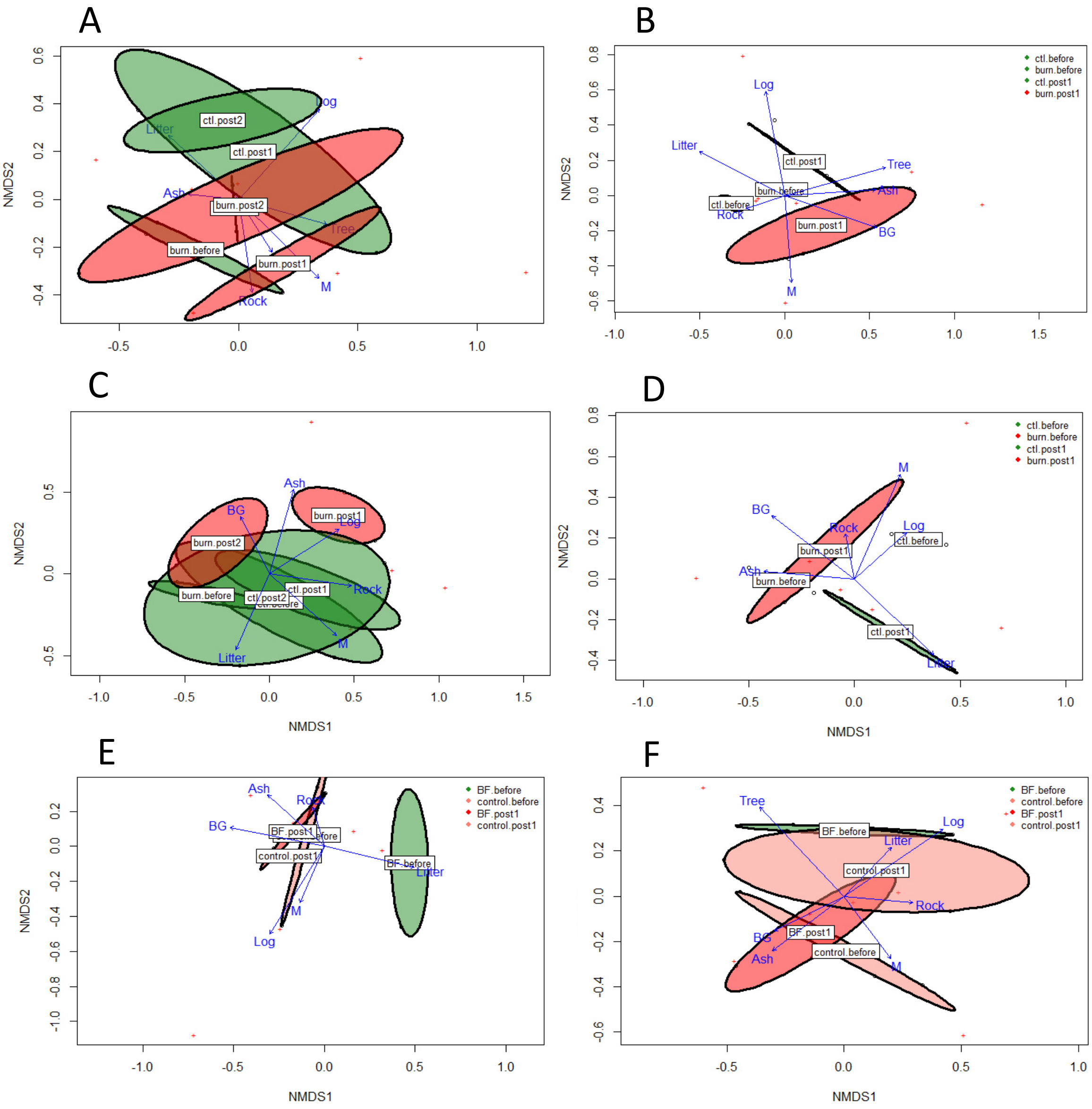


Fig. 2-5: MDS ordination of the relative abundance of different ground covers (Ash, litter, rock, moos, bare ground, log or tree), before, one year as well as two years post prescribed burning in control and burn sites for a prescribed burn. Fire affected sites are red shaded, control sites and unburnt sites are green. A: Prescribed spring burn in in Para Wirra (NMDS stress: 0.18). B: Prescribed spring burn in Kersbrook (stress: 0.11). C: Autumn burn in South Para (stress: 0.12). Wildfire affected sites: E: Millbrook (stress: 0.041), F: Kersbrook (stress: 0.15).

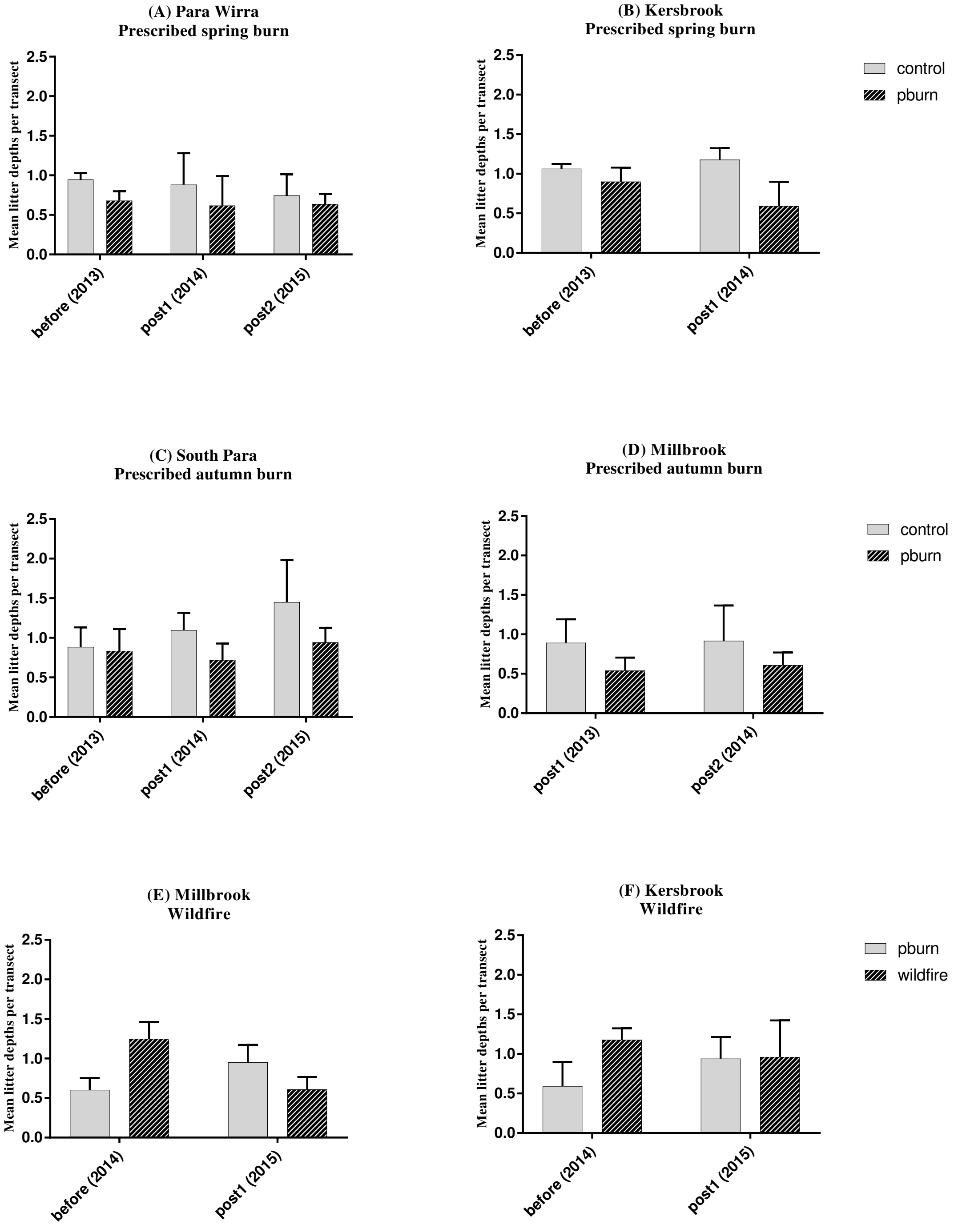


Fig. 2-6: Anova boxplot of mean litter depth (in cm) for different field sites. (A), (B) show sites affected by prescribed spring burning. (C), (D) show field sites affected by prescribed autumn burning. (E), (F) show field sites affected by wildfire.

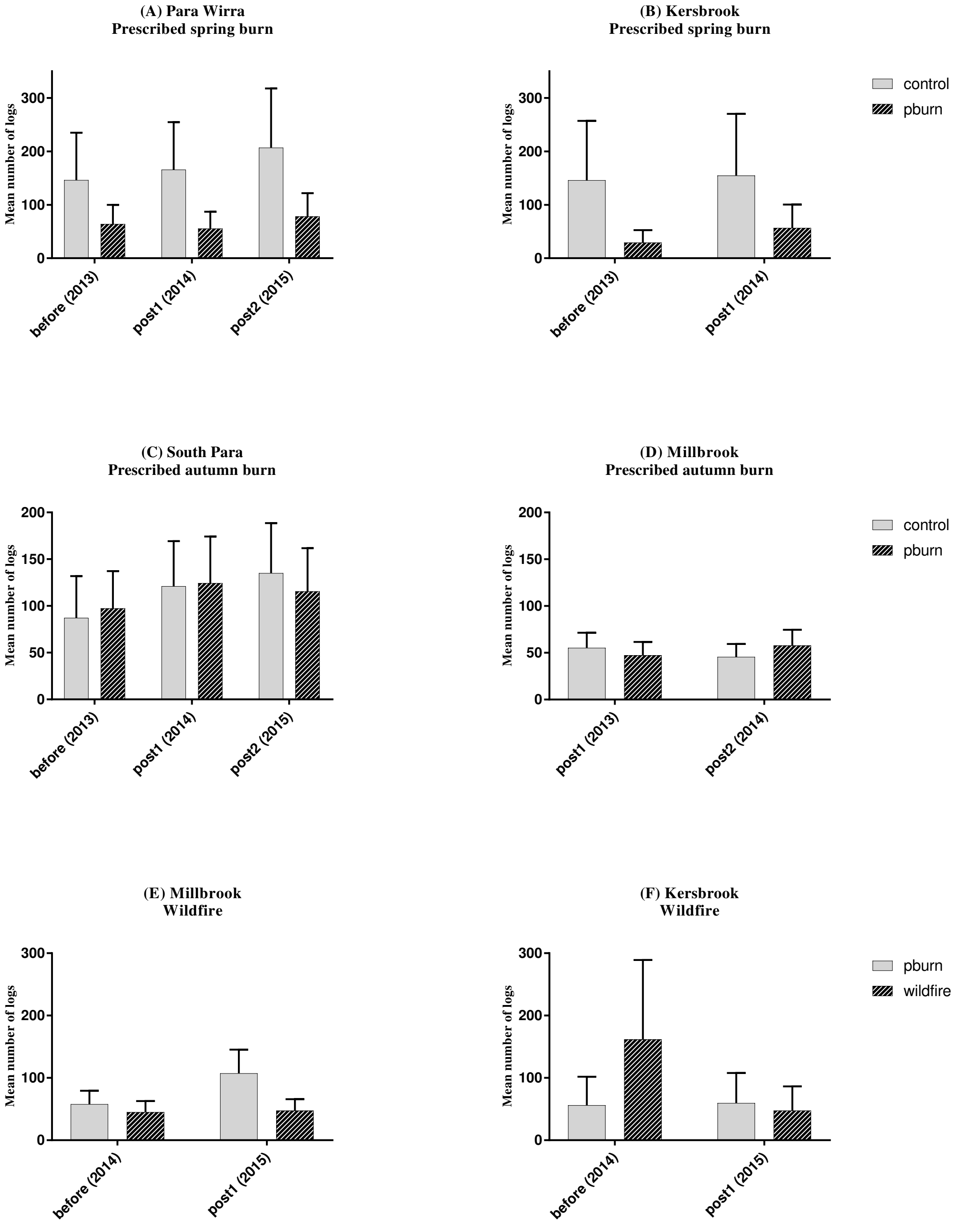


Fig. 2-7: Table of mean number (nb GLMM model) of logs per control and burn site. (A) - (D): Impacts of prescribed burning on log abundance. The ‘before’ period marks data collection before the fire, post1 data was collected in the first spring after prescribed burning and post2 marks the second spring after burning. (E) - (F): Impact of a wildfire on log abundance. Period ‘before’ marks data collection in spring before a wildfire occurred in January 2015. Period ‘post1’ marks data collection on the first spring after the wildfire.

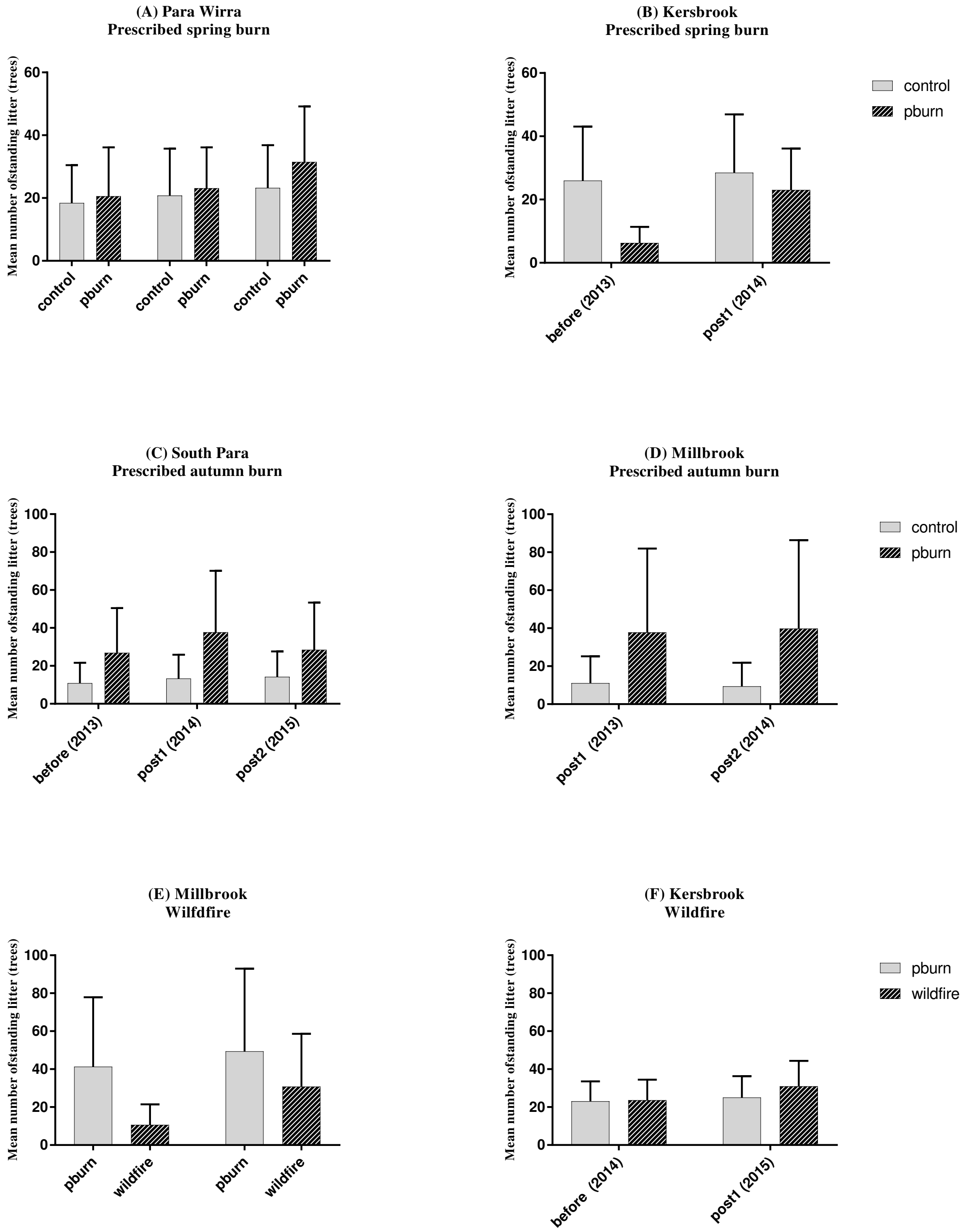


Fig. 2-8: Table of mean number (nb GLMM model) of standing litter (trees only) per control and burn site. (A) - (D): Impacts of prescribed burning on standing litter abundance. The 'before' period marks data collection before the fire, post1 data was collected in the first spring after prescribed burning and post2 marks the second spring after burning. (E) - (F): Impact of a wildfire on standing litter abundance. Period 'before' marks data collection in spring before a wildfire occurred in January 2015. Period 'post1' marks data collection on the first spring after the wildfire.

2.6. Discussion

2.6.1. Effects of fire on insect habitat

This study documented a decrease in floral abundance and vegetation density after both prescribed burns and wildfires. The loss of floral resources could lead to changes in pollinator communities (Potts et al., 2003b). Furthermore wildfires were found to have a severe ecological impact on other insect habitat characteristics by reducing litter depths as well as the abundance of logs and standing litter, which showed only minimal changes following prescribed burns.

Contrary to expectations, no changes in plant species composition were observed in prescribed burn sites and neither in wildfire affected sites. The plant composition in early successional stages after a fire was similar to before the fire. The results also demonstrate that in the observed study system, fire does not affect the number of species present (species richness). These findings are supported by other research conducted in eucalypt forest of southern Australia (Purdie and Slatyer, 1976, Gill, 1999). It seems that most species in Australian woodlands which are present before fire events quickly re-establish in the first months after the fire (Gilbert, 1959, Cremer, 1965, Gill, 1975). These findings were expected for prescribed burning, where the low fire intensity was, presumably, not sufficient to induce germination of dormant seeds (Keeley, 1986, Saharjo and Watanabe, 1997, Penman et al., 2007) and less likely to seriously damage present plants. The observed consistent species richness before and after prescribed fires also supports research that questions the benefits of prescribed burning for biodiversity (Penman et al., 2007, Pastro et al., 2011). For wildfire affected sites, the results do not support previous studies conducted in other Australian forests/habitats which demonstrated an increase in species richness after fire events or reported a temporary increase in species richness through specialised short-lived species (Ashton, 1981, McMahon cited in Bradstock et al., 2012). These results emphasize that eucalypt woodlands of South Australia seem to differ in their response to wildfires from other Australian forests.

Only one location affected by prescribed autumn burning showed a trend towards changes in species composition. Although the result was not strongly significant, it might still indicate an important interactive effect (Facelli and Facelli, 2002), suggesting that the season of the burn could have influenced the species composition. The regeneration after a fire is known to be dependent on the burn season (Gill, 1975) and on post fire conditions, but also on the pre-burn composition of the plant community (Purdie and Slatyer, 1976), which can differ between seasons but could also have differed between the field sites observed in this study. Another explanation might be the shorter recovery time for plants from the burn in April to the surveying period in spring (5 months), compared with a spring burn conducted in November and also surveyed in spring (9 months after burning).

Interestingly, no plant species were found to be negatively associated with recent fire. Although the plant community did not change in the first two years after a fire event, it may still be affected by long term successional processes. Three plant species were found to be positively associated with fire regimes. The weed *Senecio pterophorus* was increasingly recorded in sites affected by wildfires. This result is not surprising, as *S. pterophorus* is an invasive pioneer plant that grows on bare ground after disturbances of the soil or vegetation and can become dominant in the first years after fire (Heddle, 1974). The other observed species positively associated with fire (*Stackhousia* sp., *Cheilanthes austrotenuifolia*) were all resprouters which are known to thrive in post fire conditions (Jon and Zedler, 1978).

Both prescribed spring burning and wildfire were found to reduce vegetation density. In contrast to spring burns, autumn burns had less impact, which may be also related to the time since fire at sampling, which was shorter compared to spring burns, or the different life stages of the plants during the fire (Gill, 1975). Perennial lilies (e.g. *Arthropodium strictum, Caesia calliantha)* that were quite abundant in some of the locations go dormant in late summer and may be less vulnerable to a fire happening in autumn.

The results on the effect of fire on vegetation structure were inconsistent across sites and may indicate dynamic processes of vegetation regrowth after fire events. Vegetation in the height class 0.5 – 1 m was most affected by prescribed fires and it is plausible that changes in vegetation density in the height below 0.5 m were not observed at the time of sampling due to vegetation regrowth since the time of fire. No pattern could be observed when comparing autumn burns to spring burns. In the wildfire affected sites the vegetation in the 1 – 2 m height was the most affected by fire. This result suggests that wildfire may have also affected taller vegetation than prescribed burning, probably due to the higher intensity of the fire and higher reaching flames. It must be noted that also the short time period between fire and sampling may not have allowed the higher vegetation to recover. The different responses found between the different locations of field sites imply that fire can affect similar plant communities in different ways and fire responses can vary between sites, which is consistent with previous research (Keith et al., 2002).

A decrease in floral abundance after recent fire events was observed at all sites affected by prescribed burning and wildfire. These results do not support that fire stimulates flowering as observed by Lamont and Downes (2011), but concur with findings from Potts et al. (2003b) who observed a decline in floral abundances shortly after wildfires in Israel. Fire did not have an impact on the composition of the floral community and the majority of flowering species were still present, but flowering of some species was reduced. This was most likely plants being in regenerative life stages allocating resources to re-growth at the expense of flowering. The results indicate that the year of sampling also plays an important role in shaping the floral community. Changes in floral abundance among the years are likely to be influenced by the climatic conditions.

Further findings of this study indicate that the effect of fire on potential insect nesting resources can differ, depending on the nature of the fire (prescribed burn or wildfire), the season of the prescribed burn and between locations. Prescribed spring burning was not found to decreases litter depths. Litter on the ground could have been reduced directly after the fire, but would have increased again after deposition of canopy leaves killed by the fire. Interestingly, litter depths were reduced by prescribed autumn burning, but the shorter time interval between fire and sampling period is likely to have restricted leaf litter accumulation. As expected a decrease of litter depths after wildfires was recorded. The canopy in wildfire-affected sites was mostly consumed by the fire, preventing accumulation of new leaf litter. These results only take into account the short term effects of fire (up to 24 months after a fire), but litter in eucalypt woodlands is expected to rise above the pre-fire results at 4 - 5 years after fire events due to reduced decomposition rates (Raison et al., 1986).

The abundance of dead branches and logs increased after prescribed burning in most of the field sites probably due to limb or tree mortality and fall. In contrast, wildfire affected sites showed a decrease in logs, presumably due to fire consuming the material. No effects of fire on standing litter in the form of dead trees were observed. The prescribed burn as well as the wildfire resulted in little mortality of fire resistant species with thicker bark such as *Eucalyptus* species. A wildfire-induced increase in the abundance of dead smaller shrubs was documented. Species like *Calytrix tetragona, Hakea spp.* and *Spyridium parviflorum* had most of their aerial parts killed, but not all of them were consumed by the fire (personal observation). Some of these plants are expected to resprout in later years.

Most of the variation in our results is likely to be related to the intensity and patchiness of the fire. Wildfires are likely to consume more litter, logs and standing litter than prescribed fire. Importantly, this study indicates that prescribed burning does not significantly reduce the fuel load (in form of log abundance, litter depth and standing litter) which contradicts previous studies that reported a decline of fuels (Carrington, 1999, Hubbert et al., 2006). Low intensity prescribed burns consume less biomass and have lower mortality of limbs and branches. The prescribed burns conducted in this study were mostly patchy, especially because transects with abundant grass cover or transects with lower fuel load did not burn very well. Even though efforts were made during burns to ensure that areas containing the transects were burnt, some parts of some transects were unburnt. This might explain the unexpectedly few changes in insect habitat associated with prescribed burning and indicates that patchy prescribed burns are likely to have a lower ecological impact than a wildfire (Penman et al., 2007).

2.6.2. Implications for insect pollinators

The floral composition after both prescribed burns and wildfires mirrored closely the pre-fire composition and no plant species were found to be negatively affected by recent fire regimes. These findings suggests that pollinators relying on specific plant species, for nectar or pollen resources are unlikely to lose their food plant. In contrast, the observed decrease of flower abundance may still lead to changes in insect communities as the abundance of pollinating insects is strongly connected to the abundance of their resources. A decline in floral abundance could potentially lead to a decline in bee abundance and diversity (Ne'eman et al., 2000, Potts et al., 2003b) and potentially to a negative impact on insects with smaller foraging ranges.

Both prescribed fires and wildfires induced changes in pollinator nesting resources that can have important implications for insect survival and reproduction (Fig. 2-9). The increased availability of bare ground observed after both fire types can be an important resource for ground nesting insects, such as native bees that burrow nests in the soil, and could enhance insect abundance and diversity in burnt habitat (Potts et al., 2005). Also the reduced density of the vegetation due to fire is likely to favour heliophile insects that prefer open spaces, such as hoverflies and wasps (Gittings et al., 2006).

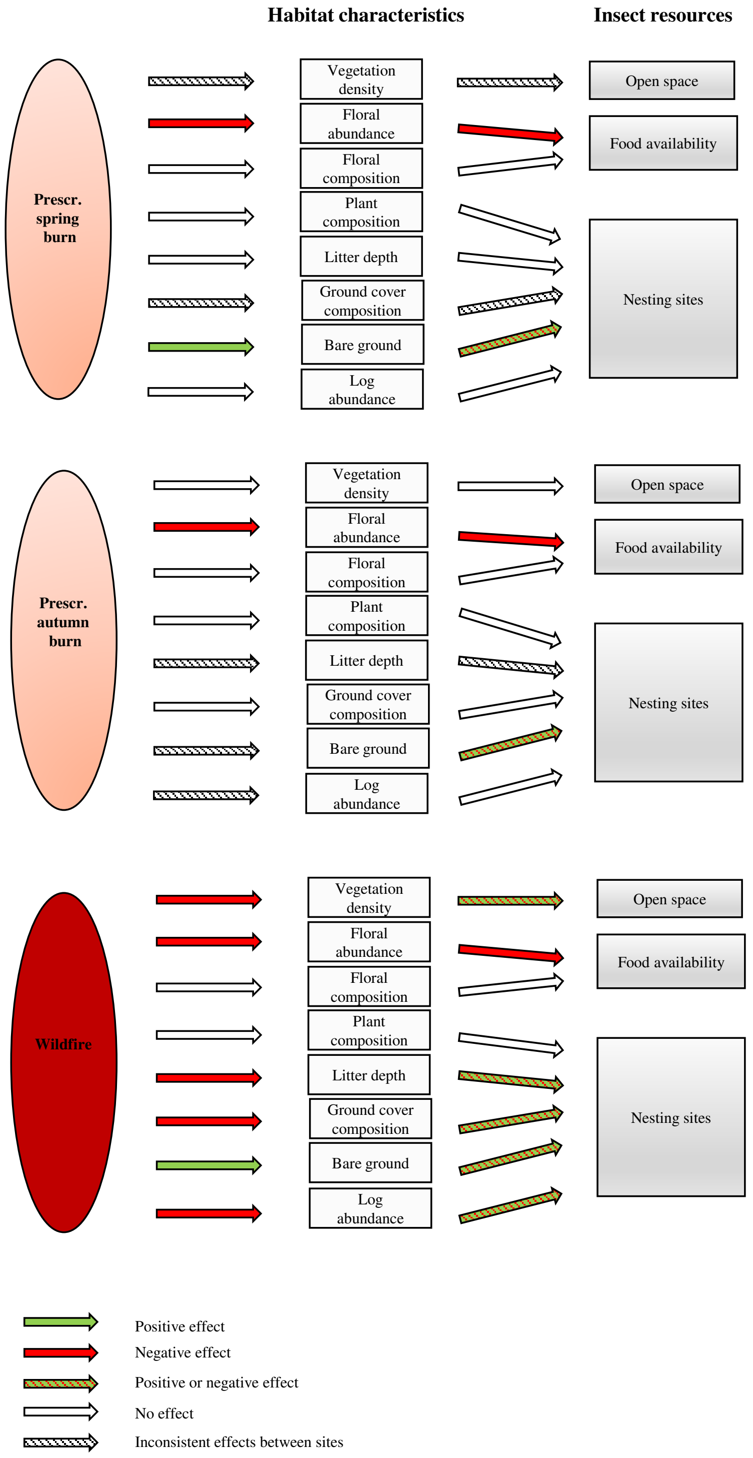


Fig. 2-9: Proposed graphic model summarising the effects of the three different fire events (prescribed spring burn, prescribed autumn burn, wildfire) on habitat characteristics and the potential impact on insect resources.

