Introduction

Seed banks play a critical role in the persistence of plant populations in variable environments (Thompson 2000). In the context of fire, seed banks are a particularly important source of seed of species killed by fire which rely on seed stored in the canopy (Bradstock and Myerscough 1981; Zammit and Westoby 1987; Bradstock and O'Connell 1988) or in the soil (Auld 1986; Parker and Kelly 1989) to regenerate and persist in the landscape following such disturbance events.

Fire severity can vary, depending on a number of factors including intensity, duration of the fire, fuel loads, fuel and vegetation type, topography, climatic conditions, soil texture and moisture, soil organic content, time since last fire and area burnt (Whelan 1995; Neary *et al.* 1999). Fires consuming a large amount of fuel can result in greater heat penetration of the soil profile, greater soil temperatures and longer residence times during the passage of fire (Bradstock and Auld 1995; Neary *et al.* 1999). This is particularly important for species with a soil-stored seed bank, as heating of soil may promote or inhibit the germination of seeds in the soil and affect the regeneration of species following fires of different severities.

Fire can trigger the germination of many species from the soil seed bank through the impact of factors such as heat (Auld and O'Connell 1991; Enright *et al.* 1997; Read *et al.* 2000), smoke (Brown 1993; Dixon *et al.* 1995; Keeley and Fotheringham 1998), and their interaction (Kenny 2000; Thomas *et al.* 2003), as well as by the influence of other factors such as charred wood (Keeley and Fotheringham 2000) and ash (Enright *et al.* 1997). Responses to fire cues may vary in different plant communities, with species in more fire-prone communities generally having a positive response to different cues (Enright *et al.* 1997; Hanley *et al.* 2001), whereas species in more mesic communities, which experience infrequent fire, may have a negative response to different fire cues (Hopkins and Graham 1984). With fire severity, fire-driven local extinction of species relying on soil seed banks may be a result of the death of seeds due to high temperatures during hot fires or the failure of seeds to germinate as a result of insufficient heating during cool fires (Keith 1996). This PhD project is part of a larger ARC Linkage Grant (LP0775145) and was initiated to assess the persistence of species with soil-stored seed banks in the context of fire severity.

Fire is recognised as an important disturbance in the Australian landscape, however changing fire regimes pose a threat to plant populations by disrupting the lifecycle of vulnerable species (Whelan 1995; Keith 1996). Where the effects of fire frequency on plant biodiversity have been modelled at landscape scales, the predictive ability of models is limited by the unknown effect of fire severity. The major aim of the ARC Linkage project is to determine the effect of plant persistence from plot to landscape scale. This larger project has three main themes: 1) spatial analysis of fire severity, 2) the persistence ability of soil-stored seed, and 3) the persistence ability of sprouting species in relation to fire severity and intervals. The PhD project is associated with the second theme of