The changes on pollinator habitat were not always consistent between prescribed fires and wildfire. For instance, a reduction in logs was only recorded after wildfires which can have a potential negative influence on insect communities. Logs play not just an important role for the creation of patches and can enhance plant species richness and diversity as well as the emergence of seedlings (Bowman and Facelli, 2013) but they also serve many insects as microhabitats or nesting sites (Vanderwel et al., 2006). Insects or their larvae living directly in logs could be killed by the fire and recolonization can be difficult if many logs are destroyed by the fire. The wildfire observed in this study were very homogenous and left no unburnt vegetation patches, which lead to more drastic changes in insect habitat and could potentially lead to more changes in pollinator communities. The patchy nature of prescribed burns can instead provide safe “islands” or “refuges” for insect pollinators to survive the direct effects of fire (Dosso et al., 2011). Unburnt patches or patches of reduced fire intensity may not just allow for an increased survival of individuals during the fire, they also allow insects to find food resources unaffected by the fire and give opportunity for dispersal into and re-colonisation of the burned areas following the fire event (Bradstock et al., 2005, Robinson et al., 2013).

However, this study found the effects of fire on insect habitat to be highly heterogeneous at various scales which may certainly lead to heterogeneous responses of the pollinator community.

2.7. Conclusion

The results of this study show that fire causes profound changes in the structure of South Australian eucalypt woodlands. Both prescribed spring burns and wildfires impacted floral abundances and reduced vegetation density. Of particular importance is the observed decrease in floral abundance, which may negatively impact foraging success of pollinating insects (Ne'eman et al., 2000). The increased availability of bare ground after fire events might on the other hand benefit many Australian ground nesting bee species. The findings of this study also suggest that prescribed burning does not significantly reduce logs, litter and standing litter, which may be a favourable outcome for insect pollinators that rely on these for nesting opportunities. This however, raises questions about the rationale behind the prescribed burns, which are assumed to reduce fuel to prevent intense wildfires. As expected, the impact of a high intensity wildfire was more severe than the impact of heterogeneous prescribed burns and further lead to significant changes in habitat characteristics like litter depth, logs abundance and the composition of the groundcover. The important changes in pollinator habitat after a wildfire have potential to cause significant changes in pollinator communities.

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CHAPTER 3

Reliable identification of orchid pollinators using DNA barcoding methods combined with morphological identification

The native bee *Lasioglossum Chilalictus lanarium* with orchid pollinia attached. Photo by Ben Parslow.

Statement of Authorship

Title of Paper	Reliable identification of orchid pollinators, using DNA barcoding methods combined with morphological identification
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Publication Details	Conducted all lab work, conducted all statistical analyses and wrote the manuscript

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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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3. Reliable identification of orchid pollinators, using DNA barcoding methods combined with morphological identification

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3.1. Abstract

DNA barcoding using the *CO1* gene can provide a reliable method to identify pollinating insects. To date, the Australian insect fauna has not been well sampled and very few sequences are available in online data banks. This study aimed to identify insect specimens from the families Apidae s.l., Syrphidae and Tiphiidae, collected for an ecological field studies, with a combined approach of DNA barcoding and morphological identification. Barcoding worked well for distinguishing between species, especially for hoverflies and native bees. In particular families with lower species diversity such as hoverflies could be reliably distinguished, using a cost effective subsampling approach. Subsets of species separated by barcoding could then be forwarded to expert taxonomists for morphological identification. However, DNA barcoding of large samples, for example, bees encountered in this long term ecological study, was time and cost intensive. Ecologists should consider using new and cost saving approaches such as next generation sequencing. Furthermore, barcoding of thynnine wasps only using one gene (*CO1*) was problematic and the presence of the endosymbiontic bacterium *Wolbachia* potentially caused further difficulties that resulted in specimens unable to be identified using standard amplicon Sanger sequencing approaches. Most specimens analysed in this study could be assigned to species which provided a good base for further ecological studies. The results of this study will further contribute to the representation of Australian insect fauna in the online databases.

Keywords*:* *CO1* gene, barcoding, insects, bee, wasp, phylogenetic tree

3.2. Introduction

DNA barcoding is the use of a short DNA sequence from a standard genetic locus and has been applied to identify species in a wide variety of taxonomic groups (e.g. Diptera, Hymenoptera; Hebert et al., 2003b). Several authors have also recommended the use of barcoding for taxonomy (Hebert et al., 2003b, Blaxter et al., 2005, Schindel and Miller, 2005). Unknown specimens can be allocated to species that have previously been described and barcoding can further help with the discovery of new species (Meyer and Paulay, 2005). The identification system used most often for insects is a short DNA sequence fragment of ~ 600 bp from the mitochondrial gene, cytochrome c oxidase subunit 1 (*COI*). Many studies have previously shown that the *COI* identification system provides a reliable, cost-effective and accessible solution to the current problem of species identification (Hebert et al., 2003a).

Barcodes obtained from unknown specimens can be compared to online data banks such as GenBank ® (https://www.ncbi.nlm.nih.gov/genbank) which is a genetic sequence database, an annotated collection of all publicly available DNA and protein sequences (Benson et al., 2012). It also hosts the program Basic Local Alignment Tool (BLAST), which finds regions of maximum similarity among target sequences and the Genbank database and ranks them using a statistical score. Another collection of reference sequences is the Barcode of Life (BOLD, http://www.boldsystems.org/) databank, which is eukaryote-specific and only contains barcoding sequences (e.g. *COI* sequences for animals). The BOLD databank also contains non-public private sequences which are not in Genbank, but are still included in the identification process. However, Genbank sequences often need to be treated with caution, as anyone can submit sequences and there is a chance of misidentified taxa or erroneous sequences and there is no requirement for voucher deposition. In contrast the BOLD sequences are mostly reviewed by specialists (BOLD handbook) and they also have strict requirements for submission, including digital images and chromatogram files for all sequences.

A barcoding approach could be particularly helpful in regions of the world where the diversity of pollinator remains poorly studied (Brown and Paxton, 2009, Eardley et al., 2009) and where keys are incomplete - for example, for native bees in Australia (Batley and Hogendoorn, 2009). It has been shown that DNA barcoding can help to resolve difficult issues in bee taxonomy and can therefore be a useful tool in ecological studies (Sheffield et al., 2009).

DNA barcoding has already been successfully used to identify pollinating insects. It has recently adopted for species identification of bees (Murray et al., 2009, Sheffield et al., 2009, Davies et al., 2013, Groom et al., 2013), flies (Masetti et al., 2006, Ståhls et al., 2009, Renaud, 2011) and wasps (Griffiths et al., 2011b, Quicke et al., 2012) and also for the discovery of new species (Gibbs, 2009, Magnacca and Brown, 2012). The most common primers used for *CO1* barcoding for insect specimens are the so called universal Folmer primers (Folmer et al., 1994). Also ‘Lep primers’ which were originally developed for lepidopterans (Hebert et al., 2004) have shown great success for barcoding insects from other orders (Hajibabaei et al., 2006, Smith et al., 2006, Quicke et al., 2012).

However, several studies have also reported restricted success for the identification of some insect taxa using DNA barcoding, for example (Meyer and Paulay, 2005) showed low identification and high intraspecific variability while testing and identifying 449 dipteran species. Another limitation of DNA barcoding success could be the infection of target specimens with the endosymbiotic bacterium *Wolbachia*, which has been reported to interfere with barcoding success for dipterans (Whitworth et al., 2007), wasps (Klopfstein et al., 2016) and bees (Gerth et al., 2011).

While many European insects, especially hoverflies and bees, have been *COI* - barcoded in the past and sequences can be found in online databases, most South Australian pollinators have not been sequenced. In particular, barcode data for many native bees, hoverflies and for thynnine wasps are lacking. It is difficult to identify species using traditional morphological methods because of a lack in taxonomist specialists and identification keys. There are very few keys for Australian native bees, wasps and flies (but see Stevens et al. (2007) and all three groups have a large number of undescribed species. The majority of Australia’s over 1,500 native bee species are solitary and show considerable morphological diversity which requires a high level of taxonomic expertise for their identification (Sheffield et al., 2009). Identification keys often rely on quantitative characters, while more than 57 % of Australian bees are not covered by available keys (Batley and Hogendoorn, 2009). Approximately 25 % of Australian bees are undescribed and around 50 % of described species need revision (Batley and Hogendoorn, 2009).

Identification of native wasps is even more difficult. In particular, the identification of thynnine wasps requires specialist expertise and currently only one taxonomist in Australia is able to identify these wasps using morphological characters. Wingless female wasps are rarely collected and species identification is based on male genitalia (Brown, 2005). In Australia, there are around 600 described species of thynnine wasps in 48 genera (Brown and Phillips, 2014) and Brown (2009) estimates that a further 1000 species are still undescribed. In this respect, DNA barcoding could provide a powerful tool to help identify Australian species.

This project aims to identify insects collected in an ecological field study, using a combination of DNA barcoding and morphological identification. The results of this research will provide essential information for further research, investigating the effect of fire on orchid pollinating insects. The focus of this study was on specimens collected from three families, hoverflies (Syrphidae), native bees (Apidae s.l.) and thyninne wasps (Tiphiidae: Thynninae) and aimed to barcode specimens captured in field surveys.

3.3. Methods

Over 3500 specimens were collected over three years in spring (for more information on insect collecting techniques, see Chapters 4, 5 and 6). Insects were either collected in traps with collection bottles containing 100 % denatured ethanol or were directly transferred from vane or pan traps into vials filled with 100 % ethanol. After every collection period, trapped insects were first sorted to Hymenoptera (bees and thynnine wasps) and Diptera (syrphid flies). All specimens were given a unique number, referring to a data sheet including collection date, collecting method (trap type) and location (field site, burn treatment, trap number, GIS coordinates). This study focussed on identifying species belonging to five orchid pollinator taxa, divided into three main groups: hoverflies (Syprhidae), native bees (with Halictinae, Collectinae, Apinae) and tiphiid wasps (Thynninae). Not all insects collected in our study could be barcoded due to the high number of insects trapped and the limited time frame and budget of the project.

3.3.1. DNA extractions

DNA was extracted from a single mid leg to preserve insect specimens as intact as possible. We followed standard protocols and used a lysis method and CCDB Glass Fibre Extraction plate. Insect legs were washed with 10 mM Tris twice before 100 µl of insect lysis buffer (97 % lysis solution with the combination of NaCl, Tris-HCI, EDTA, SDS plus 3 % Proteinase K) was added per sample. Samples were digested overnight at 56° C. DNA was precipitated with a binding mix (containing 96 % ethanol, guanidine thiocyanate, EDTA, Tris-HCI, Triton X-100) and transferred to an Acroprep Filter plate (PALL Acroprep ADVANCE 96-Well Filter Plate, 1ml 3.0 µm Glass Fibre/0.2 µm Supor). The lysate was washed twice (protein wash buffer, containing binding buffer, 96 % ethanol and water) and DNA was resuspended/eluted in 50 µl TLE by a final elution centrifugation. The DNA was amplified using PCR and different primers were tested and developed, to target the *COI* gene. Early tests showed that the commonly used Folmer primers (Folmer et al., 1994) showed better results than the universal Lep primers LepF1/LepR2 (Hebert et al., 2004), which have been successfully used for sequencing Fijian halictine bees and thynnine wasps in earlier studies (Groom et al., 2013). Therefore, we used Folmer primers (LCOI490, F 414 and R 423) and cycling parameters from Hebert et al. (2003a), slightly modified by extending the final cycle from 5 min to 10 min. Each PCR product was then tested for the amount of DNA content by running 1.5 % agarose gels with DNA markers (Hyperladder IV). Subsequently, different amounts of PCR products were sequenced in one direction, using sequencing reactions containing 1 µl Big Dye, 3.5 µl 5XBD, 1 µl Primer and the required amount of DNA and water. Due to time and cost considerations, unpurified sequencing reactions were submitted to the Australian Genome Research Facility (AGRF). The sequencing results were analysed using the barcoding software Geneious, version 5.4.2 (Drummond et al., 2011). The *COI* sequences were initially aligned, using global alignment with free end gaps (cost matrix: 65 % similarity) and then modified to remove ambiguously aligned regions. Shifted codon positions or intron/exon boundaries in native bee sequences were aligned to fit voucher sequences from Danforth and Ji (2001).

3.3.2. Species delamination analysis

We used Geneious, version 5.4.2 (Drummond et al., 2011) to analyse species relationships by using *COI* sequence alignments. Maximum Likelihood (ML) phylogenetic trees (PhyML, Guindon and Gascuel, 2003) were obtained for each taxon group and analysed using an online version of the program bPTP (http://species.h-its.org/ptp/) to generate species delimitation hypotheses. We also used the PhyML tree to calculate pairwise average ML distances with 10 % cut offs to identify the divergent monophyletic groups of individuals. The alignment of the *CO1* data for the PhyML tree was also analysed using the program Automated Barcode Gap discovery (ABGD; http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html; Puillandre et al., 2012) with the following parameters: Pmin = 0.001, Pmax = 0.1, steps = 10, X (relative gap width) = 1 and the Kimura 2 parameter distance model (TS/TV = 2.0). The resultant tree was used to measure phylogenetic support of species, using the species delimitation plug in (Masters et al., 2011) of the Geneious software, which applies the method of Rosenberg (2007) and calculates the probability of reciprocal monophyly under the null model of random coalescence. We used a Rosenberg cut off of P (AB) of < 0.05 for testing the species hypotheses distinguished by ABGD.

Selected high quality sequences from distinctive linages supported by Rosenberg statistics (< 0.05 cut off) were then compared to online databanks (BLAST and BOLD). If they were identical or showed only minor differences (Max Identity over 98 %) it was assumed that the sequence was likely to belong to the same species. Nevertheless, all bee and hoverfly specimens from species separated by species delimitation (Rosenberg cut offs), disregarding matches in the databanks, were given to specialists (see Acknowledgements) for morphological identification and to test for congruities with the sequence databank results.

3.3.3. Combining morphological and genetic approaches: Selecting specimens (subsets) for barcoding analysis to obtain voucher specimens for morphological identification

Syrphidae

Hoverflies were first sorted based on obvious morphological differences; “yellow-black” colour (wasp-mimicry) and the remaining “others” being black or red. The few specimens sorted to “others” were identified to genus level. To ensure that species were not missing in the large “yellow-black” group, a total of 100 of the “yellow-black” syrphid flies were randomly chosen and DNA barcoded, targeting *CO1*. A PhyML of *CO1* sequences was then generated and analysed using the three different species delimitation approaches. The resulting sequences separated by the analysis were first compared to the Genbank database and were later identified morphologically by hoverfly specialists (see Acknowledgements). Detailed photographs were taken of morphological distinctive features of the different flies. Photos were taken using a Visionary Digital BK+ imaging system with a Canon EOS 7D 18 megapixel camera. Several photos at different focal planes were stacked using Zerene Stacker, Zerene Systems LLC, PMax software and resized and edited in Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA). All “yellow-black” hoverflies were finally identified based on their morphology, using the voucher specimens and photographs from the different species separated by DNA barcoding.

Apidae s.l.

All native bees from the first collection year were barcoded and phylogenetic trees obtained. Specimens collected in the two following periods were pre-sorted, separating and identifying morphological distinctive species, such as *Paraphecodes* spp., *Amegilla* spp. species as well as *Lasioglossum* (*Chilalictus) clelandi* and *L. C. lanarium* from the remaining unknown species. All unknown specimens were barcoded as well as randomly chosen specimens (subsets of 10 specimens) from the morphologically identified specimens. Barcoding was repeated if the first attempt failed to produce good sequences. Finally, all obtained sequences ($n = 462$) were aligned and phylogenetic trees were generated. Due to limitations in the Geneious species delimitation plug in (inbuilt limit for the number of nodes) the number of *CO1* sequences had to be reduced (to $n = 176$) for building the final tree and applying Rosenberg statistics. This was done by removing sequences from abundant species which showed very distinctive lineages in the PhyML tree which were also supported by ABGD. Duplicates were also identified (number of identic sequences is indicated in brackets) and sequences with low quality were excluded from the analysis. Sequences with lower quality were loaded into the tree later to obtain grouping information. Furthermore, different sequences from South Australian bees, such as the nine *Chilalictus* species barcoded by Bryan N. Danforth (1999) were loaded into the dataset to gain more insight into the grouping of *Chilalictus* specimens. The processed sequences for single species were compared with sequences saved in existing databanks (Genbank, Bold) or with bee sequences obtained from other research projects within the University. Voucher specimens from species separated by species delimitation analysis (Rosenberg statistics, 0.05 cutt offs) were later identified morphologically by bee specialists (see Acknowledgements). Species were given a unique “working name”. Species which could not be identified, either with matching sequences in existing databanks or morphology kept their working name for further ecological research.

Tiphiidae (Thynninae)

A total of 196 thynnine wasps were available from field collections. Considering time and cost limitations as well as further use of the data, only a subset of specimens was barcoded. Wasps were sorted into groups (from now on called "morpho-groups") based on morphological characters such as colour patterns, pilosity, and size (no genitalia dissection/examination was conducted), using a 10 x binocular microscope. Random specimens of those morpho-groups were barcoded, with sample size depending on the size of the group. All females not physically attached to male wasps were chosen for barcoding. Overall, 110 specimens were barcoded from 12 clearly distinguishable morpho-groups, excluding the females which could not be morphologically assigned to groups. A ML phylogenetic tree (PhyML) of *CO1* sequences was obtained using mostly clean sequences (over 75 % HQ). Sequences with lower quality were loaded into the tree later to obtain grouping information. The genetic information obtained on species delimitation, using Rosenberg statistics was then compared with the morphological groupings.

3.4. Results

3.4.1. Syrphidae

The majority of flies captured ($n = 2{,}864$) had the typical hoverfly appearance, showing the wasp mimicry colours of black and yellow. Different lineages could be clearly distinguished within the "yellow-black" group ($n = 85$) using DNA barcoding. The PhyML tree showed clear distinctions between three putative species (Fig. 3-2).

COI barcode data for the three species showed little similarity with sequences in Genbank after a BLAST analysis in 2014. The proportion of the alignment that involved identical nucleotides'(Max Ident) never reached the nominated 98 % threshold and the results for lower proportions (Max Ident: 96/97 %) showed different Syrphidae species for the target sequences.

A later BLAST analysis in 2017 found close matches for two of the three species. One species closely matched a sequence for *Simosyrphus grandicornis* (Macquart, 1842), with a Max Ident of 99 %. The other species also had a 99% identity match with *Melangyna collatus* (Walker, 1852). The third species had an identity match of 96 % with a sequence of the species *Meliscaeva* sp. (accession: KM270872.1). Sequences are available on request.

Distinctive specimens and voucher specimens of species separated by DNA barcoding were morphological identified by hoverfly specialists (see Acknowledgements). Overall, six different species could be distinguished (Fig. 3-3). The specimens separated by DNA barcoding could be identified as *Melangyna collatus* and *Simosyrphus grandicornis* (Fig. 3-1), which confirms the BLAST results. The other species identified included an undescribed *Meliscaeva* spp. (n = 5), and the genera *Eumerus* and *Psilota* which could not be identified to species level (Fig. 3-3). Another distinctive species *Melangyna viridiceps* (n = 1) was also morphologically identified but was not available for sequencing.

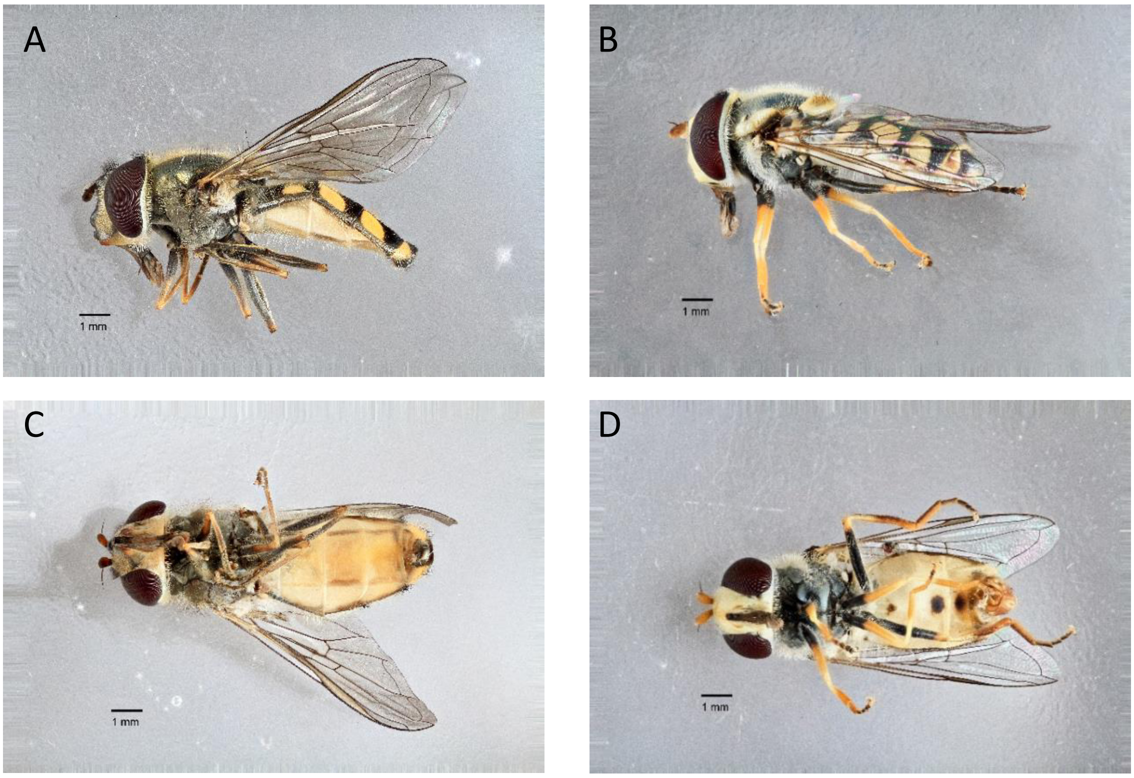

Fig. 3-1: Lateral/ventral view of A/C: *Melangyna collatus* and B/D: *Simosyrphus grandicornis*. Photos by author.

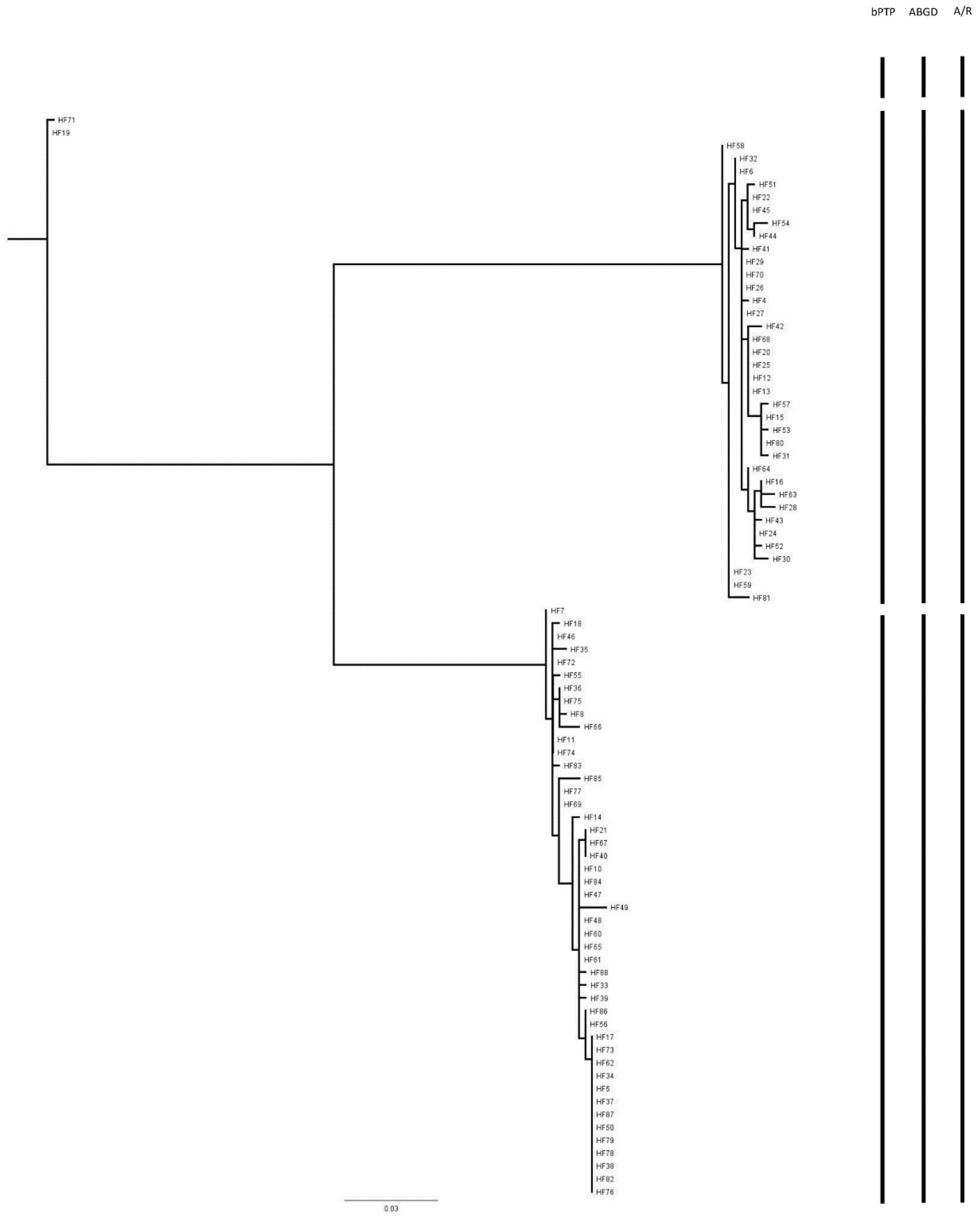

Fig. 3-2: PhyML tree of CO1 data of "yellow-black" hoverflies (n = 85 specimens), showing results from species delimitation analysis. PTP, ABDG analysis and ABDG analysis with Rosenberg statistics**.**

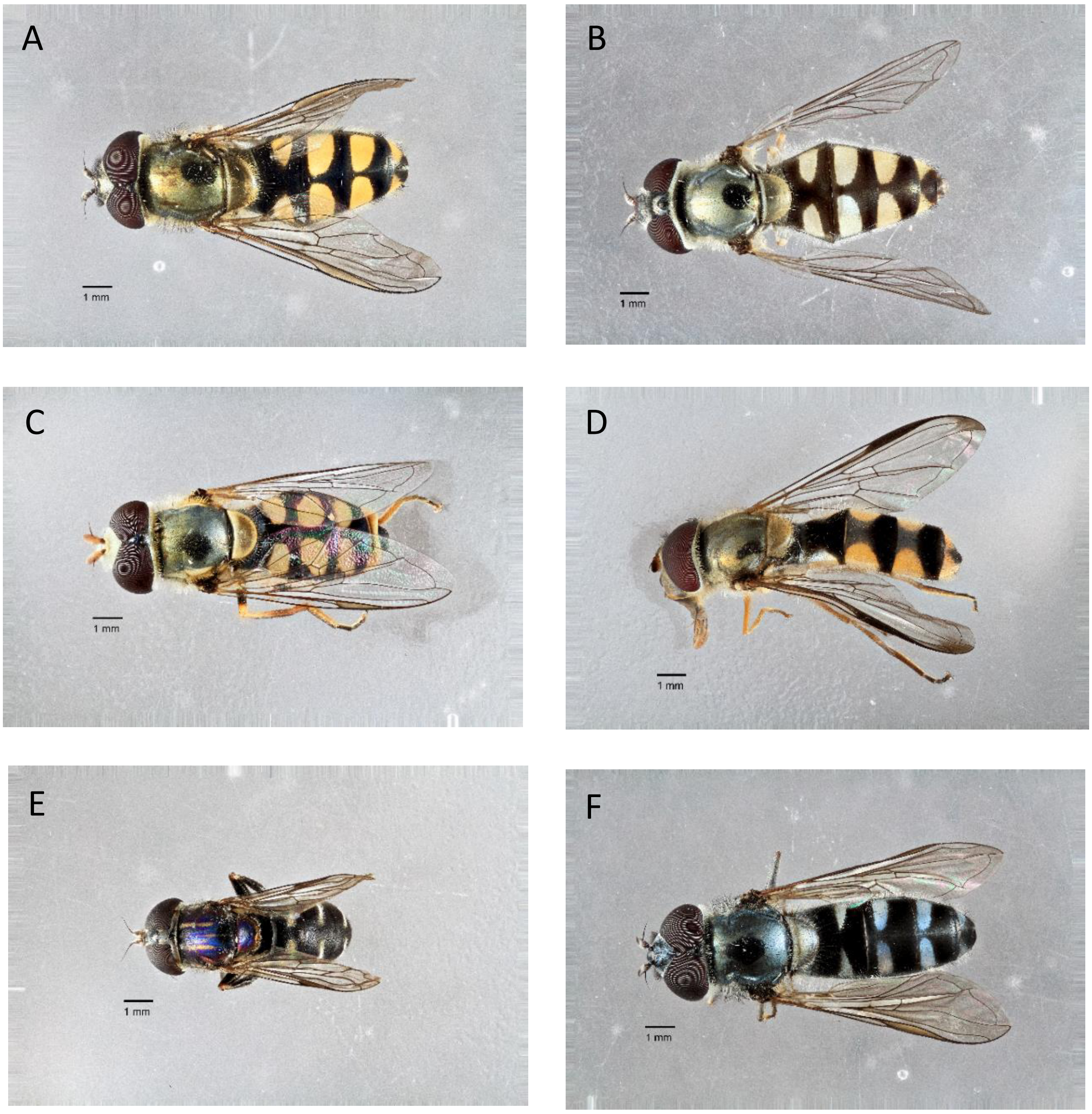

Fig. 3-3: Specimens of identified Syrphidae (dorsal view). A: *Melangyna collatus* (bright colours), B: *Melangyna collatus* (lighter colours), C: *Simosyrphus grandicornis,* D: *Meliscaeva* spp., E: *Eumerus* spp., F: *Melangyna viridiceps*. Photos by author.

3.4.2. Apidae s.l.

DNA barcoding analyses separated the 462 bee specimens into 27 different species, using Rosenberg statistics (0.05 cut off). Thirty-eight species were detected using the bPTP method and 37 species by the ABGD method. For a small number of specimens barcoding failed even after several attempts, but were later morphologically assigned to species.

Very few sequences showed matches over 98 % identity (proportion of the alignments that involved identical nucleotides') with existing sequences in Genbank (Table 3-1). Only one sequence showed a 100 % match for a target sequence, which was for

Brevineura xanthoclypeata, which also matched the BOLD sequence search and the morphologically identification (*Brevineura* sp.). Target sequences for NB9.2 showed two matches to two different species (*Exoneura nigrescens and Exoneura robusta*) with identical Max and identity scores in Genbank. The same target sequence showed a close match (> 98 %) with a sequence from *Exoneura robusta* in the BOLD database.

Searches for other target sequences in the BOLD databases often showed no matches (Table 3-1). Nevertheless, voucher specimens from all species separated by barcoding were identified morphologically. The Genbank sequence AF103956 for *Lasioglossum Chilalictus lanarium* is actually *L. C. clelandi,* according to morphological identification. However, some species separated by barcoding could not be identified to species level, but were given a genus description and working name. This was the case for three *Leioproctus* spp. and several *L. Chilalictus* species. NB9.2 was only morphologically identified to genius level *(Exoneura* sp.*),* but it is most likely *Exoneura robusta* as suggested by Genbank and BOLD searches.

Barcoding separated individuals morphologically identified as *Lasioglossum C. erythrurum* into two different species, *Lasioglossum C. cf. erythrurum* (NB2.1) and *Lasioglossum C. cf. erythrurum* (NB17). The term cf. between species and genus name is further also used to express a possible identity of a species.

Barcoded subsets supported the previous morphological groupings apart from minor errors for some specimens (*n* = 3) of *Lasioglossum Chilalictus lanarium* which were confused with *L. C. clelandi*.

Sequences are available from the first author upon request.

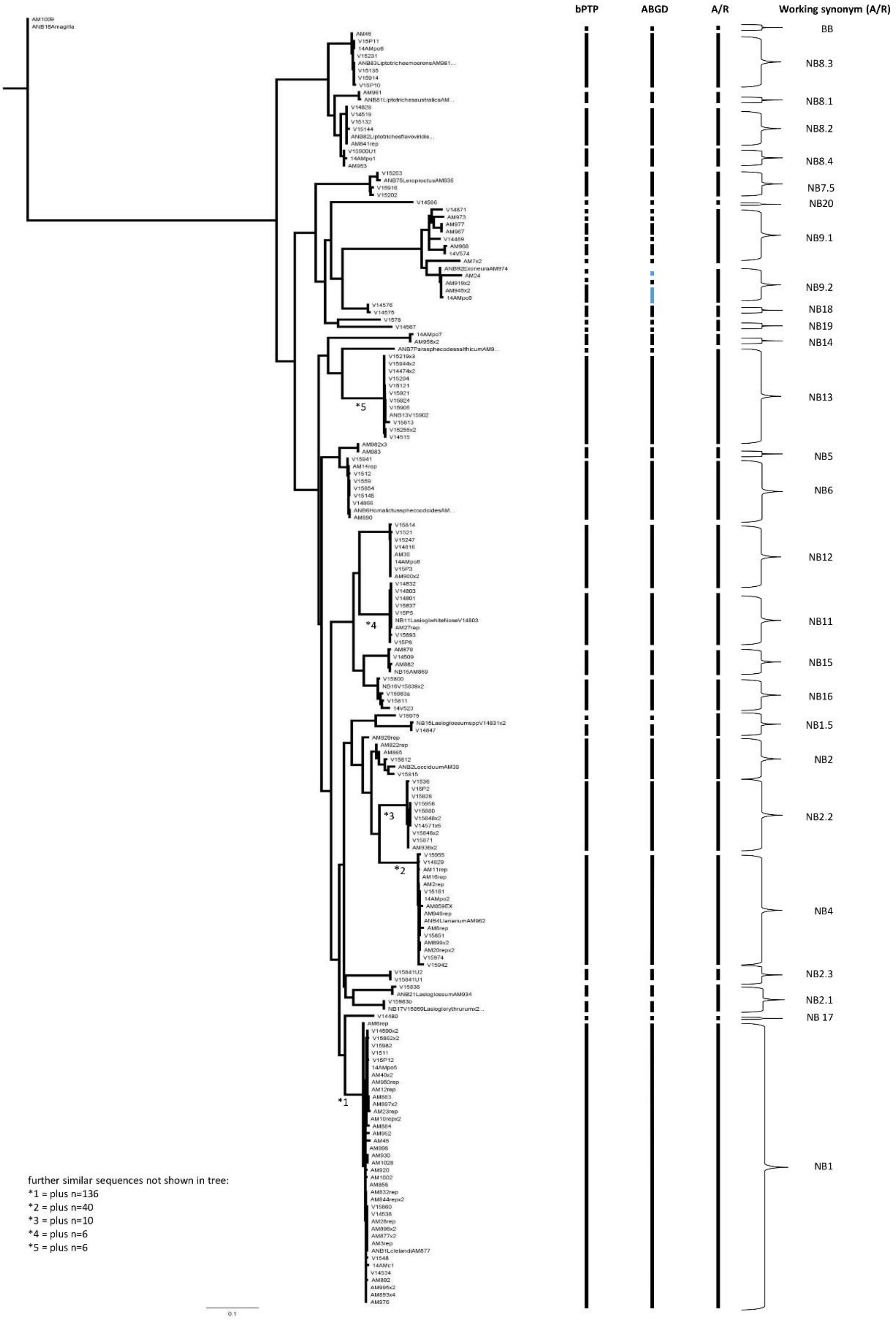

Fig. 3-4: PhyML tree of CO1 data of native bees (n = 176 specimens), showing results from species delimitation analysis: bPTP, ABGD analysis (ABGD), ABGD analysis with Rosenberg statistics (A/R) and working synonyms for species separated on A/R basis.

Table 3-1: Translation of working synonyms (see tree Fig. 3-4) into species names obtained by morphological identification compared to BLAST (Genbank) and BOLD results from selected sequences (high quality) of each species.

Working synonym	**Species (morphological ID)**	**Voucher sequence used for BLAST**	**ID recording to BLAST results (% Max Ident), sequence ID**	**BOLD search species level (% Similarity)**
NB1	*Lasioglossum (Chilalictus) clelandi*	AM877	*L. lanarium* (99 %), AF103956.1	*L.lanarium* (99.53 %),
NB1.5	*Lasioglossum C.* sp. *(*NB1.5)	V14831	*Lasioglossum xanthopus* (89 %), KT164683.1	N/A
NB2	*Lasioglossum C. occiduum*	V15851	*Lasioglossum villosulum* (88 %), KT074062.1	*Lasioglossum lanarium (100 %)*
NB2.1	*Lasioglossum C. cf. erythrurum* (NB2.1)	AM934	*Lasioglossum xanthopus* (89%), KT164683.1	N/A
NB2.2	*Lasioglossum C. cambagei*	V15P2	*Lasioglossum pectorale* (90 %), FJ582297.1	N/A
NB2.3	*Lasioglossum C.* sp. (NB2.3)	V15841	*Lasioglossum hemimelas (89 %),* KR782951.1	N/A
NB4	*Lasioglossum C. lanarium*	V15846	*L. xanthopus* (89 %), KT164683.1 ; *L. pectoralae* (89 %), FJ582297.1	N/A
NB5	*Homalictus punctatus*	983	*Lasioglossum pectinatum* (98%), KF199973.1	N/A
NB6	*Homalictus sphecoodoides*	866	*Lasioglossum lustrans* (98 %), FJ663075.1	*Homalictus sphecoodoides (98.6 %)*
NB7.5	*Leioproctus* sp. (NB7.5)	V15203	*Colletes coriandri* (88 %), EF028497.1	N/A
NB8.1	*Lipotriches australica*	AM996	*Lipotriches yasymatsui* (91 %) KY072343.1	*Lipotriches* AUS01 *(98.92 %)*
NB8.2	*Lipotriches flavoviridis*	14828	*Lipotriches sp.* (89 %), KY072135.1	*Lipotriches flavoviridis (98.17 %)*
NB8.3	*Lipotriches moerens*	AM981	*Lipotriches sp.* (90 %), KY072135.1	N/A
NB8.4	*Lipotriches cf. flavoviridis* (NB8.4)	AM953	*Nomia incerta* (91 %), KY072660.1*; Lipotriches yasymatsui* (91 %) KY072343.1	*Lipotriches flavoviridis (98.86 %)*
NB9.1	*Exoneura (Brevineura) xanthoclypeata*	14489	*Brevineura xanthoclypeata* (100 %), DQ149672.1	*Brevineura xanthoclypeata* (97.82 %)
NB9.2	*Exoneura robusta*	14AMpo9	*Exoneura nigrescens* (99 %), DQ149662.1; *Exoneura robusta* (99 %), DQ149661.1	*Exoneura robusta* (98.59 %)
NB10	*Lasioglossum C. cognatum*	Barcoding failed	-	-
NB11	*Lasioglossum C. cf. seductum*	V14803	*L.xanthopus* (89 %), KT164683.1	*Lasioglossum seductum (97.24 %)*
NB12	*Lasioglossum C.* sp.	V15p3	*Lasioglossum villosulum* (89 %), KT074062.1	N/A
NB13	*Parasphecodes cf. salthicum*	V15121	*L. smeathmanellum* (89 %) KT074061.1	N/A
NB14	*Parasphecodes hiltacum*	958	*Lasioglossum xanthopus* (87 %), KT164683.1	N/A
NB15	*Lasioglossum C.* sp. (NB15)	AM869	*Lasioglossum xanthopus* (89 %), KT164683.1	N/A
NB16	*Lasioglossum C.* sp. (NB16)	15839	*Lasioglossum marinum* (89 %), FJ663080.1	N/A
NB17	*Lasioglossum C. cf. erythrurum* (NB17)	V15859	*Lasioglossum villosulum* (90 %), KT074062.1	*Lasioglosum erythrurum (99.53%)*
NB18	*Pseudohylaeus* sp. (NB18)	14576	*Hylaeus dilatatus* (90 %), KT164646.1	N/A
NB19	*Leioproctus* sp. (NB19)	V1578	*Hylaeus punctulatissimus*, (89 %) KJ837727.1	N/A
NB20	*Leiprotctus* sp. (NB 20	V14596	*Leioproctus sp.* (85 %), EF028473.1	N/A
BB	*Amegilla* sp. (BB)	AM1009	*Smittia edwardsi* (85 %), KU373560.1	N/A
NB21	*Hylaeus* sp. (NB21)	Barcoding failed	-	-

3.4.3. Thynninae

From 110 specimens, only 62 good quality sequences were obtained. Additionally, six specimens showed BLAST matches to the bacterium *Wolbachia*, without picking up the wasp sequences. The three species delimitation methods gave three different results for the number of species encountered (n = 62). Thirty-two different species were detected for the bPTP method, 33 species for the ABGD method with 20 species when Rosenberg probabilities were calculated.

Species delimitation using the Rosenberg cut offs did support most of the 12 morphological groupings, but 8 additional species (n = 20) were identified using this approach. Twenty-five specimens, all within the black pilose wasp morpho-group (Fig. 3-5), which were either not chosen for barcoding or where barcoding did not work, could not be clearly assigned to species. Species names could not be assigned to the species separated by barcoding, as none of the sequences showed a match Max Ident over 98 % in Genbank or similarities to species sequences in BOLD.

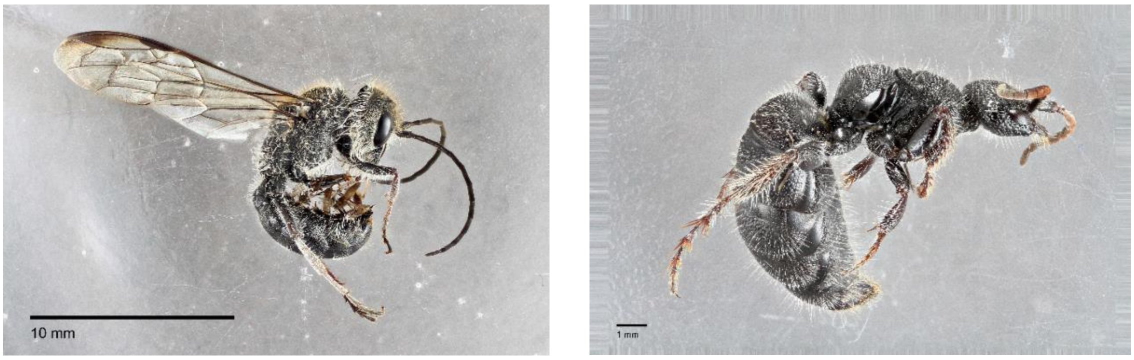

Fig. 3-5: Example of sexual dimorphism in thynnine wasps. Left: Male. Right: Female. Photos by Ben Parslow.

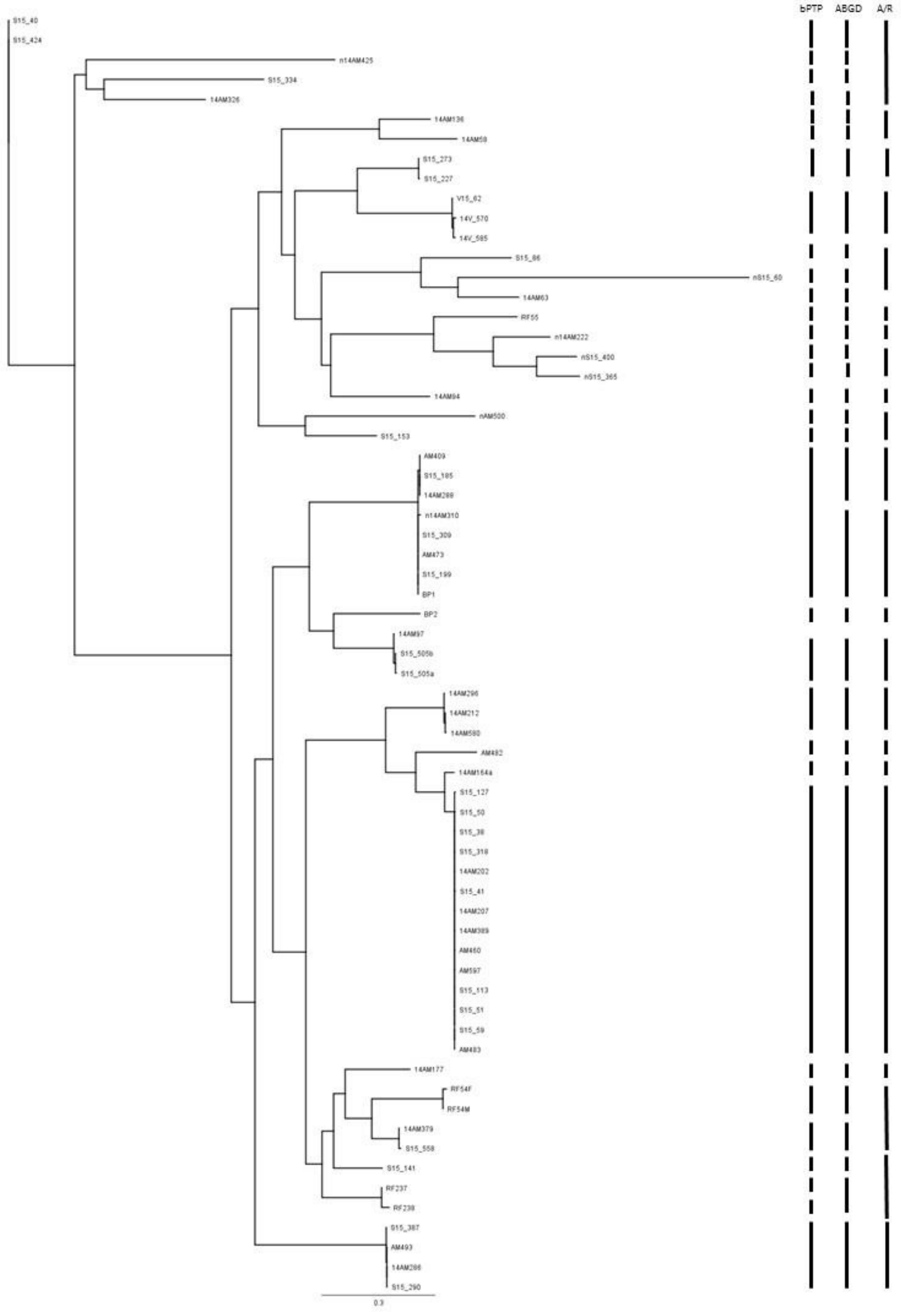

Fig. 3-6: PhyML tree of selected barcodes of thynnine wasps, showing results from species delimitation analysis. bPTP, ABDG analysis and ABDG analysis with Rosenberg statistics (A/R).

3.5. Discussion

3.5.1. Syrphidae

A combination of *COI* barcoding and morphological identification was a successful method for the reliable identification of hoverfly specimens. The majority of specimens could be relatively easily distinguished using morphology. For ecologists without taxonomic expertise, barcoding a subset of specimens to obtain voucher samples for morphology is recommended. Nevertheless, colour and sexual dimorphisms can complicate the identification and some species; for example, *Eumerus* spp. require genitalia dissection for morphological species identification. For the latter specimens DNA barcoding is likely to be useful. Based on the ecological aims of this study and the low numbers of *Eumerus* spp. and *Psilota* spp specimens present, the morphological distinguished genus was sufficient for further data analysis. Also *Eumerus* spp. can only be distinguished using the male sex for identification but only females were captured during this study. The genus *Psilota* is currently being revised and identification to species level was not possible for this study. One other distinctive hoverfly was identified as *Melangyna viridiceps*, however, it is unclear if the species for the genus will hold up (Andrew Young, pers. comm.).

It is clear from this study that using DNA barcoding only for the identification of syrphid flies is reliable, but certainly not a cost effective method. Hoverflies usually occur in large numbers and the identification of thousands of specimens exclusively using DNA barcoding with traditional Sanger sequencing approaches is financially and, considering the effort, not a recommended method. Alternatively, we recommend analysing DNA from subsamples, as successfully applied in the current study, or the use of an alternative approach based on next generation DNA sequencing or metabarcoding analyses (e.g. Meyer and Kircher, 2010, Clarke et al., 2014).

3.5.2. Native Bees (Apidae s.l.)

Morphological identification of South Australian bees is extremely difficult and pre-sorting of specimens was only possible for a few species due to a lack of identification keys for most genera. Unfortunately, not all specimens could be barcoded due to time and cost management, but grouping specimens based on morphology worked well as barcoding confirmed the morphological groups. The only exemption was the species *L. Chilalicts clelandi* and *L. C. lanarium* which were not always successfully pre-sorted using morphology. The PCR amplification process was successful for the majority of specimens analysed. Voucher samples from the different species identified by DNA analysis could be forwarded to morphology specialists and this helped to validate the delineation of species. The majority of sequences showed very few congruities with data from existing species in the available online databases, which was expected given the Australian native bee fauna has not been well sampled for genetic analyses. Furthermore, one wrong voucher sequence in GenBank as well as in the BOLD database was identified. The sequence for the native bee *Lasioglossum C. clelandi* is erroneously recorded as a *L. C. lanarium* sequence. In addition, searches for the same target sequences often revealed contradicting results for species identities between Genbank and the BOLD database.

These findings lead us to the conclusion that data from online sequence databanks still need to be treated with caution when used for taxonomic identifications of Australian bees. However, not all species delimitated in the PhyML tree could be assigned species names, because of the limited availability of sequences in the databanks, difficulties in morphological identification and also because of incomplete taxonomies (i.e. many undescribed taxa). However, the exact species name was not essential for use of these barcoding data in ecological studies, but the main requirement of separating specimens collected into species was achieved. The two *Chilalictus* species where barcoding revealed discrepancy with the morphologically pre-sorting were grouped together for further ecological analysis, which was sufficient for the purpose of the study.

3.5.3. Thynninae

Morphological identification of wasps is generally difficult, as it is mainly based on male morphology and requires genitalia examination and considerable taxonomic knowledge (Brown, 1998, Brown, 2005). The approach of sorting wasps into broad morpho-groups without examining genitalia was a good starting point, but insufficient to distinguish among species. The following approach of barcoding subsets of those morpho-groups provided further insight into species delimitation, but lacked completeness as sequences could only be obtained from ca 60 % of the specimens' barcoded, which was an unexpectedly low success rate. Griffiths et al. (2011a) have previously reported problems when sequencing *CO1* in thynnine wasps and reported failure to obtain barcodes within an entire clade, suggesting mutation at the priming site(s) as the cause. Another problem could lie in the infection of thynnine wasps with the endosymbiont *Wolbachia*. There was little evidence in the literature that thynnine wasps are potentially *Wolbachia* infected, although over half of all insect species are thought to be infected by the bacterium (Hilgenboecker et al., 2008). Although previous studies suggest that DNA barcoding is not compromised by *Wolbachia* infections (Smith et al., 2012), six of the wasps specimens did not show any host DNA amplification, which made genetic identification of those wasps impossible. A study by Klopfstein et al. (2016) investigated the success of DNA barcoding on ichneumonid wasp species infested with *Wolbachia* and found a significant correlation between *Wolbachia* infection status and the failure of barcoding. It remains unclear if *Wolbachia* infect thynnine wasps and this influenced the failure of obtaining or the contamination of sequences in our study, as the other specimens were not scanned for further *Wolbachia* infections.

Nevertheless, DNA barcoding of thynnine wasps gave a good overview of the diversity of species present in the habitat and a majority of species could be distinguished by morphological characters, which was supported by the DNA data. Not all specimens could be assigned to species and significant additional work (e.g. new primer design) would need to be done to link species to DNA barcodes. However, the DNA results were beneficial for distinguishing between certain species and can still partly be used for further ecological investigations. Due to the low numbers of wasps captured, only overall thynnine wasp abundance was used for the following ecological studies (Chapter 6) without distinguishing between species.

Contribution to build a reference library of orchid pollinators

Insects belonging to the three main groups identified in this study are widely known to pollinate orchids occurring in eucalypt woodlands in South Australia (Bates 2011; Faast et al., 2009). Nevertheless, detailed information about specific pollinating species is often missing and for some orchid species, for example *Glossodia major*, pollinators are still unknown. In this study, some of the identified insects had orchid pollen (pollinia) attached. For example, a hoverfly with pollinia attached was identified as *Melangyna collatus,* which can now be added to the database of orchid pollinating species. Around 20 further insect specimens with pollinia attached were also identified. Future research is needed to develop DNA methods which allow to match these pollinia to orchid species. This will provide an opportunity to link pollinators to their respective orchid species. In this respect, the accumulated insect sequence data in this study provides a reference library of *COI* barcodes for future research. Importantly, this library can be expanded over time to include orchid pollinators associated with species of orchids as well as other flowering plants, not just for South Australia but worldwide.

3.6. Acknowledgements

Special thanks to Dr. Remko Leijs from the South Australian Museum for the morphological identification of bees and to Dr. Jeff Skevington and Andrew Young from the Canadian Insect Collection for the morphological identification of syrphid flies. Also thanks to Ben Parslow for photographing bees and wasps. Thank you to the Holsworth Wildlife Research Endowment, the Lirabenda Endowment Fund and the Nature Foundation SA Inc. for funding this study. This study is a contribution to a larger ARC linkage project, investigating the effects of fire on the reproductive ecology of terrestrial orchids and on their pollinators in fragmented landscapes of southern Australia.

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CHAPTER 4

Hoverfly (Diptera: Syrphidae) communities and their response to fire in selected landscape fragments of South Australia

The author during a prescribed burn in Para Wirra (November 2013). Photo by Renate Faast.

Statement of Authorship

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Name of Principal Author (Candidate)	Anita Marquart		
Contribution to the Paper	Conducted all field and lab work, conducted all statistical analyses and wrote the manuscript		
Overall percentage (%)	75 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

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4. Hoverfly (Diptera: Syrphidae) communities and their response to fire in selected landscape fragments of South Australia

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4.1. Abstract

Hoverflies, also known as flower flies (Diptera: Syrphidae) play a significant role visiting flowers and providing pollination services (Jauker and Wolters, 2008, Rader et al., 2009). Nevertheless, syrphids are often overlooked and are extremely understudied in Australia, especially when it comes to (fire) ecology. In this study, a faunistic survey of hoverfly abundance and diversity was conducted before and after prescribed burns for environmental management. Two of the study sites were additionally affected by a large summer bushfire, which allowed for a comparison of the effects of prescribed burning with the impacts of a bushfire. Four eucalypt woodland locations with similar habitat characteristics in the Adelaide Hills, South Australia were used for the study. Hoverflies were sampled during spring using SLA - Malaise traps and vane traps. Prescribed spring and autumn burns were investigated to determine whether the seasonality of burning changed hoverfly communities differently. DNA barcoding of the *COI* region and morphological comparison were used to identify syrphid species. Five genera and six species were recognised, with hoverfly communities being strongly dominated by two common species, *Melangyna collatus* (Walker, 1852) and *Simosyrphus grandicornis* (Macquart, 1842), contributing 99 % of all captures. Very few specimens (37 out of 2892) were recorded from other three genera, *Eumerus*, *Meliscaeva* and *Psilota*. A negative effect of prescribed burning or bushfires on hoverfly abundance was not detected, although floral abundance significantly decreased after each fire event. Hoverfly abundance differed significantly among years of sampling. Our results clearly indicate that studies on hoverflies should be always carried out over several years and employing a number of different traps per sampling site to provide a representative sampling of the community composition and abundances.

Keywords: Prescribed burning, bushfire, fire regime, Syrphidae (hoverfly), Malaise trap (SLAM trap), insect abundance, Flower Fly

4.2. Introduction

Fire regimes in Australia are a major environmental factor (Gill, 2008) and control species composition of almost all components of the Australians biota (e.g. Noble and Slatyer, 1980, Coates et al., 2006, Keeley et al., 2011). Fire regime variables, such as the intensities of fire and their seasonality have a direct influence on species' survival (Gill, 1994). The use of prescribed burns to reduce bushfire risk has become more frequent in southern Australia and, since 2004, the South Australian Government has increased the frequency of prescribed burning and the level of bushfire suppression capacity. However, the consequences for the associated plant and animal communities are often poorly understood. Pollination is an important factor in ecosystems as it constrains reproductive success and influences population dynamics in plants (Price et al., 2008). Declines in insect abundances can lead to reduced pollination and may result in a lower fruit set in small plant populations (Cunningham, 2000). Approximately 90 % of all flowering plants depend on insects and other animals for pollination (Ollerton et al., 2011), with a minority utilising abiotic pollen vectors, mainly wind. A recent decline in pollinators has been reported in several studies (Potts et al., 2010, Vanbergen, 2013) where the reasons remain often unknown. However, results point to animal-mediated pollination being disrupted by a range of environmental factors (Potts et al., 2010), such as habitat changes, resource availability and fire.

Hoverflies (Syrphidae) form one of the largest families of Diptera with about 6200 species worldwide (Pape and Thompson, 2013). Although Diptera is the second most important order providing pollination services after hymenopterans in both natural and agricultural ecosystems (Rader et al., 2016) hoverflies are often overlooked as pollinators. Syrphids are found throughout Australia and may appear in large numbers during hot weather. Adults pollinate many plant species, while larvae (particularly Syrphinae and Pipizinae) are significant predators of sternorrhynchan Hemiptera. With the decline in bee numbers (Potts et al., 2010), hoverflies might play an important role as alternative pollinators. Adult syrphids feed upon both nectar and pollen as well as on honeydew, but rely on pollen as a protein source (Schneider, 1948, Gilbert, 1981), whereas nectar mainly consist of carbohydrates and is essential for their energy requirements (Haslett, 1989).

Information about European hoverflies has increased in the recent years e.g. Syrph Net database (Speight, 2012), whereas the Australian syrphid fauna is still poorly investigated, and comprises many undescribed species. Information on species microhabitats, distribution and mortality factors including fire is missing and there is little information on dispersal and migration behaviours.

The direct survival of insects after fire is related to the fire regime itself, the location of the individuals at the time of the fire and on their mobility, depending on their stage of development (e.g. stationary egg, larva, pupa versus the adult stage, Swengel, 2001). Fire may affect insects either directly by fire-related mortality or indirectly by changing plant communities, reducing floral resources (Hermann et al., 1998, Potts et al., 2003) and nesting sites (Potts et al., 2005). Some hoverflies are dependent on dead or dying trees for some stage of their life cycle (Nyoka, 2010) and most Syrphidae lay their eggs in or on plants, thus a reduced floral stem density due to fire possibly might decrease their oviposition grounds. Fire might have negative short-term effects on pollinator communities, however Pryke and Samways (2012) suggest that burning and long-term recovery is important for overall pollinator diversity. To date, few studies have been undertaken on the effects of fire on insect, and particularly on syrphid fly, abundance and diversity and these have all been from the America and Europe. Studies from North Carolina and southern Brazil suggest that the creation of open spaces, especially a reduced density in overstory trees, and an increased herbaceous plant cover (Campbell et al., 2007), as well as frequent disturbances, might favour syrphid diversity and abundance (Gittings et al., 2006, Campbell et al., 2007, Souza et al., 2014). Fire often results in communities of herbs, shrubs and trees that favour heliophilous insects like hoverflies. Furthermore, not only the location, but also the seasonality of the fire has been reported to play an important role in insect survival and on changes in abundance and diversity. Moretti et al. (2004) reported positive effects on syrphid species richness of winter forest fire events in the Southern Alps of Europe. On the other hand, negative effects of fire on pollinators were observed after summer fires in Israel (Ne'eman et al., 2000). Additionally, the intensity of the fires and the surrounding, unburnt vegetation, especially the presence and abundance of food resources/flowering plants might influence syrphid abundances. Hoverflies are also known to be sensitive to habitat changes (Sommaggio, 1999a) and it can be expected that they could easily leave an unrewarding environment with low resources, such as a post-fire landscape.

The initial objective of this study was to gain a deeper understanding of hoverfly communities in the South Australian bushland. The specific aims are:

1) to determine species present in the field sites observed in the Mt. Lofty ranges

2) to assess if syrphid communities are affected by one of the main ecological factors in Australia – fire (prescribed burns and bushfire)
3) to assess fire induced changes in floral abundance, which could influence hoverfly abundances

4.3. Material and Methods

4.3.1. Study Sites

The study was carried out in the Mt. Lofty Ranges, to the east of Adelaide, South Australia. The area has a Mediterranean - type climate, with moderate rainfall (600 - 1000 mm/year mostly in winter), and hot summers and mild winters. The field site selection was based on availability of field sites scheduled for prescribed burning and fire season. Four locations were selected (Table 4-1) to assess the occurrence of hoverfly species and their respective response to prescribed burns. These were at Parra Wirra Recreation Park (PW), in the South Parra Reservoir (SP), Millbrook Reservoir (M) and Kersbrook Native Forest Reserve (K) (see Chapter 2 for a detailed map).

Table 4-1: Field sites in the Mt. Lofty Ranges with coordinates of an approx. centre point of the site, site size, burn time and quality estimates of the burn.

	Field site	Coordinates (approx. centre of field site)	Size	Prescrib. burn	Patchiness	Bushfire
1 - burn	Kersbrook Native Forest Reserve	138°50'16.412"E 34°43'11.971"S	Ca 28 Ha	Spring 2013	Medium patchy (ca 70 % burnt)	
1 - control	Kersbrook Native Forest Reserve	138°50'11.227"E 34°43'36.655"S	Ca 30 Ha			January 2015
2 - burn	Millbrook Reservoir	138°49'39.874"E 34°48'48.801"S	Ca 19 Ha	Autumn 2013	Well burnt (85%)	
2 - control	Millbrook Reservoir	138°49'44.153"E 34°48'29.622"S	Ca 15 Ha			January 2015
3 - burn	Para Wirra Recreation Park	138°49'10.545"E 34°41'45.284"S	Ca 35 Ha	Spring 2013	Very patchy (50% burnt)	
3 - control	Para Wirra Recreation Park	138°49'29.626"E 34°41'48.45"S	Ca 35 Ha			
4 - burn	South Para Reservoir	138°52'31.472"E 34°40'35.612"S	Ca 27 Ha	Autumn 2014	Very patchy (ca 60% burnt)	
4 - control	South Para Reservoir	138°52'9.396"E 34°40'25.268"S	Ca 35 Ha			

All sites are within conservation parks; three sites have no public access and are managed either by SA Water or Forestry SA. Each location contained one burn ("burn") and one adjacent control ("control") area with similar habitat attributes, such as vegetation density and plant species present. Control and burn sites were neighbouring each other, but separated by wide fire tracks (see below). Surveys were carried out over three years, with four burn and four adjacent control areas. The sites are characterised by woodlands of *Eucalyptus* species i.e. *Eucalyptus goniocalyx*, *E. obliqua, E. leucoxylon,* and *E. fasciculosa*, mixed with golden wattle trees (*Acacia pycnantha*) on the lower slopes. The understory is frequently dominated by *Pultenea* spp., *Hibbertia* spp., *Gonacarpus* spp., *Lepidosperma* spp., *Arthropodium* spp. and *Xanthorrhoea* spp..

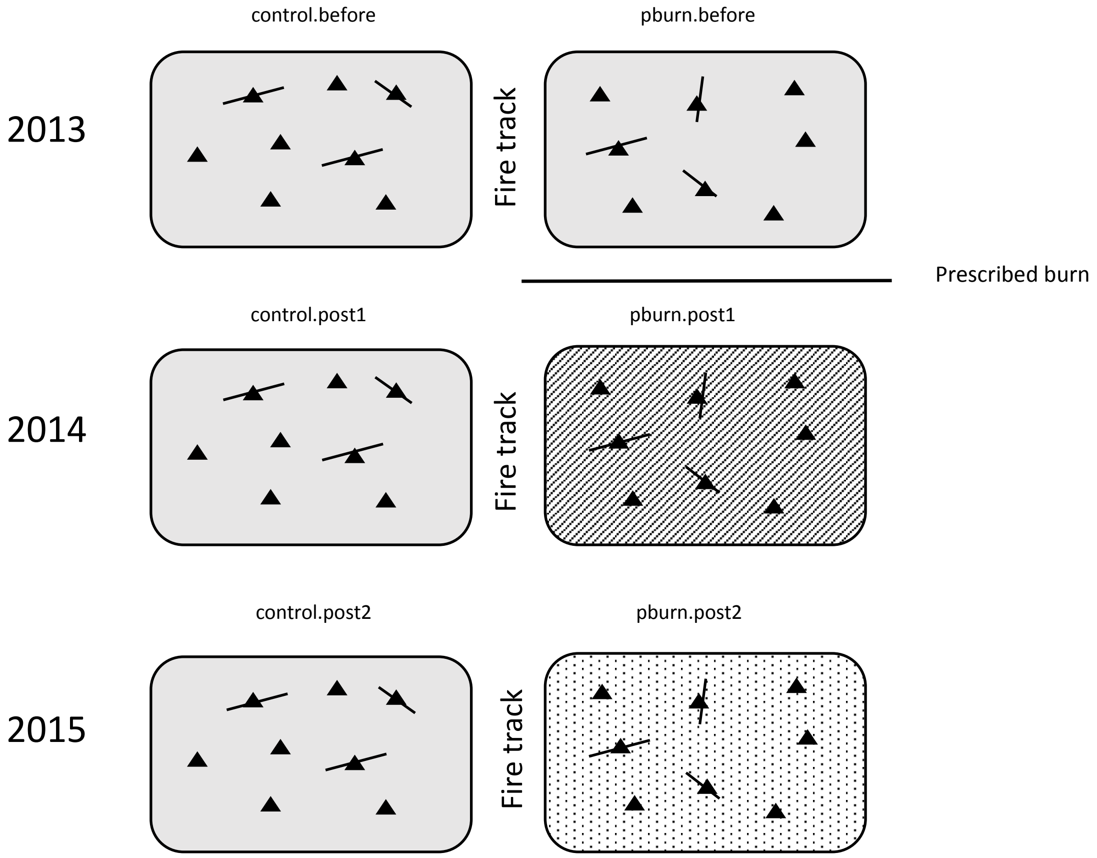


Fig. 4-1: Schematic of BACI design, showing treatment sites (control and burn) with 8 SLAM traps (triangles) and 3 transects (lines) per site, sampled over 3 years.

4.3.2. Prescribed burns and bushfires

Two spring and two autumn burns carried out by the South Australian government were chosen. The spring burns in Para Wirra and Kersbrook occurred in November, 2013. The prescribed burn in Para Wirra was relatively patchy, and some grassy parts remained unburnt. Kersbrook was well burnt, with very few unburnt patches. The field site in the Millbrook was already well burned in April 2013. Another autumn burn happened in April 2014 in South Parra, which was very patchy with large areas of the site being only slightly burnt or unburnt. The burn patchiness (indicated in Table 4-1) was estimated (as percent of surface) visually by walking through the entire field site.

The "Sampson Flat Bushfire" was a series of bushfires from 2nd to 9th January 2015 that affected 20,000 hectares of the Mt. Lofty Ranges and burnt two of the four field sites. The control sites in Millbrook and Kersbrook were completely burned out with overstorey trees and leafage being affected. These control sites were treated as "Bushfire" sites for syrphid abundance and floral abundance analysis and were analysed separately, with the previous prescribed burn site serving as control site.

4.3.3. Transects

Six permanent belt transects were set up in each field site to assess floral abundance. Transects were placed at least 200 m from the edge of each site and at least 400 m from the respective treatment site. This distance between transect was considered sufficient to guarantee independence. Each transect was set up next to a single Malaise trap. Vane trapping (four traps/transect) was also conducted along each transect. Locations were chosen to ensure good availability of transects, but also to represent the general habitat and ecological characteristics across each site. Transects were 50 m long x and 10 m wide and were spaced more than 300 m apart. It was ensured that transect in burn treatment sites got burnt by using hand flame throwers when they were not ignited by the original fire treatment on the same day.

4.3.4. Study design and data collection

The impact of fire was assessed using a BACI (Before-After, Control-Impact) design (Stewart-Oaten et al., 1986, Stewart-Oaten et al., 1992, Smith, 2002), by estimating the changes at an unaffected control site and comparing them to fire affected sites. Sampling was carried out in spring, starting in early September to late November. Each Field site was sampled for three seasons, spring 2013, 2014 and in spring 2015. Collection periods were categorised into: before prescribed burning ('before'), one year post-prescribed burning ('post1') and two years post-prescribed burning ('post2') (see Fig. 4-1). A special case was the field site in Millbrook Reservoir, where a prescribed burn happened before this study was undertaken. Data on hoverfly abundance collected "before" the prescribed burn in Millbrook Reservoir (restricted to 4 traps per site) was forthcoming, but no data on floral abundance. The Sampson Flat Bushfire in early 2015 did not change the data collection approach.

Collection methods

Three insect trapping methods were initially used, as adopted in previous studies (Juillet, 1963, Westphal et al., 2008).

Vane traps (SpringStar^{TM}, Stephen and Rao, 2005) are special plastic traps attracting pollinating insects using colour. Trapping was carried out on sunny days over 20° C. At each transect two pairs of Vane traps, (two blue and two yellow) were installed approximately 1 m off the ground, on a stake. Burn and adjacent control sites were surveyed on the same day. Sunny spots were chosen and each pair of traps was placed at least 5 m apart. Vane traps were installed at about 10:00 am and brought in at sunset in order to standardise total trapping time for all transects. Vane traps were set out on no fewer than four trapping days at each site. Collected insects were transferred directly to vials and stored in 100 % ethanol.

SLAM (Sea Land & Air Malaise) traps are modified Malaise traps, providing a permanent and passive intercept method for collecting insects. They are a standard method for hoverfly sampling (Sommaggio, 1999b) and have been used in similar studies (Castella and Speight, 1996, Fayt et al., 2006, Ouin et al., 2006). EM508 Standard SLAM traps, 110 x 110 x 110 cm were used (Australian Entomological Supplies Pty. Ltd.) These traps were installed in random locations and were spaced more than 200 m apart to make them largely independent. Traps were installed in sunny locations across possible flight paths of insects and, when possible, placed to block a corridor (e.g. a path in the forest) or placed perpendicular to a barrier (van Achterberg, 2009) in order to maximize trap yield. The traps were permanently installed throughout the entire insect capturing season. They were placed in the same location in the subsequent years as small changes in the placement of traps can influence catching efficiency (Matthews and Matthews, 1983).

The collecting head was always placed towards the sun (van Achterberg, 2009). Collection bottles, approximately half full with 100% undenatured ethanol, were changed every four weeks in the first year, every three weeks in the second year (required due to high temperatures) and every 4 weeks in the third year. A total of 64 SLAM traps were installed, with 16 traps per field site.

Pan traps were also used initially in the study. Yellow and white bowls were filled with approximately 200 ml water, with a small amount of unscented detergent to reduce surface tension. After the first field season it was already apparent that pan trapping is not effective for capturing syrphid flies for this project. Therefore it was discontinued after the first year and will not be further considered in this study.

4.3.5. Insect Identification and DNA Barcoding

Hoverflies were first sorted based on obvious morphological differences; “yellow-black” colour (aposematic colouration, wasp-mimicry) and the remaining “others” being black or red. To ensure not to miss out on species in the large “yellow-black” pool, a total of 100 of the collected “yellow-black” syrphid flies were randomly chosen and DNA barcoded, targeting the mitochondrial cytochrome oxidase I (*COI*) gene, using Folmer primers (LCOI490) and cycling parameters adapted from (Hebert et al., 2003). Phylogenetic trees were generated from sequence alignments, and species were thought to be independent when similarity was below 98 %. Selected sequences from the separated species were compared to the BOLD database, with those not getting a match in the database being identified morphologically by hoverfly specialists (Andrew Young; Canadian National Collection, Ottawa) as well as the few specimens sorted to “others”. All “yellow-black” hoverflies where finally identified based on their morphology, using the voucher specimens and detailed photographs from the different species separated by the barcoding approach. For detailed information on the identification process see Chapter 3.

4.3.6. Data analysis

Insects collected in SLAM traps were used exclusively to analyse changes in hoverfly abundance, as catching success in Vane traps was too low. Each field location was analysed independently to account for site effects, such as geographical differences, differences in plant compositions and the different burn seasons. Generalised Linear Mixed Models (GLMM's, package glmmADMB, Fournier et al., 2012) were used to examine differences in syrphid abundance between 'control' and 'burn' treatment sites, and how these changed after burning (i.e. pre-burning ('before'), and at two times post-burning ('post1', 'post2') in the BACI design). Syrphid abundance was used as response variable, burn treatment and time period were treated as fixed factors, and individual traps, which were revisited at each time point, were treated as a random effect in the analysis. Abundance counts were modelled using a Poisson distribution and a log link function using an interaction model (abundance ~ treatment : period). Likelihood ratio tests were conducted to obtain analysis of deviance tables (Anova function, package car, John and Sanford, 2011). The estimates and 95 % confident intervals of the mean number of syrphid flies collected per treatment and period for each location were generated for graphing. Changes in hoverfly abundance in Kersbrook Native Forest were analysed comparing before and one period post prescribed burning only, as the third data collection period was affected by a bushfire. The locations Millbrook and Kersbrook which were affected by a bushfire were analysed separately, investigating changes in hoverfly and floral abundance before and after the bushfire ('before', 'post1'). Sites previously affected by prescribed burning served as control sites.

4.4. Results

4.4.1. Species composition and insect trapping

A total of 2,892 individual syrphid specimens were captured in 64 Malaise traps and yellow/blue vane traps across the four field sites over three years. The two trap types showed significant within and between variance in numbers of hoverflies caught. Numbers varied from 0 to 123 hoverflies caught per SLAM trap across the different sampling periods, and from 1 to 123 hoverflies per trap within the same field site (Millbrook Reservoir). Only 53 hoverflies were captured in vane traps: the majority (50 flies) were caught in blue vane traps and only three in yellow vane traps. Interestingly, no hoverflies were found in vane traps in 2013 ('before') and only one hoverfly was found in the Millbrook control site in 2014 ('post2'). Due to the very low catching success in vane traps, only data from SLAM traps were used for statistical analysis of syrphid abundance and its dependency on fire.

Five genera of hoverflies were identified, using DNA barcoding and morphological identification (see Chapter 3 for detailed barcoding results, phylogenetic trees and photographs of species). The majority (99 %) of flies captured were *Simosyrphus* *grandicornis* and *Melangyna collatus,* which show the typical hoverfly appearance with aposematic black and yellow colouration. These two dominant species were present at all field sites over all three years (Fig. 4-2). Only 37 individuals from other species were captured: 24 individuals from the genus *Eumerus,* three individuals from *Psilota*, nine individuals of an undescribed species of *Meliscaeva* and one specimen of *Melangyna viridiceps*. Specimens of *Eumerus* and *Psilota* were not identified to species level mainly because of their low catch rates.

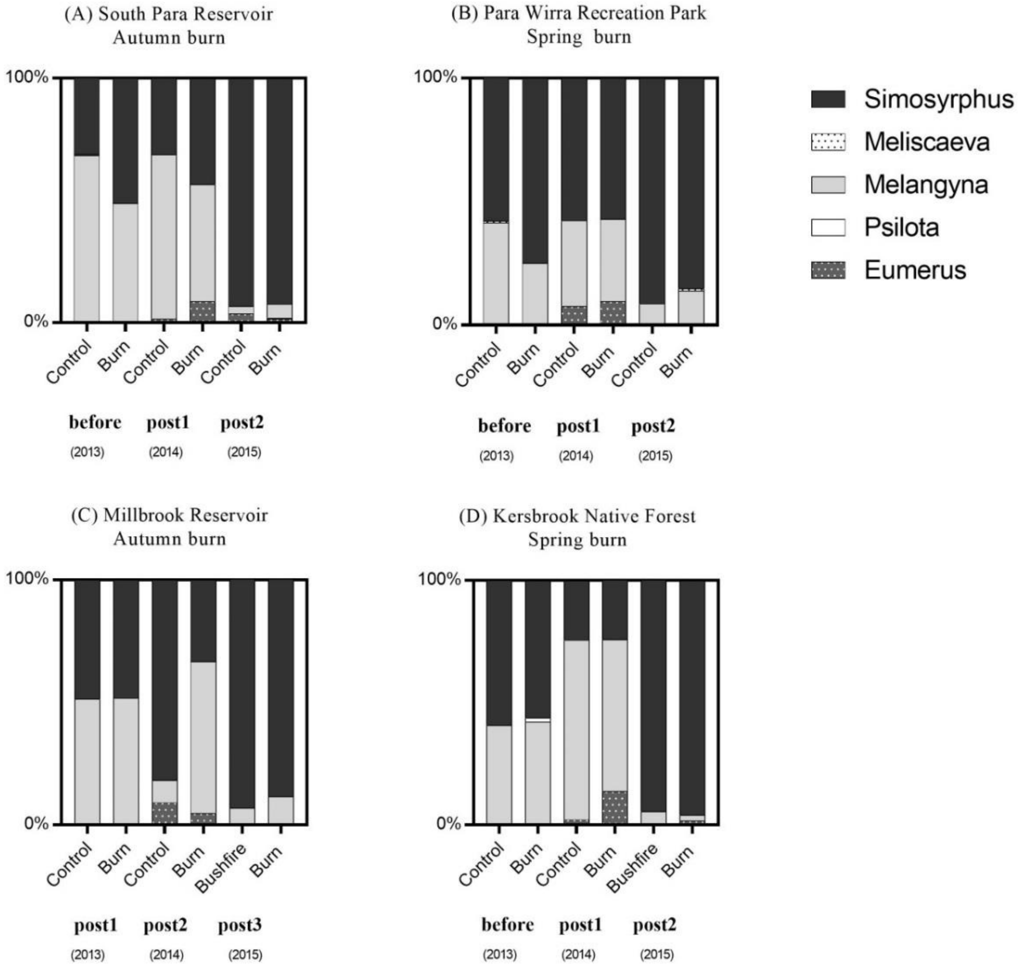


Fig. 4-2: Relative species contribution of Syrphidae genera in four different field sites over three periods of sampling (before, post1, post2). (A) South Para Reservoir, (B) Para Wirra Recreation Park, (C) Millbrook Reservoir, (C) Kersbrook Native Forest. Period 'before' stands for data collection before prescribed burning, in either autumn (A) und (C) or spring (B) and (D). Post1 and post2 represent one year after prescribed burning and respectively two years after prescribed burning. (C) and (D) were furthermore affected by a natural bushfire in the post2 period in 2015.

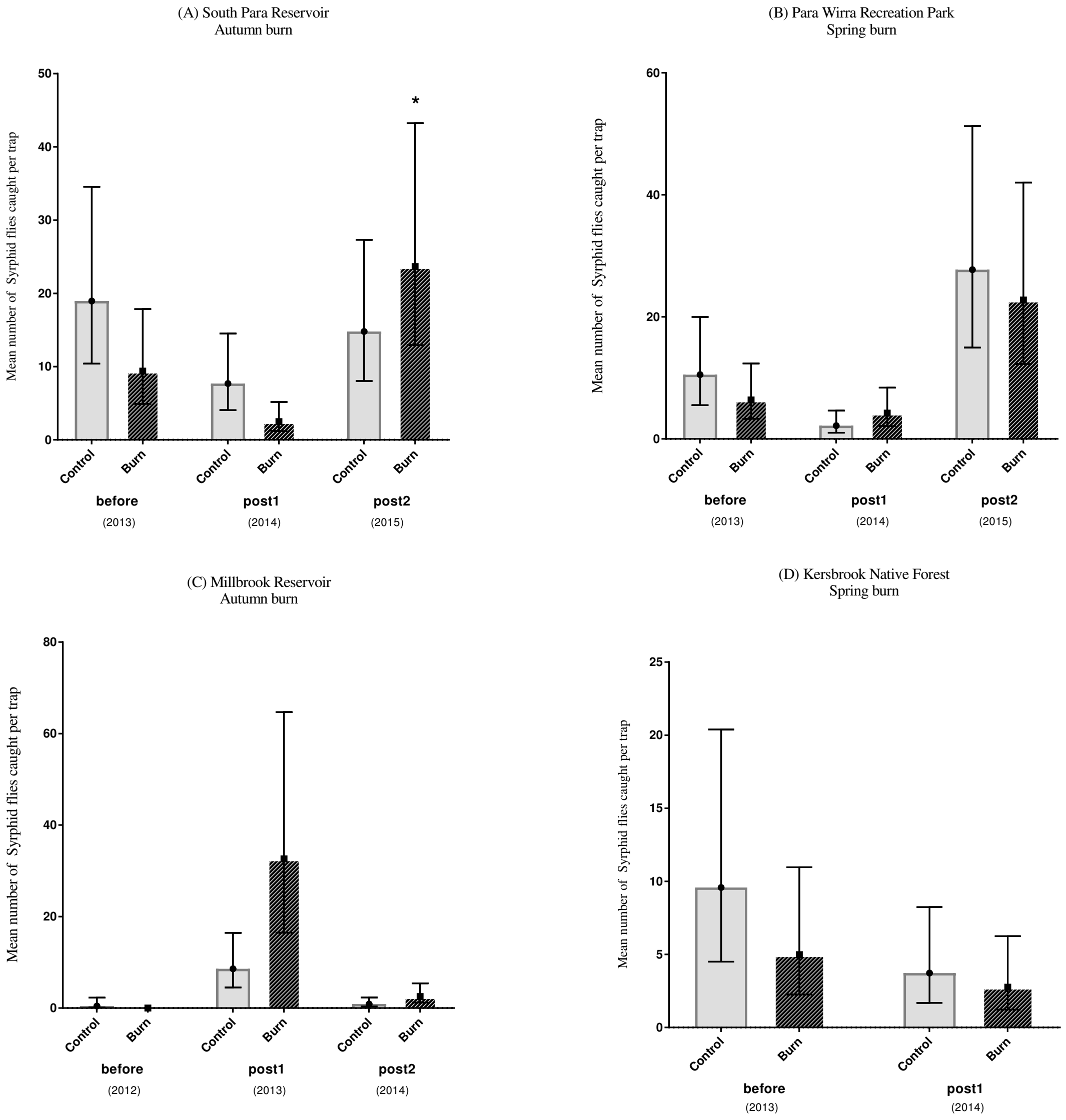
4.4.2. The effect of fire on Syrphidae abundance

Syrphid abundance was not affected by prescribed spring burning. Multiple comparison testing revealed a significant effect of fire on fly abundance in Para Wirra when comparing before and one year after the prescribed burn ($p = 0.017$), but this was not reflected by the result in Kersbrook (Table 4-2).

Table 4-2: : The effect of prescribed burns and bushfires on hoverfly abundance, shown is an analysis of deviance table for a GLMM (abundance ~ period : treatment). Shown are degrees of freedom (*Df*), Chisquare calculations, *p* (Pr>Chi) values for significance of factors.

Prescr. spring burns		**Para Wirra**			**Kersbrook**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	110.96	**<0.001**	1	8.72	**0.003**
treatment	1	0.39	0.532	1	0.86	0.354
Period:treatment	2	4.74	0.093	1	0.81	0.368
Prescr. autumn burns		**South Para**			**Millbrook**	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	2	11.09	0.004	1	123.68	**<0.001**
treatment	1	0.24	0.623	1	5.59	0.015
Period:treatment	2	7.42	**0.024**	1	-	-
Wildfire		**Kersbrook (WF)**			**Millbrook** (WF)	
	Df	Chisq	Pr(>Chisq)	*Df*	Chisq	Pr(>Chisq)
period	1	22.05	**<0.001**	1	32.88	**<0.001**
treatment	1	1.52	0.22	1	1.22	0.27
Period:treatment	1	1.48	0.22	1	0.93	0.3

Interestingly, the autumn burn in South Para showed a significant increase in syrphid abundance among the treatments over the three periods. Millbrook could not be analysed with an interaction model due to very small variation in the treatments, probably due to insufficient data from the first data collection period (only 4 traps per site). Abundance fluctuated dramatically among years, with 2013 and 2015 being years with generally high numbers of syrphids and 2014 having low syrphid abundance across all treatments and all field sites (Fig. 4-3).


(A) South Para Reservoir
Autumn burn

(B) Para Wirra Recreation Park
Spring burn

(C) Millbrook Reservoir
Autumn burn

(D) Kersbrook Native Forest
Spring burn

Fig. 4-3: Mean-model (nb GLMM) of syrphid abundance in different field sites. A prescribed autumn burn was performed between 'before' and 'post1' in (A) South Para Reservoir and (C) Millbrook Reservoir. A prescribed spring burn was performed between 'before' and 'post1' in (B) Para Wirra Recreation Park and (D) Kersbrook Native Forest. Results show hoverfly abundance before prescribed burning ('before'), one and two years post prescribed burning ('post1', 'post2'), apart from (D) Kersbrook with data for one year post prescribed burning only**.**

The bushfire result indicates that the bushfire did not affect hoverfly abundance significantly (K: $p = 0.33$, M: $p = 0.24$) but the year of sampling played an important role in overall hoverfly abundance, indicated by strongly significant p values for the factor period in the analysis of deviance (see Table 4-2). Numbers increased significantly in both control (previously prescribed burn) and burn site in the post1 period (Fig. 4-4).

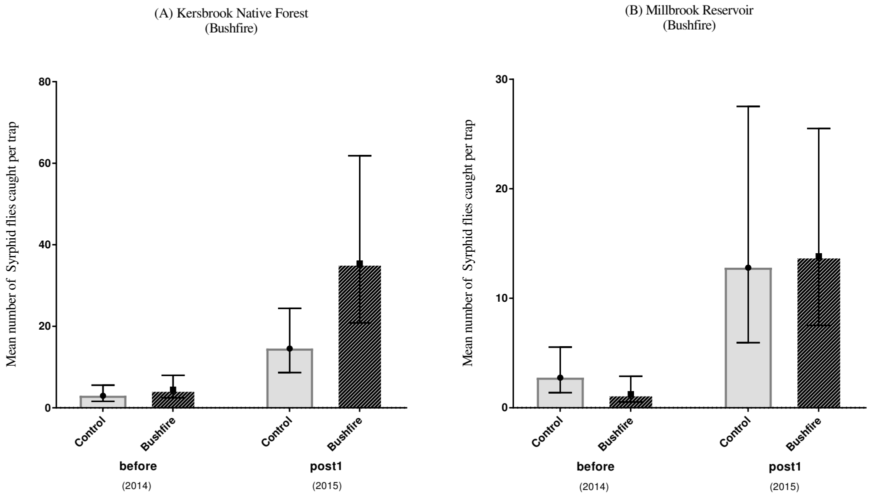


Fig. 4-4: Mean-model (nb GLMM) of syrphid abundance in different field sites. Graphs show hoverfly abundance before ('before') and one year ('post1') after a bushfire.

4.5. Discussion

4.5.1. Species composition and insect trapping

With only six different taxa (including four species and *Eumerus*_spp. and *Psilota* spp., which were only identified to genus level) the species diversity encountered was surprisingly low in the four field locations. Species composition was strongly dominated by *S. grandicornis* and *M. collatus*, which are native to Australia (Pape and Thompson, 2013). Hand catching in spring 2015 indicated the presence of *Psilota* specimens and great abundances could be observed on *Calytrix* spp. bushes, but only very few specimens were found in traps. Results show that SLA - Malaise trapping captured many specimens, although the trapping numbers varied between traps, even in the same field site and habitat. To obtain more information about species diversity, vane and pan trapping was also conducted. Pan trapping was conducted in the first year, but discarded in the following years due to very low catching success. Very few syrphid flies could be caught in vane traps, although many studies (e.g. Finch and Collier, 1989, Hoback et al., 1999) show that syrphids are attracted to the yellow and blue colours of the traps used in this study. Interestingly, more hoverflies were attracted by blue vane traps and just very few (3 specimens) could be found in yellow vane traps, which contradicts Soleyman-Nezhadiyan (1997), who stated that yellow colours are preferred by Syrphidae. A possible reasons for the overall lower vane trapping rates is that the traps were only out for one day at a time. This was necessary as insects needed to be collected shortly after capture to be conserved in 100 % denatured ethanol for DNA barcoding. Net trapping is also often used to collect hoverflies, which gives information about species presence or absence but is not very useful for quantitative information or abundance studies. Future studies should use as many malaise traps as financially and logistically possible and not rely on single malaise traps as a representative for hoverfly abundance studies. The 16 traps per location used in this study still produced very wide confidence intervals, even in adapted negative binominal GLMM's which include the random effect of trap. It can also be hypothesized that future studies on hoverfly diversity should use a broader range of different methods, especially hand netting and sticky traps as recommended by several authors before (Sutherland et al., 2001, Chen et al., 2004) which were not suitable for this study with the main focus on the effect of fire on hoverfly abundance.

4.5.2. Effect of fire on Syrphidae abundance

The results here indicate that syrphid abundance is not negatively affected by any of the fire events included in this study, but they rather responded to a prescribed autumn burn with a slight increase in numbers.

Adult hoverflies might have found shelter in unburnt patches of the prescribed burn sites. Australian hoverflies seem to be able to adapt their short life cycle to the post fire conditions and when the weather is suitable, several generations can occur within a few months (Soleyman-Nezhadiyan, 1997). The prescribed burn in autumn, did not affect the leaves of eucalypt trees (personal observation) and is therefore unlikely to have killed resting/overwintering hoverflies or larvae, which are often found in leaf folds and curls underneath open tree leaves (Rotheray and Gilbert, 1989).

Hoverflies are able to travel long distances (up to hundreds of kilometers, Lack and Lack, 1951, Snow and Ross, 1952, Rotheray and Gilbert, 2011) and were therefore expected to leave unrewarding habitats. The results show that hoverflies were still abundant in fire affected sites, indicating that that hoverflies seem to cope well with a habitat altered by fire events (Chapter 2)

It was anticipated that prescribed burns and bushfires would produce a decline in floral abundance, with the lowest abundance one year after the fire (Potts et al., 2003). This in turn would lead to declines in hoverfly abundances (Meyer et al., 2009) as hoverflies rely on energy from nectar resources for their energy-consuming flight, and pollen is required by females for egg production (Haslett, 1989). Although hoverflies can move long distances from feeding to egg laying sites, they prefer them close together (Schneider, 1969). The number of flowers in the study sites was found to be reduced after fires but floral diversity was not affected (Chapter 2). The decrease in floral abundance did not seem to affect hoverfly abundance negatively. The prescribed burns were rather patchy and left some parts of the plant community unaffected, providing food resources unaffected by fire. Most Australian hoverflies have a very broad diet and indeed the two common species are known generalists. Favored plant species are cup shaped flowers or small tubular flowers with easy accessible nectar like buttercups, daisies, thistles and calendulas (Larson et al., 2001). Some of those plants and numerous other flowering plants favored by Australian hoverflies such as *Goodenia* spp. and *Acacia* (Soleyman-Nezhadiyan, 1997) were recorded on fire affected sites, indicating that food resources were still available. It is also possible, that foraging areas of hoverflies are larger than those affected by fire.

The relatively stable numbers of hoverflies observed after a fire and the increased abundance after an autumn burn indicate that a fire affected landscape can still be an attractive habitat, even though floral abundance may be reduced. Other factors might have even favored hoverfly abundance. Vegetation density was found to be reduced in most habitats affected by fire (as shown in Chapter 2), and a reduced vegetation density after fire is likely to provide attractive open spaces for the insects (Gittings et al., 2006), which prefer feeding in the sun (Gilbert, 1985). Another factor may be predation. Common natural enemies of hoverflies are songbirds (Kristin, 1991), other insects (Rotheray and Gilbert, 2011) and pathogens (Nakata, 1995). Reported larval enemies in Australia are predatory wasps and flies, spiders, beetles, birds, lizards and bacterial/fungal diseases (Rotheray and Gilbert, 2011). A fire affected landscape could host a reduced number of hoverfly enemies, which could then favor hoverfly abundance.

Neither of the two dominant syrphid species declined significantly after a fire event. *S. grandiscornis* and *M. collatus* were abundant in control and burn sites but, nevertheless, with significant changes in abundance from year to year. It can be assumed that both species have a similar ecology and a similar diet. Their appearance is highly alike, as well as their body size and the typical wasp mimicry. This could explain, that both species react similarly to changes in their environment (Ricklefs and Miles, 1994) and in response to fire.

4.5.3. Fluctuation in syrphid abundance among the years

The most notable variation in syrphid abundance was not associated to fires but to specific years. Abundance indeed varied significantly between the years among the treatments within all four field sites. These findings indicate that year-to-year variation, which has been previously observed for hoverfly communities in a Mediterranean study system (Petanidou et al., 2011), was much more important in influencing syrphid abundance than variation associated with fire. It is hard to ascertain the drivers that lead to an increase in syrphid abundance, but it can be assumed that climatic factors, such as temperature, rainfall and even wind velocity are likely factors that influence the activity of insects (Andrewartha and Birch, 1954). Syrphids adjust their number of eggs laid to the available prey, and even synchronize their life stages with them (Van Emden, 1965). Also, the presence or absence of syrphid predators could further influence syrphid abundance. Ultimately, it is most likely that syrphid abundance is strongly connected to lerp/psyllid insect abundance. Unfortunately, there are no records of psyllid abundance or reported outbreaks in the years of this study. The question remains whether burning in years with low hoverfly abundances could have a negative effect on hoverfly abundance in the following years. A modelling approach would help to answer this questions.

4.6. Conclusion

Very little information on Australian hoverflies is available, in spite of their abundance and their effectiveness as pollinators (Rader et al., 2016). This study tested the effect of fire on hoverfly communities and showed that there were no negative effect of fire on hoverfly abundance or species composition, neither for prescribed burning nor for a natural bushfire. The most dominant species were S*imosyrphus grandicornis* and *Melangyna collatus.* Results suggest that both species are well adapted to South Australian fire regimes and cope well with the challenges of a fire affected landscape. Nevertheless, there are still many unanswered questions about the ecology of Australian hoverflies in regard to fire. An unanticipated finding of this study was that hoverfly abundance significantly changed among the years independently of fire. The reasons for this are unclear, but are likely to include changes in climate or the availability of food for larvae. Burning in years with low hoverfly abundances could have a significant effect on hoverflies in the following years. Further work should be undertaken to determine key aspects of hoverfly ecology, particularly flight ranges as well as on feeding habits as the role of hoverflies in pollination services may get more important.

4.7. Acknowledgements

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CHAPTER 5

Native bee response to fire events in South Australian eucalypt woodlands: linking bee community changes to habitat modification

A prescribed spring burn at twilight in Para Wirra (November 2013). Photo by author.

Statement of Authorship

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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5. Native bee response to fire events in South Australian eucalypt woodlands: linking bee community changes to habitat modification

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5.1. Abstract

Native bees in South Australia are generally understudied, with limited information on bee community composition and little information available on bee ecology. This study investigated the effects of prescribed burns and wildfires on communities of solitary bees in a eucalypt forest of South Australia. Bees were sampled over three years during spring time, using Malaise and Vane traps, with Vane traps capturing the majority of bees (712 out of 729 specimens). The findings contribute to the current limited knowledge on species abundance and species richness of Australian native bees and furthermore provide important information on bee community responses to fire regimes. The results of this study showed that abundance and diversity of spring active bees are not negatively affected one year following fire. However, bee abundance showed a marginal decline two years following prescribed burning, probably due to a loss in floral abundance. Species richness and species composition were never negatively affected by fire. Furthermore locations affected by recent wildfire showed a higher bee diversity than unburnt sites.

Keywords: native bee, pollinator, prescribed burn, wildfire, fire, Vane trap

5.2. Introduction

Australia has a fauna of around 1660 native bee species (AFD, 2017), nearly all of them solitary and showing high morphological diversity (Sheffield et al., 2009). Native bees play an important role in many ecosystems, especially by contributing to the pollination needs of more than 80% of flowering plants (Hogendoorn, 2016) Nevertheless, there is a primary concern regarding the worldwide decline of bee populations, which was first reported almost 20 years ago (Allen-Wardell et al., 1998, Kearns et al., 1998) and is still alarmingly present, as bee populations are still declining worldwide for reasons often unknown (Potts et al., 2010, Vanbergen, 2013). The main drivers for changes in bee communities are a wide range of environmental changes headed by habitat loss (Winfree et al., 2009), and the availability of resources and general disturbance regimes (Potts et al., 2010). Disturbance in the form of fire has played an important role in changing ecosystems worldwide (Peterson et al., 1998), and this has been altering the composition of Australian flora and fauna for many years (Coates et al., 2006) but many effects on biodiversity are still unclear. Very little research has been carried out on the effect of fire on native bee communities and their habitat in Australia.

Fire is already known to be a driving factor in changing wild bee communities in Mediterranean type ecosystems (Moretti et al., 2006) and can affect bees on different spatial and temporal scales. Bee communities in general display spatial heterogeneity, explained by several habitat factors such as flower availability and geographical patterns (Torné-Noguera et al., 2014), with fire events playing a further role in producing various heterogeneous habitats, mainly depending on fire intensity, fire patchiness, size (Gill, 1975, Gill et al., 1981) or even the vegetation type (Whelan, 1995). The direct effects of fire (flames, smoke and heat) can lead to a decrease in bee diversity and abundance through immediate mortality (Warren et al., 1987). Indirect effects of fire on bee communities could be due to changes in the habitat and might depend on the temporal differences in vegetation recovery. Bee diversity can decrease due to habitat changes such as limited nectar resources (Ne'eman et al., 2000) or by the destruction of nesting sites (Williams et al., 2010) in the early post-wildfire period. However, the majority of studies (Moretti et al., 2006, Winfree et al., 2009, Rubene et al., 2015) found no significant effects of fire on bee abundance, although Potts et al. (2003) reported an increase in bee abundance and diversity due to a diverse floral community after wildfires in Israel. Several other studies reported increased bee diversity in the early post-fire years, mainly driven by a diverse range of food resources and an increase in nesting resources, such as bare ground and dead wood in the post-fire habitat (Potts et al., 2005, Campbell et al., 2007, Grundel et al., 2010).

Many recent studies have recommended the use of functional traits to investigate bee communities and their response to disturbances (Grundel et al., 2010, Williams et al., 2010, Mateos et al., 2011, Lazarina et al., 2016). A study by Lazarina et al. (2016) found that general bee species diversity and abundance did not differ between fire history regimes, but detected a significant effect of different functional traits on wild bees, investigating nesting preferences, trophic specializations and body size. These three traits seem to be the most commonly used traits and the driving factors for bee responses to fire (Lazarina et al., 2016).

Nesting preferences, especially the availability of bare ground and the occurrence of pre-existing burrows, are important factors for bee communities, guild composition and species abundance (Potts et al., 2005). Bee species richness is positively related to the abundance of potential nesting resources (Grundel et al., 2010). Not only the nesting preferences, but also the location of bees of different life stages during the fire can play an important role for bee survival.

Ground nesting species are considered to be safe during a fire when they are in the nest, especially those with burrows deeper than 10 cm, whereas shallow nesters are at risk of suffering lethal heating during an intense wildfire (Cane and Neff, 2011). Intense fires are thought to heat soil confined to 10 - 20 cm depth (Christensen, 1994), although not many wild bee species nest in shallow nests. Lazarina et al. (2016) furthermore reported a positive effect on ground nesting bees associated with recently burnt sites. Species that nest above-ground, such as twig and stem nesting species and males of ground nesting species which do not sleep in their nests but in the above ground vegetation, are less likely to survive the direct impacts of a fire, but might survive by escaping or hiding in unburnt patches (Cane and Love, 2016). These findings support a study by Williams et al. (2010), who showed that nesting preferences significantly affect the response of bees to fire, where below ground nesting species were not affected in contrast to above ground nesters which showed negative responses to recent burns.

Nesting resources for stem-nesting guilds could be highly reduced by fires due to the loss of potential nesting habitats but, in contrast, it has been shown that fire promotes the abundance of nest sites for the stem nesting bee *Exoneura nigrescens*, nesting in *Xanthorrhoea* stems (Stow et al., 2007). These studies indicate that the availability of nesting requirements needs to be considered when investigating pollinator responses to disturbance or habitat fragmentation (Neame et al., 2013) as various bee guilds can respond differently to disturbances. Around 70 % of Australian native bees are ground nesting species, digging burrows between 2 cm and 180 cm in different types of soil (Smith and Heard, 2016). The fire induced availability of bare ground as nesting sites and also the fire safety of underground burrows could therefore play an important role for many Australian bee species and could favour ground nesting groups (e.g. Halictidae), while having negative effects on above ground nesting bees, such as stem nesting allodapine bees (e.g. *Exoneura*).

The functional traits considering trophic specialisation might also play an important role in the response of bee communities to fire. Polylectic bees which forage on a broad range of flowering plants might be less susceptible to fire regimes, whereas oligolectic bees might be at risk of losing food resources when specific plants are negatively impacted by fire regimes. In contrast, Grundel et al. (2010) found that the abundance of oligolectic bees was positively associated with open habitats, which are often found following fire.

Body size might also influence bee response to fire as it is a predictor of foraging range (Gathmann and Tscharntke, 2002). As such it is an important determining factor for the distribution of native bees, especially for species relying on woody vegetation for nesting substrates (Hogendoorn, pers. Comm.). Some large bees are known to have long flight ranges (e.g. 200 - 800 m for solitary bees; Wesserling and Tscharntke, 1995, Gathmann and Tscharntke, 2002, Greenleaf et al., 2007) which might also be an advantage in fire affected habitats and could allow them to use the fresh floral resources from surrounding unburnt patches. Several studies support that the honeybee *Apis mellifera*, with relatively large foraging ranges (up to 2 km), is less sensitive to habitat changes than many smaller native species (Steffan-Dewenter and Kuhn, 2003, Ricketts, 2004) and might be less affected by the loss of nectar resources. Other large native bees of similar size to honeybees, such as *Amegilla* species (blue banded bees) or *Lipotriches* species can be expected to travel similar distances as honeybees. However, information on dispersal distances is mostly lacking, but it can be assumed that bees with larger body sizes can travel longer distances and might be favoured when it comes to foraging, or migrating into a new habitat, as they are well known for their high mobility and can rapidly recolonise freshly available habitats (Michener, 1979). In contrast, small and medium-sized solitary bees have only short foraging flights of around 150 - 500 m e.g. *Osmia* and *Andrena* species (Dafni and Shmida, 1996, Gathmann and Tscharntke, 2002), and it is more likely that they are absent in post-fire environments (Ne'eman et al., 2000). Therefore, the short foraging range of small solitary bees and their slow invasion rate into burned areas could explain their absence in post-fire areas (Ne'eman et al., 2000). Nevertheless, very small bees might only forage 50 - 250m away from their nest and might be restricted by limited floral resources, but are also known to have low food requirements (Müller et al., 2006) and can possibly use wind to drift longer distances.

When investigating bee responses to fire, we generally need to take into account the different nature of fire events and distinguish between the effects of low intensity prescribed burning and high intensity wildfires. Both are known to have different fire severities Debano and Conrad, 1978, Keeley, 2009) and may influence pollinator communities and their habitat differently (as shown in Chapter 2) Prescribed burning affected insect habitat only marginally whereas the impact of a wildfire was more severe, affecting a variety of insect habitat characteristics, especially potential insect nesting sites.

Most of the studies previously discussed sampled bee communities in wildfire affected sites, but without having investigated the bee communities before the fire disturbance (Ne'eman et al., 2000, Moretti et al., 2006, Rubene et al., 2015, Cane and Love, 2016, Lazarina et al., 2016). Very little research has been carried out on the short-term effects of fire, focussing on the first one or two years following a fire event (but see Peralta et al., 2017). There is also a lack of studies focussing on the short-term effects of low intensity fires, and only two studies (Campbell et al., 2007, Rubene et al., 2015) are known to have investigated the response of bee communities to prescribed burning.

This study is the first to investigate the effects of fire on native bee communities in *Eucalyptus* dominated woodlands of Australia and provide insight into the changes in bee communities in response to both prescribed burning and wildfire. Results will be compared to previous research on fire induced changes in pollinator habitat characteristics such floral abundance and nesting resources in the same study system.

This study aimed to answer the following research questions:

a) Does fire change bee abundance, species richness or species composition?
b) Can changes in the bee community be related to fire induced changes in their habitat?
c) Do prescribed burns and wildfires have a different effect on bee communities?

5.3. Methods

5.3.1. Study sites

The impact of fire on bee communities was assessed using a BACI ("Before - After, Control-Impact") study design (Stewart-Oaten et al., 1986, Smith, 2002) by recording changes in bee communities at unaffected sites ('control') and comparing them to prescribed burn ('pburn') or wildfire ('WF') sites in four separate forests (Table 5-1).

Table 5-1: Set up of study sites. Four locations were used to investigate the effects of prescribed burning. Two out of four sites were affected by a wildfire in January 2015, and were analysed separately to investigate wildfire effects.

LOCATION	TREATMENT SITES	FIRE EVENT	MONITORED TIME PERIODS/YEAR		
			2013	2014	2015
PARA WIRRA	pburn control	prescribed spring burn	before	post1	post2
KERSBROOK (PB)	pburn control	prescribed spring burn	before	post1	
SOUTH PARA	pburn control	prescribed autumn burn	before	post1	post2
MILLBROOK (PB)	pburn control	prescribed autumn burn	post1	post2	
MILLBROOK (WF)	pburn WF	prescribed autumn burn (post1) summer wildfire		before	post1
KERSBROOK (WF)	pburn WF	prescribed spring burn (post1) summer wildfire		before	post1

The study was conducted in the Mt. Lofty Ranges, east of Adelaide, South Australia. The area has a Mediterranean - type climate, with moderate rainfall brought by south-westerly winds, hot summers and mild winters.Four locations were selected to assess the effect of fire on native bee communities present during springtime. Field sites (Table 5-1) were set up in Para Wirra Recreation Park (PW), in the South Para Reservoir (SP), in the Millbrook Reservoir (M) and in the Kersbrook Native Forest Reserve (K). Each location contained one fire affected site ('pburn' or 'WF') and one adjacent unaffected site ("control") with similar habitat attributes, such as vegetation density and plant species composition. Control and burn sites were neighbouring each other, but separated by wide fire tracks. The field sites are characterised by woodlands of *Eucalyptus* species, mixed with golden wattle trees (*Acacia pycnantha*) on the lower slopes. The understory is frequently dominated by *Pultenea* spp., *Hibbertia* spp., *Gonacarpus* spp., *Lepidosperma* spp., and *Arthropodium* spp. For more information on the study design and a detailed map of the study sites see Chapter 2 and Chapter 4.

5.3.2. Insect trapping

Insect collection surveys were carried out from 2013 to 2015, always during springtime (early September to late November) in all field sites at all four locations. Each field site contained six permanent transects. At each transect two pairs of Vane traps (2 blue and 2 yellow Vane traps), were installed approximately 1m off the ground, on a stake. Due to higher bee activity on warm and sunny days (Westphal et al., 2006), Vane traps were set out on sunny days over 20° C, with no fewer than four trapping days at each site. Vane traps were installed at about 10:00 am and brought in at sunset, so as to standardise total trapping time for all transects (Roulston et al., 2007). Collected insects were transferred directly to vials and stored in 100% ethanol.

SLAM (Sea Land Air Malaise traps) traps were set up during spring and were placed in the same locations in subsequent years. Traps were installed in sunny locations, across possible flight paths of insects. Sixteen traps were set up at each site (64 SLAM traps overall), with always one SLAM trap per transect and the remaining traps randomly spread through the field site. Traps were set up in late August/early September and dismantled during mid-November. SLAM trapping data was not included in the statistical analysis, due to very low catch success (< 10 native bee specimens per year for all traps). Pan trapping was also conducted in the first year of surveying but not continued due to very low catch success. For more detailed information on insect trapping surveys, see Chapter 4.

5.3.3. Data analysis

Bee identification

Native bee specimens were identified using DNA barcoding approaches and/or morphological identification. The majority of the bees were identified using DNA barcoding targeting the mitochondrial cytochrome oxidase I (*COI*) gene, using Folmer primers (414 and 423), LCOI490) and cycling parameters from Hebert et al. (2003). All collected bees were pre-sorted, separating distinctive taxa, such as *Amegilla* spp., *Parasphecodes salthicum*, *Lasioglossum, Chilalictus lanarium* and *L. C. clelandi* from the remaining unknown species. All unknown specimens were barcoded for randomly chosen specimens (subsets of 10 specimens) from the morphologically identified taxa. Unpurified sequencing reactions (CS+) were submitted to the Australian Genome Research Facility (AGRF). The sequencing results were analysed using the software Geneious, version 5.4.2 (Drummond et al., 2011), and Maximum Likelihood (ML) phylogenetic trees (PhyML, Guindon and Gascuel, 2003) were generated to obtain relationship information among specimens. Support for species was calculated using the species delimitation plug in (Masters et al., 2011) of the Geneious software, which implements the Rosenberg (2007) method and calculates the probability of reciprocal monophyly under the null model of random coalescence. Specimens separated by DNA barcoding were then identified either by comparing them to sequences in online databases (Genbank, BOLD) or by morphological identification.

For more detailed information on the barcoding approach and for the resultant phylogenetic trees, see Chapter 3.

Statistical analysis

All four field sites were analysed separately to account for spatial differences, such as in vegetation, altitude or climate conditions. Prescribed burn sites in South Para and Para Wirra were analysed for three periods (‘before’, ‘post1’ and ‘post2’). The two wildfire affected sites, Millbrook Reservoir and Kersbrook Native Forest, were analysed for only two periods (‘before’ and ‘post1’) for prescribed burning, excluding the year of sampling after the wildfire. The wildfire affected sites were then analysed separately to assess the effect of wildfires on native bee abundance, with only two periods (‘before’ and ‘post1’ wildfire) included in the analyses (Table 1). The sites which were previously prescribed burn treatment sites were used for comparison.

Vane trap data were used to analyse the effect of fire on bee abundance and species composition.

Generalised Linear Mixed Models (GLMM’s) were used to analyse the effect of fire on the abundance of overall numbers of native bees as they allow for count data and a negative binominal distribution (package glmmADMB, Fournier et al., 2012). Negative binominal models were chosen after testing Poisson models, which were over dispersed. Fixed factors included time period (‘before’/’post1’/’post2’), treatment (‘pburn’/’control’/’WF’) and transect were chosen as random effects. Normality of data was assessed using histograms and qq plots of residuals (Zuur et al., 2010). Estimates and 95 % confident intervals of the mean number of native bees collected per treatment and period along multiple transects were generated, using the interaction model of the fixed factors treatment and period (bee abundance ~ treatment : period) with the random effect of transect. Likelihood ratio tests were conducted to obtain analysis of deviance tables (Anova function, package car, Fox et al., 2017).

Species richness was calculated by counting the number of different species recorded per Vane trap and site. The effect of fire on species richness was assessed using GLMM’s as above (response variable: species richness). Then, a community matrix was generated including bee species and their respective abundance counts. Species diversity was obtained for every treatment site per time period, using the Shannon-Wiener diversity index with:

$$H' = -\Sigma\, p_i \ln p_i$$

and was calculated using the function ‘diversity’ with ‘index = shannon’ of the Vegan package.

A PERMANOVA (function adonis of the Vegan package, Anderson, 2001) based on dissimilarity matrices with Bray-Curtis distances was performed to test for an effect of fire on bee community composition (response variable: bee community matrix, fixed factors: time period, site treatment, random effect: trap).

A non-metric multidimensional scaling ordination (nMDS) was performed to illustrate the results. The significance of variables (different bee species) was tested using the envfit function (Vegan package) with a permutation test (Oksanen et al., 2013) to obtain Pr values for different vectors. The bee species *Lasioglossum C. clelandi* (NB1) and *L. C. lanarium* (NB4) were analysed together (NB1_NB4), as they could not be reliable distinguished (see Chapter 3). Indicator species were detected using multi-level pattern analysis (Dufrene and Legendre, 1997, De Cáceres et al., 2010) from the indicspecies package. All analyses were conducted using R studio (R© 3.1.2).

Functional trait assignment

Nesting preferences, trophic specialization and body size of the different bee species was assigned using literature (Halcroft, 2016, PaDIL) and expert advice. Bees were categorised into two different nesting preferences: ground nesting and stem/twig nesting. Diet requirements were categorised into two trophic specializations: polylectic bees which are generalists and forage on a broad range of plant species and oligolectic bees which only prefer a small number of plant species. Classification of bee species into polylectic and oligolectic was assigned using the information provided in Halcroft (2016) and PaDIL. While diet requirements are often discussed controversially, most bee species encountered in this study have well documented trophic specialisations. Body sizes were categorised into small (< 7mm), medium (5-12mm) and large bees (> 13mm), according to the average size of the genus stated in the literature (Halcroft, 2016, PaDIL). This approach has limitations but was chosen to allow for a simple size categorisation which will help to interpret results regarding maximum flight distances, which are strongly connected with body size (Greenleaf et al., 2007).

5.4. Results

5.4.1. Bee surveys

Over three seasons a total of 729 native bees were captured in Vane trap surveys and only 17 native bees in SLAM trapping surveys. The majority of native bees were collected using Vane traps coloured blue (82.5 %) and a small proportion in yellow traps (15 %). Bees captured in SLAM traps (2.4 %) were excluded from the abundance and diversity analyses due to the low success in catching rates.

Captured were 28 different species of native bees, 3 species of Apidae (33 individuals), 20 Halictidae (682 individuals), and 5 Colletidae (14 individuals). At genus level 13 species of *Lasioglossum* (*Chilalictus*) (580 individuals), 4 species of *Lipotriches* (46 individuals), 2 species of *Homalictus* (17 individuals), 1 *Parasphecodes* (17 individuals), 3 *Leioproctus* (8 individuals), 2 *Exoneura* (27 individuals), 1 *Amegilla* (6 individuals), and 2 *Hylaeus/Pseudohylaeus* (2 individuals) were captured. The most abundant species (65 % of all individuals captured) comprised *L. C. clelandi* and *L. C. lanarium*.

Some taxa could not be identified to species as they had no matches (> 98 % similarity) in DNA databanks (Genbank, BOLD 2017) and could not be morphologicaly identified. These species were assigned a working name (Table 5-2).

Table 5-2: Table of bee species (or synonyms) collected in the study with respective nesting preferences (BG=Bare ground, S/T = Stem/Twig); diet preferences (P= Polylectic, O= Oligolectic); body size (S = Small (< 8 mm), M = Medium (8 - 12 mm) L = Large (> 12 mm). Nesting preferences, trophic specialization and body size of the different bee species from Halcroft, 2016, PaDIL, expert advice and personal observations.

Identification code	Species (DNA match or morphological Id)	Nesting preferences	Trophic specialisation	Body size code
NB1	*Lasioglossum (Chilalictus) clelandi*	G	P	M
NB1.5	*Lasioglossum C.* sp. *(*NB1.5)	G	P	M
NB2	*Lasioglossum C. occiduum*	G	P	M
NB2.1	*Lasioglossum C. cf. erythrurum* (NB2.1)	G	P	M
NB2.2	*Lasioglossum C. cambagei*	G	P	M
NB2.3	*Lasioglossum C.* sp. (NB2.3)	G	P	M
NB4	*Lasioglossum C. lanarium*	G	P	M
NB5	*Homalictus punctatus*	G	P	S
NB6	*Homalictus sphecoodoides*	G	P	S
NB7.5	*Leioproctus* sp. (NB7.5)	G	O (Mytraceae)/P	M
NB8.1	*Lipotriches australica*	G	P	M
NB8.2	*Lipotriches flavoviridis*	G	P	M
NB8.3	*Lipotriches moerens*	G	P	M
NB8.4	*Lipotriches cf. flavoviridis* (NB8.4)	G	P	M
NB9.1	*Exoneura (Brevineura) xanthoclypeata*	T/S	P	S
NB9.2	*Exoneura robusta*	T/S	P	S
NB10	*Lasioglossum C. cognatum*	G	P	M
NB11	*Lasioglossum C. cf. seductum*	G	P	M
NB12	*Lasioglossum C.* sp. (NB12)	G	P	M
NB13	*Parasphecodes cf. salthicum*	G	P	M
NB14	*Parasphecodes hiltacum*	G	P	M
NB15	*Lasioglossum C.* sp. (NB15)	G	P	M
NB16	*Lasioglossum C.* sp. (NB16)	G	P	M
NB17	*Lasioglossum C. cf. erythrurum* (NB17)	G	P	M
NB18	*Pseudohylaeus* sp.	T/S?	P?	M
NB19	*Leioproctus* sp. (NB19)	G	P	M
NB20	*Leiprotctus* sp. (NB 20	G	P	M
BB	*Amegilla* sp. (BB)	G: Burrows, bricks, sandstone	P	L
NB21	*Hylaeus* sp. (NB21)	T/S	P	S

5.4.2. Effect of fire on native bee abundance

Bee abundance was found to be negatively impacted following both prescribed spring and autumn burning in three of the four forest locations (Fig. 5-1).

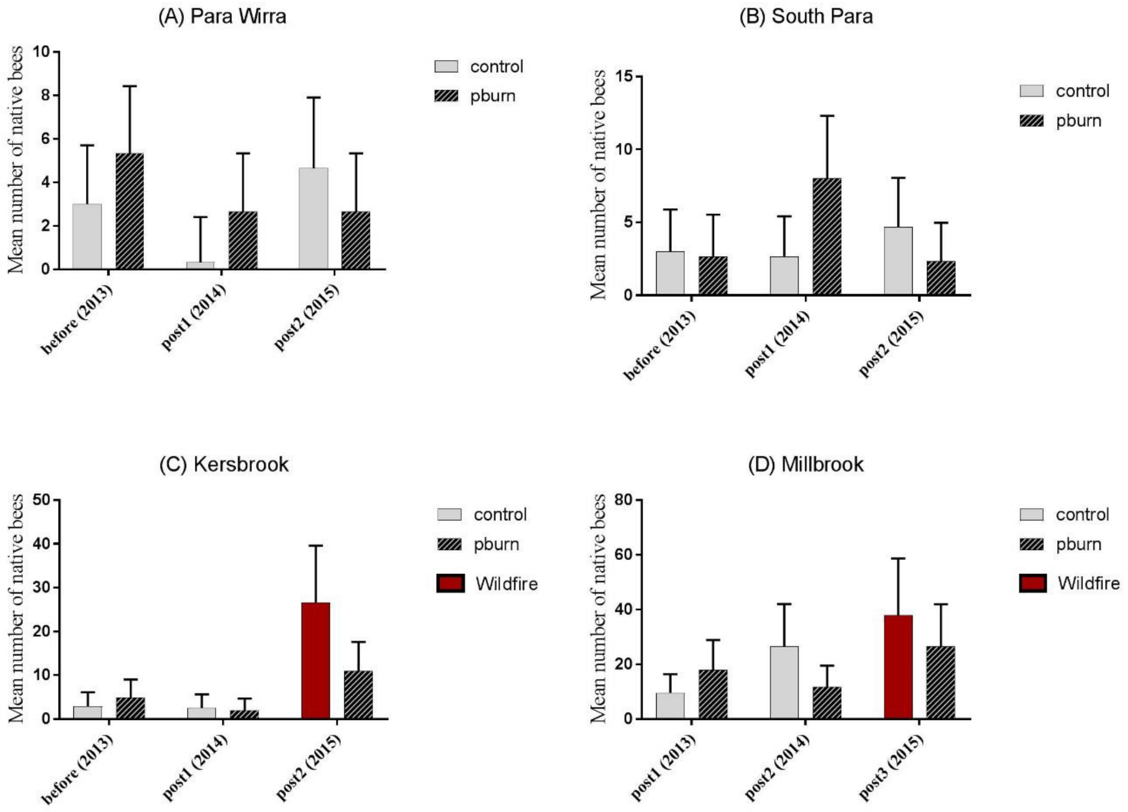

Fig. 5-1: Mean-model (nb GLMM) of bee abundance with 95 % confidence intervals in different field sites. A prescribed autumn burn was performed between 'before' and 'post1' in (B) South Para Reservoir and (D) Millbrook Reservoir (only sampled for the periods post1 and post2). A prescribed spring burn was performed between 'before' and 'post1' in (A) Para Wirra Recreation Park and (C) Kersbrook Native Forest. A wildfire in January 2015 affected the control sites in (C) Kersbrook and (D) Millbrook.

Multiple comparisons showed that negative effects never occurred in the first sampling period following the fire (Table 5-3). Bee abundance sampled 9 months following prescribed spring burning was not found to be altered by fire. Further, an increase in bee numbers was observed 4 months after a prescribed autumn burn. However, bee abundance was negatively affected in the burn treatment site in the second period following prescribed fires with a lower abundance compared to before fire and to the first year post fire (Paired contrast investigation, Table 5-3).

Table 5-3: Effect of fire on native bee abundance, showing results of GLMM's and paired contrast analysis. Abundance declines (-) or inclines (+) are indicated in brackets. (N/A) = not applicable i.e. no data/site was wildfire affected.

Prescribed spring burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Para Wirra	0.19	<0.001 (-)	<0.001 (-)
Kersbrook	0.45	N/A	N/A
Prescribed autumn burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
South Para	0.063 (+)	0.001 (-)	<0.001 (-)
Millbrook	N/A	N/A	<0.001 (-)
Wildfire	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Millbrook	0.294	N/A	N/A
Kersbrook	0.241	N/A	N/A

No significant effect of fire on bee abundance was recorded. However, the data indicate (Fig. 5-4) that the period of sampling significantly influenced bee abundance in both wildfire affected sites.

Table 5-4: Effect of fire on bee abundance in the different field locations. Likelihood ratio tests (displayed as table of deviance for every field location) for individual levels of factors in the generalised linear mixed model (abundance ~ treatment : period, random effect of trap).

	Para Wirra			**Kersbrook (PB)**		
Prescr. spring burns	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	2	3.833	0.147	1	0.983	0.322
treatment	1	0.456	0.499	1	0.087	0.768
Period:treatment	2	6.852	**0.033**	1	0.584	0.445
	South Para			**Millbrook (PB)**		
Prescr. autumn burns	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	2	4.888	0.086	1	4.074	0.045
treatment	1	0.480	0.488	1	0.117	0.753
Period:treatment	2	8.396	**0.015**	1	21.952	**<0.001**
	Kersbrook (WF)			**Millbrook (WF)**		
Wildfire	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	1	52.086	**<0.001**	1	6.317	**0.012**
treatment	1	11.374	<0.001	1	7.532	0.006
Period:treatment	1	1.103	0.294	1	1.375	0.241

5.4.3. Effect of fire on bee community composition

Species richness was unexpected low in all field locations. The lowest number of species was observed in South Para, with only three species recorded. The highest species richness was observed in Millbrook, with 12 native bee species. Field sites affected by a wildfire showed the highest species richness (11 and 12 species) and diversity (Table 5-5). Interestingly, species richness was not affected by fire, but by the year of sampling in wildfire locations (GLMM analysis and maximum likelihood test, Table 5-6). Not only the fire affected site but also the adjacent control site showed a high number of species and an increased diversity (H') compared to the sampling period before the wildfire. Field sites affected by a prescribed burn showed lower species richness and diversity (H'), especially in the first sampling period after the burn, but this was also observed in the adjacent control sites (Table 5-5).

Table 5-5: Species richness (SR) and Species diversity (H') of native bees in four different field sites, sampled over three years. Sites in Kersbrook and Millbrook were affected by a Wildfire in January 2015.

		Spring burn Para Wirra			Spring burn + WF Kersbrook	
Year	**Treatment: period**	**SR**	**(H')**	**Treatment: period**	**SR**	**(H')**
2013	pburn.before	5	1.13	pburn.before	6	1.45
2013	control.before	6	1.58	control.before	3	0.96
2014	pburn.post1	2	0.38	pburn.post1	2	0.45
2014	control.post1	2	0	control.post1	3	0.73
2015	pburn.post2	1	0	pburn.post2	5	1.13
2015	control.post2	6	1.47	**Wildfire.post1**	**11**	**1.52**
		Autumn burn South Para			Autumn burn + WF Millbrook	
Year	**Treatment: period**	**SR**	**(H')**	**Treatment: period**	**SR**	**(H')**
2013	pburn.before	1	0	pburn.post1	8	1.15
2013	control.before	2	0.35	control.post1	9	1.45
2014	pburn.post1	3	0.34	pburn.post2	5	0.93
2014	control.post1	2	0.38	control.post2	12	1.22
2015	pburn.post2	1	0	pburn.post3	12	1.19
2015	Control.post2	2	0.26	**Wildfire.post1**	**12**	**1.38**

Permutational tests (adonis) support these results and showed no effect of fire on species composition of native bees in all field sites and across the different treatments. Locations affected by wildfire showed that the time period (year of sampling) influenced species composition marginally (M: $p = 0.063$; K: $p = 0.03$).

Table 5-6: The effect of fire on species richness. Likelihood ratio tests (displayed as table of deviance for every field location) for individual levels of factors in the generalised linear mixed model (richness ~ treatment : period, random effect of trap). Low sample variation prevented tests for the site in Millbrook (N/A).

	Para Wirra			Kersbrook		
Prescr. spring burns	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	2	4.36	0.133	1	0.89	0.345
treatment	1	0.08	0.769	1	0.00	0.999
Period:treatment	2	3.23	0.198	1	0.18	0.671
	South Para			Millbrook		
Prescr. autumn burns	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	2	0.25	0.88			
treatment	1	0.01	0.933	N/A		
Period:treatment	2	0.27	0.874			
	Kersbrook (WF)			Millbrook (WF)		
Wildfire	Df	Chisq	Pr(>Chisq)	Df	Chisq	Pr(>Chisq)
period	1	7.20	**0.007**	1	4.05	**0.044**
treatment	1	2.36	0.125	1	0.35	0.55
Period:treatment	1	0.40	0.524	1	1.12	0.289

5.4.4. Effect of fire on bee species

Multi-dimensional scaling (Fig. 5-2) showed that bee species display a great variation in spatial distribution as fire responses varied greatly between field sites. *Homalictus punctatus* (NB5) and *Exoneura robusta* (NB 9.2) showed a trend away from sites affected by prescribed spring burning (Fig. 5-2: A, B), but this result was not found in sites affected by prescribed autumn burning or wildfire. *Lipotriches moerens* (NB 8.3) was more often found in unburnt habitats than in burnt habitats and showed a similar trend across all field sites.

Prescribed spring burning (Fig. 5-2: A, B) indicated that the bee community is likely to prefer unburnt habitats, although results were not significant.

Especially *Lasioglossum Chilalictus* species seemed to not be affected by recent fire and showed a marginal, although not significant, trend towards an increased abundance in wildfire affected habitats (Fig. 5-2: E, F).

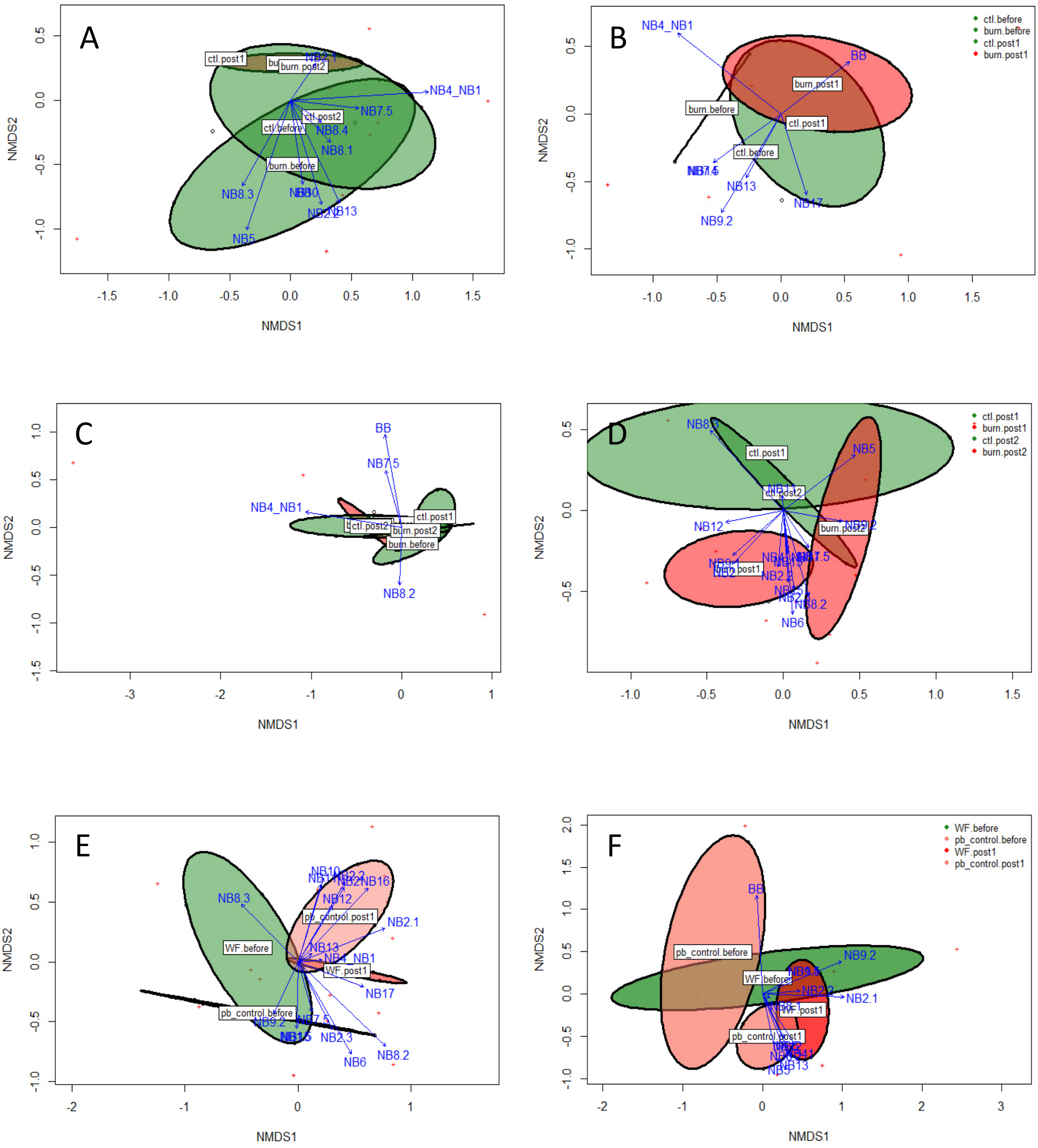

Fig. 5-2: MDS ordination of bee species composition. Vectors represent bee species (see Table 2 for synonyms). Fire affected sites are red shaded, control sites and unburnt sites are green. (A) prescribed spring burn in in Para Wirra (NMDS stress: 0.041) (B) prescribed spring burn in Kersbrook (stress: 0.096) (C) autumn burn in South Para (stress: 0.013) (D) autumn burn in Millbrook (stress: 0.119). Wildfire affected sites (E) Millbrook (stress: 0.119), (F) Kersbrook (stress: 0.076).

5.4.5. Functional traits and the effect of fire

The majority of species captured in our study are ground nesting bees, with the only exception being *Exoneura* and *Hyleaus* species, the latter with only 2 individuals recorded. The overall abundance of native bees, and therefore the majority of ground nesting bees, was either not (spring burns, wildfires) or slightly positively (autumn burns) affected in the first year following fire but abundance declined two years after prescribed burning. Wildfire had no significant effect on stem/twig (above ground) nesting bees (*Exoneura)* in the first spring after a wildfire in summer, but they were negatively affected by prescribed spring burning (Fig. 2, NB9.2 - *Exoneura*). Trophic specialization showed little variation between species, with most species being polylectic, i.e. using a broad range of flowering species. Only some Australian *Leioproctus* species, have shown trends to be oligolectic (Houston, 1991, Vieira and de Souza Lima, 1997), preferring flowers from the Myrtaceae. However, *Leioproctus* abundance was too low to perform sensitive statistical tests, but multi-dimensional scaling and indicator species analysis did not show any effect of fire on this genus. Body size of bees was associated to bee genera and will be discussed (below) within this context.

5.5. Discussion

5.5.1. Trapping methods

In this study, the bee community before fire, one year and two years following fire was sampled during springtime in a eucalypt forest in South Australia. The majority of bees were captured in coloured Vane traps. Different trapping methods were initially conducted to avoid a sampling bias (Westphal et al., 2008), but very low catching success for pan and SLAM traps was recorded. SLAM traps were probably less effective as bees are likely to easily escape the traps and as they are not an active colourful catching method (Campbell and Hanula, 2007, Campbell et al., 2007) as bees are especially attracted to certain colours that mirror floral colours such as blue and yellow (Kirk, 1984, Kevan and Backhaus, 1998). Blue Vane traps were the most successful traps in this study, which supports findings from Campbell et al. (2007) who also reported blue traps as the most successful trap colour. However, white and yellow pan traps as well as yellow vane traps showed very little trapping success in this study, although families which were present at the study sites are known to be attracted by yellow colours (Leong and Thorp, 1999). It is likely that the many yellow flowering plants in the study sites, such as *Acacia* trees and

Hibbertia shrubs which do not provide accessible nectar rewards (Keighery, 1975, Kenrick, 2003) made these colours less appealing for bees (Campbell et al., 2007). The effectiveness of pan traps in this study could also have been limited due to reduced visibility in the sometimes dense vegetation in the study site. Cane et al. (2000) have also previously reported that pan traps placed on the ground had poor success for bees visiting flowering shrubs, probably due to the height difference of trap and food resources.

However, the species diversity represents the bee community present in spring and is likely to be somewhat biased by the trapping method. Net trapping/transect walks, which are a good method to sample a better diversity of bee communities (Roulston et al., 2007, Westphal et al., 2008, Hall, 2016) were not conducted in this study as it was not compatible with the BACI design, as net trapping results are strongly dependent on the person conducting the trapping and could not have been conducted by the same person at the same time.

The colour of Vane traps could have also influenced the observed bee community composition. Some bee families are particularly attracted to specific colours. Halictidae, for example, reportedly prefer yellow and blue coloured traps (Stephen and Rao, 2007, Heneberg and Bogusch, 2014, Hall, 2016). In this study, the majority of individuals from the Halictidae family were captured in blue coloured Vane traps, supporting their preference for blue traps (Toler et al., 2005). Only few individuals of the Colletidae family were recorded in this study, which could have been less attracted by the coloured traps (Toler et al., 2005, Stephen and Rao, 2007, Hall, 2016). The abundance of bees caught in coloured Vane traps might also be influenced by the increased visibility of these active traps in sites following fire, which showed significant lower vegetation densities (Chapter 2) and might have allowed for a better detection of the traps (Fig. 5-3).

5.5.2. Functional trait approach

Functional trait approaches have been widely used to investigate the response of bees to disturbances (Moretti et al., 2009, Lazarina et al., 2016) and assigning species to traits can be helpful to understand how different species with the same traits respond to environmental change (McGill et al., 2006). However, a functional-trait based approach for analysing bee community responses to fire was not continued in this study, as most of the species collected share similar physiology, morphology and habitat requirements and are thought to utilize the same resources. All species apart from one collected in this study are generalists (polylectic) (Table 5-2), and it is expected that generalist species share their needs for similar flowers. The bees captured are also expected to have less diversity in nesting preference, as most of species captured were ground nesters with *Exoneura* spp. and *Hyleaus* spp. as the only exceptions. It may still be an advantage to investigate the respective nesting preferences of Australian bee species to understand the response of bee communities to environmental/habitat changes, despite the similar functional trait use of those recorded in this study.

5.5.3. Effect of fire on bee communities

This study demonstrated that prescribed burning affected the abundance of bees negatively two years following fire. Wildfire was not found to alter bee abundance one year following fire. Bee species composition was not altered by both prescribed burning and wildfire.

Interestingly, no decrease in bee abundance was recorded in the first spring following both prescribed burns and wildfire. These results support previous studies (Moretti et al., 2009, Winfree et al., 2009, Rubene et al., 2015) which reported no effect of recent fires on bee abundances. This is possibly not surprising, considering that most of the bees collected here are ground nesting bees, which have also been shown to be not affected by recent fire (Williams et al., 2010).

However, a decline in bee abundance after prescribed burning was observed when a second sampling period in the following spring was included into the analyses. This observation indicates that bees are likely to have survived the direct impacts of fire (Cane and Love, 2016), probably due to being safe in their underground nests, by finding shelter in unburnt vegetation or being high up in trees. However, the decline in bee abundance two years following prescribed burning suggests that burning might still hold long term consequences for bee communities, possibly due to changes in their surrounding habitat.

It has been shown previously that a loss of floral resources can have negative impacts on bee communities (Ne'eman et al., 2000, Potts et al., 2003). Although the floral composition remained unaffected by fire, the research presented in Chapter 2 found a significant decrease in floral abundance after one and two years following fire events. The reduced floral abundance might not have affected the reproduction success of bees in the first year, but could have influenced foraging success in the following years. Bees might have been forced to extend their foraging ranges and increase their flight activity to collect sufficient energy resources in fire affected sites. Longer foraging ranges can have negative consequences for bees, such as reduced progeny (Zurbuchen et al., 2010a) and even a shortened life span (Schmid-Hempel and Wolf, 1988). Reduced foraging success or an increased foraging effort in the first year following fire could have led to fewer progeny in the second year post fire. *Homalictus punctatus* and *Exoneura robusta*, which are rather small bee species, were two of the few species which were found to be negatively affected by fire. These small bees with smaller foraging ranges could have been especially disadvantaged by the reduced food availability following fire.

Nevertheless, species richness of bees was never negatively affected by fire. Furthermore, high species richness and species diversity could be observed after recent summer wildfires, indicating that a high level of disturbance might even favour bee species (Moretti et al., 2006). Some bee species could possibly have been favoured by these new post fire conditions and might have been able to occupy newly available niches in a habitat significantly altered by wildfire (see Chapter 2).

Fire often resulted in a greater availability of bare ground in the habitat (Chapter 2) which allows for better nesting resources for ground nesting bees (Potts et al., 2005) and could explain the observed increase in bee abundance in the first spring following an autumn burn. This result is supported by Lazarina (2016), who found that ground nesting bees, like the majority of species encountered in this study, are favoured by recent burns, due to the production of more nesting resources in the form of accessible soil for burrows (Potts et al., 2005, Campbell et al., 2007, Lazarina et al., 2016). The only stem/twig nesters in this study, *Exoneura* spp*.,* showed a trend away from prescribed spring burning, also possibly due to direct fire mortality (no ground nest for shelter) or due to the loss in nesting sites (less dense vegetation structure, decline in dead shrubs). These results support the findings from Williams (2010) who also showed that recent burns can affect above ground nesters negatively.

Another fire related habitat change which can favour bees is vegetation structure. Wildfire has been shown to reduce vegetation density significantly (Chapter 2) and result in more open space in the habitat, which can favour heliophilous insects (Gittings et al., 2006) and some bee species (Grundel et al., 2010) and thus might explain the observed trends here after wildfire.

Species richness was also not significantly affected by fire, but the years sampled post prescribed burning showed a low species richness in both fire affected and fire unaffected sites. This observation suggests that species richness might still be affected by prescribed burning, but species might be mixing between treatment sites. Treatment sites were thought to be independent according to maximal flight ranges of solitary bees (Wesserling and Tscharntke, 1995, Gathmann and Tscharntke, 2002), as traps in the respective sites were spaced over 1 km apart but foraging behaviour and maximum foraging ranges are still poorly understood for Australian native bees. The majority of bees captured in this study were of medium body size (5 - 14 mm). Previous studies suggest a foraging range of 200 - 800 m for solitary bees (e.g. Wesserling and Tscharntke, 1995) but bees with large body size such as *Amegilla* spp. (up to 15 mm) and larger medium sized bees (e.g. *Lasioglossum C. lanarium*, with up to 14 mm lengths) might be able to travel longer distances, of up to 1400 m (Zurbuchen et al., 2010b). However, species composition was not affected by fire, which suggests that body size of the respective species did not influence the response to fire.

Although prescribed burning was found to have a marginally negative effect on bee abundance, they were still present and diverse in all fire affected sites. Wildfire did not seem to have a negative impact on the bee community in the first year following the fire. Our findings do not support research by Ne'eman et al. (2000) who reported very few solitary bees in burnt areas. Furthermore, only a few species were negatively associated with fire, whereas some ground nesting *Chilalictus* species seem to be favoured by fire, especially in the first year following a wildfire event. The results of this study also indicate a substantial temporal and spatial variability in bee abundance and diversity among years and locations. In particular, the response of certain species to fire events differed markedly among field sites. However, the marginal decline in bee abundance in the second year following prescribed burning is still a concern and more research is needed to investigate the long-term effect of fire on bee communities.

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CHAPTER 6

Effect of fire on the abundance of specialist tiphiid wasp (Hymenoptera: Tiphiidae) orchid pollinators

Transect #74 in Kersbrook. Top: before a wildfire (Spring 2013). Right: 20 days after a wildfire (Summer 2015). Bottom: 5 months after a wildfire (Spring 2015). Photos by author.

Statement of Authorship

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Overall percentage (%)	85 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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6. Effect of fire on the abundance of specialist tiphiid wasp (Hymenoptera: Tiphiidae) orchid pollinators

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6.1. Abstract

Around 35 % of orchids in South Australia are listed as vulnerable, rare, or endangered (Barker et al., 2005). The loss of pollinators due to fire events may have an important impact on orchid populations. A decline in tiphiid wasp (Tiphiidae) abundance after fire could have devastating consequences and lead to a decline in orchids given they rely on a single species of insect pollinator. This study investigated the effect of fire disturbance on thynnine wasp abundance in eucalypt woodlands of South Australia. Wasps were sampled before fire, one year after fire and two years after fire, in control and fire treatment sites at four different locations. Results indicate that thynnine wasp abundance was not affected by either prescribed burns or wildfires. Although fire did not have a negative effect on wasp abundances, the relatively low abundance of wasps recorded in all field sites is a concern for the pollination success of native orchids and warrants further investigation.

Keywords: Prescribed burning, wildfire, pollinator, Thynninae (thynnine wasp), specialist orchid, Malaise trap (SLAM trap)

6.2. Introduction

Populations of orchids are declining throughout Australia, mainly because of habitat destruction and the effects of habitat fragmentation (Coates et al., 2002). Fire is known to be a driving factor for fragmenting, altering and changing habitats in Australia. Although flowering of some orchids can be promoted by fire (Coates et al., 2006, Coates and Duncan, 2009), the impact of fire on orchid pollinators is still unclear. It has been shown that declines in pollinators can lead to declines in the plants they pollinate (Biesmeijer et al., 2006, Geerts and Pauw, 2012). The loss of pollinators due to fire events may have an important impact on orchid populations, given that orchids are frequently pollen limited and consequently may be particularly sensitive to changes in pollinator communities (Burd, 1994, Ashman et al., 2004, Faast et al., 2011). The loss of certain pollinators could have devastating consequences and lead to decline or even extinction for those orchids that are specialists in their pollination strategy and rely on a single species of insect pollinator.

Several Australian genera of sexually deceptive orchids are solely pollinated by a wasp from the family Tiphiidae, most of them from the subfamily Thynninae (Peakall and Beattie, 1996, Phillips et al., 2009, Gaskett, 2011). A study in south-western Australia showed that sexually deceptive orchids tend to be rarer than orchids with broader pollination strategies (Phillips et al., 2011). A fire-induced decrease in thynnine wasp abundance or their disappearance within a site could have detrimental consequences for populations of orchid species relying on a one-to-one pollination relationship with a specific pollinator, by limiting both abundance and geographical range of the orchid (Phillips et al., 2014, 2015).

Thynnine wasps are solitary nectar-seeking wasps, and the few records available indicate that their larvae are parasitoids of various beetle larvae, mainly Scarabaeoidea (Smith, 1970). The winged male wasps carry their wingless females, who attract them by releasing sex pheromones, to nectar resources to provide food. Following mating, the male returns the female to the ground where she burrows down to search for ground-dwelling scarab beetle larvae, in which to lay her egg, so the wasp larvae can feed. Sexual deceptive orchids, such as *Caladenia tentaculata* mimic the female pheromones to attract male wasps for their pollination needs (Peakall and Beattie, 1996). The complexity of the wasp life cycle may make them particularly susceptible to environmental disturbances (Phillips et al., 2010), such as fire. Pryke and Samways (2012b) recently reported a significant decrease in scarab species richness and abundance one year after a wildfire in an Mediterranean ecosystem. Hence, the response of the host beetles to fire, as well as the availability of beetle nesting resources and beetle life cycle may further complicate the response of thynnine wasps to fire (Marino et al., 2006, Maleque et al., 2010).

Thynnine wasps could also be directly killed by fire, although females are less likely to be affected than males, as they spend most of their time underground. Both sexes may also be affected indirectly by changes in floral resources. Thynnine wasps mainly forage on nectar producing Myrtaceae *(Eucalyptus* spp.*, Leptospermum* spp.) but also Proteaceae *(Hakea* spp.*),* Pittosporaceae *(Bursaria* spp.*)* Thymelaeaceae *(Pimelia* spp.*)* and Xanthorrhoeoideae *(Xanthorrhoea* spp.*)* (Brown and Phillips, 2014). Many of these plants have different fire responses. Some show fire stimulated flowering, such as *Xanthorrhoea* spp. (Lamont and Downes, 2011). However, some plant taxa, especially within the obligate seeders (*Eucalyptus* spp., *Hakea* spp.*)* seem to take longer (up to 3 years) to reach flowering after a fire (Burrows et al., 2008). Reductions in flowering and therefore nectar production may have negative implications for pollinators, as nectar availability is of crucial importance to thynnine wasps (Smith, 1970). Female wasps lay only very few eggs (Smith, 1970) and rely on optimal resource availably to ensure sufficient progeny production.

The timing of fire is likely to be important for wasp survival. Fires occurring during the active flying phase of male wasps, typically between August and November (see Tomlinson and Phillips, 2015) might cause higher direct mortality in wasps, than fires occurring during dormancy periods in autumn.

Flight ranges of wasp species may also influence their response to fire, as the tolerance of a lower nectar availability after burning could be compensated by a larger flight range from/to areas with greater resource availability. Little research on the flight behaviour of thynnine wasps has been undertaken. The orchid *Drakaea glyptodon* is pollinated by sexual deception of the thynnine wasp *Zaspilothynnus trilobatus* and the mean pollinator movement recorded in a capture–recapture study was 32 m, while maximum flight distances were as far as 132 m (Peakall, 1990). Mark-recapture experiments with *Thynnoides pugionatus,* which is the pollinator of *Caladenia tentaculata,* an orchid common in the Adelaide Hills, documented travel distances between 40 m and 60 m. A recent study by Menz et al. (2013) reported much longer flight distances, up to several hundred meters (556 m) for the thynnine wasp *Zaspilothynnus gilesi*, which is a specialist pollinator of the orchid *Drakea elasitca*. It can be assumed that thynnine wasp males with larger body sizes are able to travel longer distances, as body size can be a predictor for flight ranges (Gathmann and Tscharntke, 2002). However, smaller species of thynnine wasps with shorter flight ranges might be similar to solitary bees, particularly affected by habitat loss and fragmentation due to fire impacts (Gathmann and Tscharntke, 2002). No research has been carried out on the flight behaviour of other Australian thynnine wasp species.

Despite the importance for specialist orchid species, far too little attention has been paid to the response of thynnine wasps to fire. Only a recent study by Brown et al. (2016) investigated the effect of fire history on orchid pollination, suggesting that thynnine wasp (*T. pugionatus*) visitation of the orchid *C. tentaculata* was enhanced in recently (4 - 10 years after fire) burnt and long unburnt vegetation in Victoria. No research has been carried out on the effect of fire on thynnine wasp abundance and species composition.

This study investigated the effects of recent prescribed burns and wildfires on thynnine wasp communities in eucalypt woodlands of South Australia. Wildfires are usually high in intensity and have been previously shown to alter insect habitat more significantly than prescribed burns (see Chapter 2), which leads to the hypothesis, that wildfire could cause more severe impact on wasp abundance. The effect of both prescribed burns and wildfire fire on thynnine wasp abundance was explored. Wasps were collected over three years during spring at four different locations, where orchids were present. Surveys were carried out before and after fire events using a "Before-After, Control-Impact" approach.

6.3. Material and Methods

6.3.1. Study sites

The study sites are located within the Mt. Lofty Ranges, to the east of Adelaide, South Australia. The average annual rainfall is 600 - 1000 mm/year and the area has a Mediterranean - type climate. Four locations were selected to assess the abundance of tiphiid wasp species and their response to fire. Each location contained one burn and one adjacent control area with similar habitat attributes, such as vegetation density and plant species present. Control and burn sites were adjacent to each other, but separated by wide fire tracks. Two out of the four control sites were affected by the Sampson Flat Wildfire in January 2015 prior to the third data collection period. For a map of sites and detailed coordinates of field locations see Chapter 2.

The habitat is characterised by woodlands of *Eucalyptus* species including *E. goniocalyx*, *E. obliqua, E. leucoxylon,* and *E. fasciculosa*, mixed with golden wattle trees (*Acacia pycnantha*) on the lower slopes. The understory is frequently dominated by *Pultenea* spp., *Hibbertia* spp., *Gonacarpus* spp., *Lepidosperma* spp., *Arthropodium* spp and *Xanthorrhoea* spp.

6.3.2. Study design

The impact of fire on thyninne wasp abundance was assessed with the common BACI (Before-After, Control-Impact) study design (Stewart-Oaten et al., 1986, 1992, Smith, 2002), by recording changes wasp abundance at unaffected sites ('control') and comparing them to either prescribed burn ('pburn') or wildfire affected sites. Locations affected by the wildfire were still sampled after the fire, but later analysed by comparing wasp abundance before and one year post wildfire (with previous prescribed burn affected sites serving as control sites). Data collection surveys were carried out over three periods from 2013 to 2015, always during springtime (early September to late November) in all field sites at all four locations. For more detailed information on the field sites and monitoring periods, see Chapter 2.1./2.2.

6.3.3. Thynnine survey

A total of 64 SLAM (Sea Land & Air Malaise) traps were installed at random locations, with 16 traps per field site (for a schematic of trap set up and more detailed information, see Chapter 2). Traps were permanently installed throughout the sampling season (spring), always placed in sunny locations and across possible flight paths of insects and spaced more than 200m apart. SLAM traps were installed in the same location in each sampling period. Collection bottles were half filled with 100 % undenatured ethanol, and were changed every 4 weeks in the first year, every 3 weeks in the second year (required due to higher temperatures) and every 4 weeks in the third year. Vane trapping, and using yellow and blue coloured traps (24/site) was also conducted on at least four sunny days with temperatures over 20° C (see Chapter 4 for detailed information about insect trapping surveys).

After each sampling season, all male and female thynnine wasps collected per trap were sorted and counted.

6.3.4. Statistical analysis

To account for site effects, such as geographical differences, differences in plant composition and the different burn seasons, all four field locations were analysed independently. The very few specimens collected in vane traps (*n* = 3) were allocated to the neighbouring SLAM trap for statistical analysis. The difference in wasp abundance between ‘control’ and ‘burn’ treatment sites and their response to burning was examined using Generalised Linear Mixed Models (GLMM, package glmmADMB, Fournier et al., 2012). Wasp abundance counts per SLAM trap and treatment site were modelled using a Poisson distribution and a log-link function using an interaction model (response variable: wasp abundance; fixed factors: burn treatment, time period; random effect: SLAM trap). Likelihood ratio tests were conducted to obtain analysis of deviance tables (Anova function, package car, John and Sanford, 2011). For graphing, the estimates and 95 % confident intervals of the mean number of wasps collected per treatment and period for each location were generated. Changes between the periods of sampling were investigated by multiple comparisons. Changes in wasp abundance in Kersbrook Native Forest were analysed comparing before and one period post-prescribed burning only, as the third data collection period was affected by the wildfire. The site in Millbrook was analysed comparing the first year and the second year following prescribed burning. The Millbrook and Kersbrook locations, which were affected by the wildfire, were analysed separately, with sites previously affected by prescribed burning serving as control sites. All analysis was conducted in R studio (R© 3.1.2).

6.4. Results

Overall, 193 wasps were collected from the four field locations over the three periods of sampling. The majority of wasps (*n* = 190) were captured in SLAM traps and only three specimens were found in Vane traps. Approximately 80 % of wasps collected could be identified and separated into 32 different species using DNA barcoding (see Chapter 3). The effect of fire on species diversity and on single species could not be investigated due to very low numbers of wasps collected per year and per field site, and due to the fact that not all specimens could be identified and separated into species (approximately 20 %).

Thynnine wasp abundance showed no or at best marginal changes caused by fire. Minimal changes occurred among periods in sites sampled over the three years (Fig. 6-2). One site affected by prescribed spring burning (Para Wirra) showed a decline ($p = 0.031$) in wasp abundance between the period before the burn and the first sampling period, nine months after the burn. This trend was not detected in Kersbrook. In the second year, 21 months after the burn in Para Wirra, thyninne abundance seems to have recovered. Prescribed autumn burning at South Para showed a marginal increase ($p = 0.043$) in wasp abundance from the sampling period directly after the fire (5 months) to the second sampling period 17 months after the burn, but this was not reflected by the results at Millbrook. However, wasp abundance two years following both prescribed spring and autumn burning ('post2') did not show any differences to abundance before fire (Table 6 - 1).

Table 6-1: Effect of fire on thynnine wasp abundance shown by the mixed model (GLMM) and paired contrast (PC), comparing the influence of fire among the different periods.

Prescribed spring burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Para Wirra	**- (0.031)**	0.89	0.72
Kersbrook	0.66	N/A	N/A
Prescribed autumn burn	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
South Para	0.89	**+ (0.043)**	0.15
Millbrook	NA	0.75	N/A
Wildfire	PC: before vs post1	PC: before vs post2	PC: post1 vs post2
Millbrook	0.52	N/A	N/A
Kersbrook	0.95	N/A	N/A

No fire related changes in thynnine wasp abundance were recorded at sites sampled eight months after a wildfire. The number of wasps captured varied considerably between traps which is illustrated by the large confidence intervals (2.5 % - 97.5 %, Fig. 6-1).

Although thynnine wasp abundance was not altered by fire, it was significantly affected by the year of sampling in some field sites. The analysis of deviance table of the GLMM model shows that the period had a significant impact on wasp abundance, as documented for both sites where prescribed autumn burning was conducted as well as in both wildfire affected sites (Table 6-2).

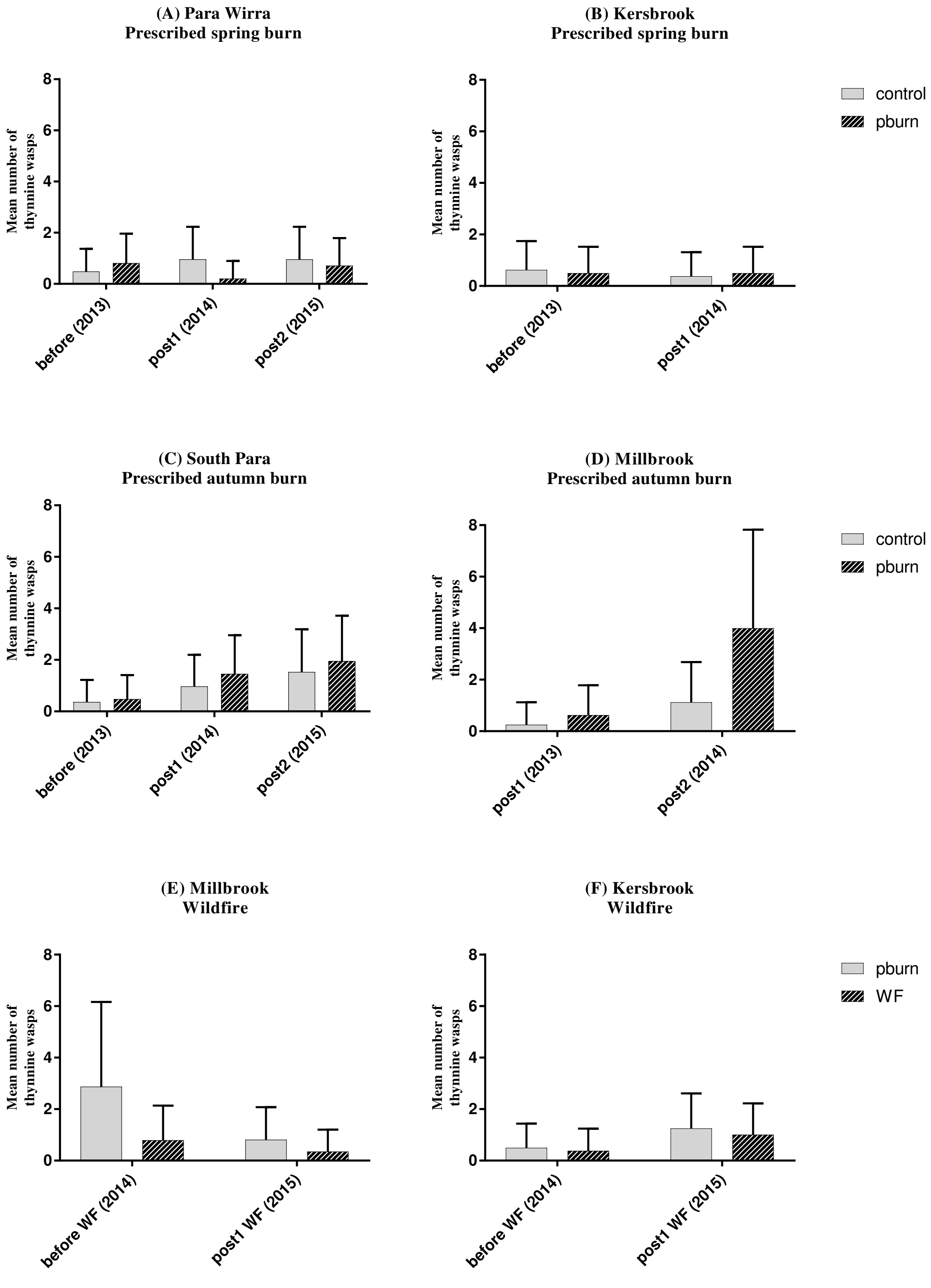

Fig. 6-1: Effect of fire on overall abundance of thynnine wasps showing mean number of wasps (GLMM results) captured per trap. (A) - (D): sites affected by prescribed burning. (E), (F): field sites affected by wildfire.

Table 6-2: Effect of fire on thynnine wasp abundance. Table of deviance. Showing degrees of freedom (*Df*), Chi-square results and p values (Pr(> Chisq), which indicate the likelihood of which factors play an important role in the model.

	Para Wirra			**Kersbrook (PB)**		
Prescr. spring burns	*Df*	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	2	0.386	0.824	1	0.187	0.666
treatment	1	0.263	0.608	1	<0.001	0.991
Period:treatment	2	4.684	0.096	1	0.196	0.658

	South Para			**Millbrook (PB)**		
Prescr. autumn burns	*Df*	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	2	9.661	**0.007**	1	11.332	**<0.001**
treatment	1	0.804	0.369	1	5.976	0.015
Period:treatment	2	0.060	0.970	1	0.105	0.746

	Kersbrook (WF)			**Millbrook (WF)**		
Wildfire	*Df*	Chisq	Pr(> Chisq)	*Df*	Chisq	Pr(> Chisq)
period	1	3.722	**0.054**	1	12.614	**<0.001**
treatment	1	0.282	0.595	1	4.109	0.043
Period:treatment	1	<0.001	0.948	1	0.413	0.521

6.5.Discussion

The abundance of thynnine wasps was not negatively affected by fire events in habitats which were sampled before fire, one year and two years after fire and compared to unburnt control sites. A decline in floral abundance could be previously reported (Chapter 2), but floral community composition in eucalypt woodlands did not change following fire. Although the abundance of potential food resources declined, none of the main food resources of thynnine wasps was eliminated by the fire. Especially flowers of plants of *Xanthorrhoea* and *Pimelia* genera which are known nectar resources for wasps (Brown and Phillips, 2014) were still present in fire affected habitats. Almost all male wasps captured in this study were of larger species (> 150 mm length) and it can be assumed that they can cover longer distances given that body size is a predictor for foraging ranges (Gathmann and Tscharntke, 2002). Thus, the reduced abundance in floral resources might not be a limiting factor when searching for nectar resources. Further, prescribed burns often leave patches of vegetation unburnt, which can also provide additional food resources after a fire.

Another important finding of this study was that neither prescribed burning nor wildfire significantly affected thyninne wasp abundance, although both impacted insect habitat differently (Chapter 2). Further, the seasonality of the prescribed burn did not play an important role in determining wasp abundance. One prescribed spring burn was found to have a marginally negative effect on wasp abundance in the first year following fire, but this was not reflected by the results at the other site affected by spring burning. Habitats affected by fire are often characterised by a lower vegetation density (see Chapter 2), which could have led to higher predation of wasps or host beetles followed by a temporal decline in wasp abundance. However, lower vegetation density was also recorded in wildfire affected sites where no changes in wasp abundance were observed. A lower vegetation density could also potentially favour thynnine wasps which are often found in open/cleared habitats (Alcock, 1981). Further, prescribed burns often produce habitats with high heterogeneity, which can also support abundance and diversity of wasps in Australian forests (Lassau and Hochuli, 2005). There is little evidence that fire had a significant influence on wasp abundance and it appears that the marginal changes are influenced by spatial and temporal variation.

Importantly, the results here indicate that the year of sampling had a much more important influence on wasp abundance. This finding concurs by other studies which also reported large variation in insect abundances among years of sampling (see Chapter 3, Pryke and Samways, 2012a). Differences among years can be caused by different climatic conditions, but can also depend on various other factors such as predator abundance and host availability.

Wasp abundance may not only be dependent on habitat and food resources such as flowering plants or vegetation density but also on the abundance of scarab beetles, serving as hosts for their larvae. The beetles are unlikely to suffer direct mortality after fire, as they mostly live underground, but a decline in species richness and abundance after wildfires has been previously reported for Mediterranean ecosystems (Pryke and Samways, 2012b). Changes in host beetle abundance could have led to changes in wasp abundance. Nevertheless, the relatively stable wasp abundance in fire affected sites suggests that fire did not have detrimental consequences on host beetle abundance.

Although overall wasp abundance was not affected by fire, individual species could still be negatively impacted by fire events. The loss of one particular species could have negative implications for specialist orchids relying on one specific pollinator, such as *C. tentaculata* which is pollinated by *T. pugionatus* (Peakall and Beattie, 1996). Unfortunately, no analysis on the effect of fire on individual species could be conducted due to the very low catch rates per field location and also because not all wasps captured could be identified to species level. This was mostly due to limited barcoding success, especially for wasps infected with the *Wolbachia* bacterium (see Chapter 3 for details). Given that different wasp species are thought to have a similar diet (Brown and Phillips, 2014) and a similar life cycle, it seems unlikely that only specific species would be negatively impacted by fire.

As thynnine wasps seem to not be affected by fire, this might even benefit orchids if they are more easily detected by their pollinators (Petit and Dickson, 2005) in a habitat with more open vegetation. Nevertheless, the relatively low abundance of thynnine wasps recorded in all four field locations is still a concern for the pollination success of native orchids and should be further investigated.

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CHAPTER 7

Discussion

Inspection of the study sites with supervisors Renate Faast (left) and José M. Facelli (right) in Millbrook, shortly after the Sampson Flat Bushfire in summer 2015. SA Water rangers clearing the track in the background. Photo by author.

7. Discussion

The main focus of this thesis has been an assessment of the impact of both prescribed burns and wildfires on terrestrial orchid pollinators and their habitat in eucalypt woodlands of South Australia.

To date, much of the research on the impact of fire on the Australian fauna has focussed on vertebrates such as small mammals (e.g. Leonard, 1973, Friend, 1993, Letnic and Dickman, 2005) or birds (Bendell, 1974, Woinarski, 1990, Taylor et al., 2012). Little attention has been paid to pollinating insects, especially insects which are important pollinators for orchids. Orchids throughout South Australia are in decline with around 35 % of species listed as vulnerable, rare or endangered (Barker et al., 2005). Insect pollinators are essential to the reproductive success of orchid species of the Mt. Lofty Ranges in South Australia (Faast et al., 2009, Faast, 2010). Until now, the effects of fire on the abundance of Australian orchid pollinators within the families Syrphidae, Apidae s.l. and Thynninae have been unknown. Information about species composition and diversity within these pollinator families in the study site were also yet to be determined.

Fire induced changes in pollinator habitat characteristics such as vegetation composition and structure, floral abundance and composition, and the availability of nesting resources can all have significant impacts on insect abundance and diversity (Ne'eman et al., 2000, Potts et al., 2003). While fire has been intensively studied in past decades, most studies have focused on ecosystems such as open grasslands, mallee-heath shrublands and sclerophyllus shrubby habitats (Bradstock et al., 2012). Very little information has been available on the short-term effects of fire in South Australian woodlands, which are often dominated by *Eucalyptus* species. These eucalypt forests are commonly targeted for bushfire management due to their proximity to anthropological assets. Hence, this thesis provides important insights into fire-induced changes in this type of ecosystem for both prescribed burns and wildfires.

In this concluding section, three main aspects of this thesis will be discussed. First, the results of insect identification are summarized and their use in ecological studies is discussed. This is followed by a section which connects the observed changes in pollinator communities with the recorded changes in insect habitat following fire. Finally, the effects of fire on pollinators and their habitat will be related to the possible implications for generalist and specialist orchids. Following each section, future research directions are discussed. Concluding remarks will summarise the importance of this thesis.

Reliable identification of orchid pollinators using DNA barcoding methods and morphological identification

To investigate the effect of fire on orchid pollinator communities, over 5000 insects collected during field surveys needed to be identified or at least differentiated into taxonomic groups. The three most common orchid pollinator families (Apidae s.l., Syrphidae, Tiphiidae) in South Australia were investigated. Morphological identification of Australian insects is often difficult, with few field guides available and identification keys missing for many groups (Batley and Hogendoorn, 2009). The availability of skilled taxonomists with the detailed knowledge required to identify the multitude of pollinator species is also very limited. To overcome these difficulties, DNA barcoding was used to separate the collected specimens to species level, with the ultimate goal of establishing a reference library for rapid identification of insect species.

DNA barcoding using the *CO1* gene proved successful for distinguishing between species, particularly hoverfly and bee species. Families with lower species diversity such as hoverflies (Syrphidae) could be reliably distinguished, using a cost effective subsampling approach. DNA barcoding revealed the presence of six hoverfly species at the study sites, with the majority of individuals (99 %) belonging to just two species. This study also offered important insights into the native bee composition and identified 27 bee species (Apidae s.l.). Furthermore, 32 species of thynnine wasps (Tiphiidae) were encountered.
Most of the sequences obtained did not match (> 98 % similarity) sequences in the online databases (Genbank, BOLD). Unfortunately, the Australian fauna has not been well sampled in the past, leading to it being greatly underrepresented in these databases. Consequently, subsets of bee and hoverfly species separated by barcoding were given to expert taxonomists for morphological identification.

Using both DNA barcoding and morphological identification, the majority of insect specimens analysed could be assigned to species which provided a good base for further ecological studies. The DNA barcodes of insects identified in this study will also be uploaded to Genbank (in preparation), improving the representation of Australian insect fauna in this database. The findings of this project also contribute to the current limited knowledge on species diversity and species richness of Australian native bees and hoverflies and further provide detailed information on community responses to fire regimes.

DNA barcoding of large datasets, like the 750 bee specimens encountered in this study, was time and cost intensive. However, the use of a single barcode marker (*CO1*) proved problematic for identifying thynnine wasps. The presence of the endosymbiontic bacterium *Wolbachia* caused further difficulties that resulted in specimens being unidentifiable using standard amplicon Sanger sequencing approaches. Future research should include the development of new primers for thynnine wasp identification. Over the past decade, developments in next generation sequencing technologies have made it possible to identify species and characterise communities from environmental DNA samples. This approach could be utilised in place of or in addition to the more traditional barcoding techniques employed here in order to further characterise these communities. Sequencing the DNA from orchid pollen (pollinia) collected by insects could further help identify the plant species they have visited. For example, extraction of DNA from pollinator and plant samples followed by next-generation sequencing could help to provide a more complete picture of pollinator-plant interactions. The accumulated insect sequence data in this study already provides a solid reference library of *COI* barcodes for insects that have carried and are likely to carry pollinia.

The effect of fire on orchid pollinator communities and their habitat

To investigate the effects of fire on orchid pollinators, insects and habitat surveys were conducted before, one year after and two years after fire and compared with surveys from control sites. Fire was found to cause profound changes in the structure of South Australian eucalypt woodlands. Wildfires affected several habitat characteristics significantly, but in contrast, results of pollinator surveys showed that even just five months after a fire there was little effect on the abundance of members of the three most common orchid pollinator families (Apidae s.l., Syrphidae, Tiphiidae). Interestingly, some hoverfly and bee species were found in higher abundances in some fire-affected sites. Also, richness and diversity of bee and hoverfly species did not appear to be negatively affected by fire, with species richness of bees even increasing after wildfires. These results support the idea that high insect species richness is associated with high disturbance rates (Moretti, 2006) and it is likely that some bee species were favoured by the post fire conditions.

Both prescribed spring burns and wildfires impacted floral abundances and reduced vegetation density. Of particular importance is the observed decrease in floral abundance, as floral resources are expected to play an important role in shaping pollinator communities. It has been previously reported that a decline in floral abundance can have a negative impact on foraging success of pollinating insects (Ne'eman et al., 2000, Potts 2003), but this was not supported by the data presented here. Instead, our results suggest that fire-induced changes in floral abundances did not have negative impacts on hoverfly or wasp abundances in eucalypt woodlands. It is likely that food resources for orchid pollinators following fire are either still sufficient, or the reduced availability can be compensated by other means. For example, prescribed burning which often results in a mosaic of burnt and unburnt areas, may still have accessible unburnt patches with flowering plants, providing abundant food resources. The wildfire, in contrast, was burned homogenously, with a lack of unburnt patches to serve as food resource islands. In these sites, it appears that other factors influence the foraging success of pollinating insects. The majority of insects studied were good fliers (hoverflies) or insects of medium to large body size (e.g. *Lasioglossum* and *Lipotriches* bee species, as well as thynnine wasps) and, given that body size is a predictor for foraging range (Gathmann and Tscharntke, 2002), they are likely to have foraging ranges that extend outside of the fire-affected areas. The abundance of bees declined marginally in the second year following prescribed burns in all monitored field sites, indicates that changes in the habitat following fire may cause negative longer term effects on bee communities. Lower floral resources in the first year following fire may have resulted in a lower reproduction success of bees in the following year.

The reduced vegetation density could have also benefited heliophilous insects by providing an increased amount of open spaces, which is known to favour syrphid flies and thynnine wasps which prefer foraging in the sun (Alcock, 1981, Gittings et al., 2006) and may explain the high numbers of syprhid flies recorded in some sites affected by fire.

The findings of this study also suggest that prescribed burning does not significantly reduce logs, litter and standing litter, which implies that insect pollinators that rely on these for nesting opportunities are unlikely to be negatively affected. On the other hand, the persistence of these fire fuel sources does pose important questions for fire management in South Australian woodlands, such as the effectiveness of prescribed burning to reduce the fuel load and the risk of wildfires. As expected, the impact of a wildfire was more severe than the impact of prescribed burns and presented significant changes in habitat characteristics such as reduced litter depth, log abundance and an increased availability of bare ground. These observed decreases are most likely due to fire consumption, since wildfires are more intense than prescribed burns.

The changes to pollinator habitat after a wildfire, such as the changes in nesting sites, were expected to have severe implications for pollinator communities. Interestingly, no pollinator species was severely affected by the fire events. Only two bee species showed a marginal decline in one location affected by prescribed burning, while other bee species showed an increased abundance in burnt sites. Bee species associated with fire affected habitats were all ground nesting species. The fire induced changes to the composition of the ground in burnt sites could have favoured these ground nesting species due to the increased availability of bare ground (Biesmeijer et al., 2006). The only stem/twig nesting bees in this study, *Exoneura* spp., were only negatively affected by one prescribed spring burn, but were not significantly affected by wildfire, despite their susceptibility to direct mortality and the potential loss of nesting resources such as standing litter.

The observed changes in fire affected pollinator habitats, especially the drastic changes in wildfire affected sites, did not lead to the expected changes in orchid pollinator communities. Also, the seasonality of prescribed burning was not found to significantly influence pollinator response. The studied eucalypt woodlands consisted of mainly native plants and seem to provide habitats very suitable for fire adapted pollinators. This study also indicates that a fire affected landscape can still be an attractive habitat for pollinators, even though several habitat characteristics were altered. It is also likely that most of the native insect species are well adapted to South Australian fire regimes and cope well with habitat changes following fire. Especially the generalist diet requirements and good flight abilities of most of the insects encountered potentially help them to survive post fire conditions.

This study documented changes up to two years after a fire. Further research should include the investigation of longer-term effects of fires and fire regimes on pollinator communities, with on-going habitat monitoring. Plant communities (Williams and Gill, 1995) as well as forest soils (Certini, 2005) are known to experience long-term changes following fire, and it is likely that the habitat will keep changing over the following years and may lead to furhter consequences for pollinating insects. For example, time since fire has previously been shown to influence species richness in bee communities, often due to a decrease in floral diversity (Moretti et al., 2009). It may also prove beneficial to monitor the recovery of the habitat in the years following fire, especially the density of vegetation, the availability of floral resources, as well as changes in important insect nesting resources such as litter and dead plant material to fully understand the ecological consequences of burns in eucalypt woodlands. In spite of these limitations, the results of this study will help inform future fire management objectives in eucalypt woodlands, particularly in terms of the importance of habitat patchiness to pollinator persistence, and the low fuel reduction observed following prescribed burns.

There are still many unanswered questions about the ecology of Australian orchid pollinators, not just in regards to fire. Further work should also be undertaken to determine key aspects of pollinator ecology, particularly in regard to flight ranges as well as feeding habits, which are often unknown for specific insect species. Further information, especially about flight ranges could benefit the management for conservation of insect pollinators; for example, by selecting appropriate area sizes for prescribed burns to ensure available food resources within foraging ranges of bees in fire affected sites.

Implications for orchid management

Fire induced changes in pollinator communities can lead to reduced pollination success and, subsequently, to a decline in populations of plants (Cunningham, 2000). However, the results presented in this thesis suggest that pollination success of orchids in South Australia eucalypt forests is unlikely to be negatively impacted by both prescribed burns and wildfires, as orchid pollinators were still abundant and diverse in fire affected habitats. In particular, orchids that are generalists in their pollination strategy are unlikely to experience lower pollination success in fire affected habitats. For example *Glossodia major* and *Caladenia rigida* are pollinated by a broad range of insects, such as hoverflies and different bee species (Faast, 2010), which were still abundant in habitats following fire.

Specialist orchids which rely on one single species of pollinator are perhaps more likely to be affected by fire events. Some species, for example, *Caladenia behrii* and *C. tentaculata* are pollinated by a single species of thynnine wasps (Bates, 2011). This view, however, is challenged by recent findings (Faast and Marquart, unpublished data), which showed that two wasp specimens captured while visiting C. *behrii* did not belong to the same species (*CO1* barcoding, R/A species delimitation analysis).

Due to low abundances of thynnine wasps in each field location, this study only investigated the effects of fire on total wasp abundance, without investigating the presence or absence of specific species in burnt habitats. The possible loss or decline in abundance of one specific thyninne wasp species due to fire regimes, could have detrimental consequences for the reproduction of a certain orchid species, potentially leading to local extinction. However, due to the expected similar diet, life cycle and the relatively large body size of all the wasps captured, it can be assumed that fire is unlikely to affect species differently. These results are supported by recent research that investigated the differences in fruit sets of the sexually deceptive orchid *C. tentaculata* and *C. behrii*, between control and fire affected sites. Faast et al. (2016a, 2016b) found no differences in fruit set between sites burnt by bushfires or prescribed spring burns, and unburnt sites. Faast et al. (2016b) also mentioned a generally low fruit set of *C. tentaculata* in study sites in the Mt. Lofty Ranges compared to other study sites in South Australia, which may be explained by the generally low thynnine wasp abundance, regardless of fire events. The low and also fluctuating numbers of thynnine wasps at the study sites are quite concerning, given that some specialist orchids rely on the wasps for their pollination and especially in the light of the current decline in orchid populations. Long-term monitoring of wasp abundance would be needed, to further investigate the stability of wasp populations in the study sites.

Although pollinating insects were not affected by burning, a fire affected habitat can still have consequences for flowering orchids. Due to reduced vegetation density, an increased visibility of orchid flowers was observed in burnt sites , which can also make them easier to detect for pollinators (Petit and Dickson, 2005). This supports recent findings from Faast et al. (2016b) who reports increased fruit set for some orchids in sites affected by wildfire. Faast et al. (2016a, 2016b) also suggests this might be due to reduced competition for pollinators with other flowering plants and related lower floral abundance following fire.

Interestingly, two generalist orchid species (*C.rigida* and *G.major*) showed an increased fruit set in wildfire affected sites, whereas the specialist orchid *C. tentaculata* did not show differences between burnt sites and unburnt sites, although very low pollination success made it difficult to assess the impacts of fire (Faast et al., 2016b). These findings suggest that bees and flies, which were still abundant in burnt sites with reduced floral diversity, are indeed using their resources more intensively. Visitation of flowers offering lower nectar rewards such as orchids was possibly increased either due to better visibility associated with reduced vegetation density or simply due to lower resource availably. Although orchids might be more easily detected by pollinators in fire affected landscapes (Petit and Dickson 2005) they also can be be more easily detected by grazing animals such as kangaroos and white winged choughs (Faast and Facelli, 2009). Grazing on orchid flowers was found to be drastically higher at some burnt sites, leading to restricted reproductive success and negating the benefits of increased pollination rates (Faast et al., 2016).

Fig. 7-1: Visibility of *Glossodia major* (left) and *Caladenia tentaculata* (right) in a wildfire affected site in Kersbook. (Photos by author)

This study also documented large temporal variation in pollinator abundances among orchid flowering seasons. Reasons for these fluctuations may differ between pollinator families, and may depend on host (Tiphiidae) or food (Syrphidae) availability for larvae or even climatic conditions. Years with increased rainfall and cooler temperatures during spring may lead to reduced pollinator activity as many pollinating insects prefer sunny and warm weather for foraging (Stoutamire 1983; Bates 1984, Beardsell et al. 1986, Westphal et al. 2006). Reduced pollinator activity during an entire season could have led to reduced capture rates and therefore a reduced estimates of abundance. Reduced activity of insects in a particular season may also result in reduced reproductive success, having knock-on effects for future seasons. These temporal differences in pollinator abundances/activity are likely to play an important role in the reproductiive success of the orchids they pollinate. For example, Faast (2010) found that the pollination success of the generalist *C. rigida* as well as of the specialist *C. tentaculata* varies significantly among flowering seasons.

The spatial and temporal variability in pollinator abundance appears to have a greater influence on pollination success of orchids than does fire. Simultaneous long-term monitoring of orchid and pollinator populations would be necessary to investigate long-term fluctuations in insect abundances among years and the resulting implications for orchid reproduction. There are still other mechanisms that can influence the effect of fire on insects and on the pollination success of orchids, such as climate parameters (rainfall, temperature, humidity) which were not assessed in this study and need further investigation.

Some orchid pollinator families were not included in this study. For example, orchids belonging to the genus *Microtis,* which were encountered in some study sites, are pollinated by ichneumonid and braconid wasps (Bates, 1981). Further, orchid pollinating flies such as Calliphoridae, Bombyliidae (blowflies), Sciaroidea (fungus gnats) and Conopidae (bee flies) are also known pollinators of orchids in the Mt. Lofty Ranges (Faast and Facelli, 2009, Phillips et al., 2009). Although these pollinator families were collected in this study, abundance and diversity were not further investigated, as this was beyond the scope of the study. However, further research is needed to investigate the effect of fire on these families to obtain a comprehensive picture of orchid pollinator ecology.

Concluding remarks

This study has made an important contribution to knowledge on the short-term effects of fire on orchid pollinating insects in eucalypt woodlands of South Australia. Pollinators are still abundant following fire despite the challenges of a fire affected landscape. The diversity of generalist orchid pollinators (e.g. hoverflies and bees) was not altered by fire events despite their habitat undergoing significant changes, especially following wildfire. The results suggest that pollination success of orchids in South Australia is unlikely to be negatively impacted by the effects of both prescribed burns and wildfires on pollinator abundance. The observed temporal changes in pollinator abundance among years are likely to have a much more severe impact on orchid pollination success. This thesis advances the understanding of the effect of fire on South Australian biota and can be used to improve management and conservation of terrestrial orchids and their pollinators. As pollinator abundance and pollination success of orchids did not seem to be severely influenced by recent fire events, other strategies for orchid conservation need to be considered, such as the reduction of potential threats including habitat loss and grazing. However, the observed decline in bee abundance two years following fire causes concern and highlights that long-term implications of fire on orchid pollinators and their habitat remain still unknown and require further research.

7.1. References

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Appendix 1

Photographs of selected orchid species/genera encountered in study sites

All photos by author.

Orchid species: *Caladenia reticulata*

Pollination strategies: Sexual deception

Pollinators: Wasps

Present at study site: South Para, Kersbrook, Millbrook

Orchid species: *Caladenia rigida*

Pollination strategies: Food deception and small nectar reward

Pollinators: Bees, syrphid flies, bee fly

Present at study site: Kersbrook, Millbrook

Orchid species: *Caladenia behrii*

Pollination strategies: Sexual deception

Pollinators: Wasps

Present at study site: Kersbrook

Orchid genus: *Caladenia tentaculata*

Pollination strategies: Sexual deception

Pollinators: Wasps

Present at study site: Kersbrook, Millbrook, South Para, Para Wirra

Orchid genus: *Caladenia leptochila*

Pollination strategies: ?

Pollinators: Possibly syrphid flies?

Present at study site: South Para, Kersbrook

Orchid species: *Glossodia major*

Pollination strategies: Unknown

Pollinators: Possibly bees and syrphid flies

Present at study site: Kersbrook, Millbrook, South Para, Para Wirra

Orchid species: *Leptoceras menziesii*

Pollination strategies: Potentially food deception

Pollinators: Possibly bees

Present at study site: Millbrook, Para Wirra

Orchid genus: *Diuris spp.*

Pollination strategies: Food deception

Pollinators: Bees, syrphid flies, wasps

Present at study site: Kersbrook, Millbrook, South Para, Para Wirra

Orchid genus: *Thelymitra spp.*

Pollination strategies: Food deception

Pollinators: Bees, syrphid flies, sawflies

Present at study site: Para Wirra, South Para, Millbrook, Kersbrook

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Appendix 2

Plant species recorded at the study sites

Genus/Species	**Family**
Acacia acinacea	Leguminosae
Acacia continua	Leguminosae
Acacia myrtifolia	Leguminosae
Acacia paradoxa	Leguminosae
Acacia pycnantha	Leguminosae
Acaena echinata	Rosacea
Acotriche serrulata	Epacridaceae
Acrotriche depressa	Epacridaceae
Aira spp.	Gramineae
Allocasuarina muelleriana ssp. muelleriana	Casuarinaceae
Amphipogon strictus	Gramineae
Anagallis arvensis	Primulaceae
Arthropodium spp.	Liliaceae
Asparagus asparagoides	Asparagaceae
Astroloma conostephioides	Epacridaceae
Astroloma humifusum	Epacridaceae
Austrodanthonia spp	Gramineae
Austrostripa spp.	Gramineae
Avena barbata	Poaceae
Blennospora drummondii	Asteraceae
Brachypodium distachyon	Gramineae
Briza maxima	Gramineae
Briza minor	Gramineae
Brunonia australis	Goodeniaceae
Bulbine bulbosa	Asphodelaceae
Burchadia umbellata	Liliaceae
Bursaria spinosa	Pittosporaceae
Caesia calliantha	Liliaceae
Caladenia behrii	Orchidaceae

Genus/Species	**Family**
Caladenia leptochila	Orchidaceae
Caladenia reticulata	Orchidaceae
Caladenia rigida	Orchidaceae
Caladenia tentaculata	Orchidaceae
Callitris gracilis	Cupressaceae
Calytrix tetragona	Myrtaceae
Cassytha glabella f. dispar	Lauraceae
Cassytha pubescens	Lauraceae
Centaurea sp.	Compositae
Centaurium erythraea	Gentianaceae
Centaurium maritimum	Gentianaceae
Centrolepis aristata	Centrolepidaceae
Cerastium glomeratum	Caryophyllaceae
Chamaescilla corymbosa var. corymbos	Liliacea
Cheilanthes austrotenuifolia	Adiantaceae
Chrysanthemoides monilifera ssp. monilifera	Compositae
Comesperma calymega	Polygalaceae
Convolvulus angustissimus var. angustissimus	Convolvulaceae
Conyza sp.	Compositae
Crassula closiana	Crassulaceae
Daucus glochidiatus	Apiaceae
Daviesia brevifolia	Leguminosae
Deyeuxia sp.	Gramnineae
Dianella revoluta var. revoluta	Liliaceae
Dichelachne crinita	Gramineae
Dillwynia hispida	Leguminosae
Disa bracteata	Orchidaceae
Diuris spp.	Orchidaceae
Dodonaea viscosisa	Sapindaceae
Drosera auriculata	Droseraceae
Drosera glanduligera	Droseraceae
Drosera macrantha	Droseraceae
Drosera peltata	Droseraceae
Drosera whittakeri ssp. whittakeri	Droseraceae

Genus/Species	**Family**
Echium plantagineum	Boraginaceae
Ehrharta longiflora	Gramineae
Epilobium sp.	Onagraceae
Eucalyptus fasciculosa	Myrtaceae
Eucalyptus goniocalyx subsp. goniocalyx	Myrtaceae
Eucalyptus leucoxylon subsp. leucoxylon	Myrtaceae
Eucalyptus obliqua	Myrtaceae
Euchiton collinus	Compositae
Exocarpus cupressiformis	Santalaceae
Galium divericatum	Rubiacea
Galium gaudichaudii	Rubiacea
Galium murale	Rubiacea
Glossodia major	Orchidaceae
Gompholobium ecostatum	Leguminosae
Gonocarpus elatus	Haloragaceae
Gonocarpus mezianus	Haloragaceae
Gonocarpus tetragynus	Haloragaceae
Goodenia blackiana	Goodeniaceae
Hakea carinata	Protaceae
Hakea rostrata	Protaceae
Helichrysum scorpioides	Compositae
Hibbertia australis	Dilleniaceae
Hibbertia crinita	Dilleniaceae
Hibbertia devitata	Dilleniaceae
Hibbertia exutiacies	Dilleniaceae
Hybanthus floribundus ssp. Floribundus	Violaceae
Hydrocotyle callicarpa	Araliaceae
Hydrocotyle laxiflora	Araliaceae
Hypochaeris glabra	Compositae
Hypochaeris radicata	Compositae
Isopogon ceratophyllus	Proteaceae
Ixodia acheillaeoides ssp. alata	Compositae
Kennedia prostrata	Leduminosa
Lachnagrostis filiformis	Gramineae

Genus/Species	**Family**
Lagenophora hueglii	Compositae
Lepidosperma semiteres	Cyperaceae
Leptoceras menziesii	Orchidaceae
Leptorhynchos squamatus	Compositae
Leptospermum myrsinoides	Myrtaceae
Leucopogon virgatus var. virgatus	Epacridaceae
Levenhookia pusilla	Stylidiaceae
Lilium sp.	Liliaceae
Lobelia gibbosa	Epacridaceae
Lomandra fibrata	Liliacea
Lomandra juncea	Liliacea
Lomandra micrantha	Liliacea
Lomandra multiflora ssp. dura	Liliacea
Lomandra nana	Liliacea
Microlaena stipoides	Gramineae
Microseris lanceolata	Compositae
Millotia tenuifolia var. tenuifolia	Compositae
Neurachne alopecuroidea	Gramnineae
Olearia ramulosa	Compositae
Olearia teretifolia	Compositae
Opercularia varia	Rubiaceae
Oxalis perennans	Oxalidaceae
Parentucellia latifolia	Orobanchaceae
Parentucellia latifoloa	Scrophulariacea
Pimelea linifolia	Thymelaeaceae
Pimelea octophylla	Thymelaeaceae
Pinus seedling	Pinaceae
Plantago sp. B	Plantaginaceae
Platylobium obtusangulum	Leguminosae
Poa clelandii	Gramineae
Poranthera microphylla	Euphorbiaceae
Poranthera microphylla	Euphorbiaceae
Prickly thistle	Gramineae
Pteridium australis	Dennstaedtiaceae

Genus/Species	**Family**
Pultenaea pedunculata	Leguminosae
Pultenea canaliculata	Leguminosae
Pultenea daphnoides	Leguminosae
Pultenea largiflorens	Leguminosae
Ranunculus sp.	Ranunculaceae
Romulea rosea var. australis	Iridaceae
Rumex brownii	Polygonaceae
Scaevola albida	Goodeniaceae
Senecio phelleus	Compositae
Senecio picridioides	Compositae
Senecio pterophorus	Compositae
Senecio quadridentatus	Compositae
Siloxerus multiflorus	Compositae
Sonchus oleraceus	Compositae
Spyridium parvifolium	Rhamnaceae
Spyridium vexilliferum	Rhamnaceae
Stackhousia aspericocca	Celastraceae
Stylidium graminifolium	Stylidiaceae
Tetratheca pilosa	Tremandraceae
Thelymitra spp.	Orchidaceae
Thysanotus patersonii	Liliacea
Tolpis barbata	Compositae
Trachymene pilosa	Apiaceae
Tricoryne elatior	Liliaceae
Trifolium arvense	Compositae
Trifolium campestre	Compositae
Trifolium glomaeratum	Compositae
Vellereophyton dealbatum	Compositae
Vulpia bromoides	Gramnineae
Wahlenbergia stricta ssp. stricta	Campanulaceae
Wurmbea dioica	Liliaceae
Xanthorrhoea semiplana	Liliaceae
Xanthosia huegelii	Umbelliferae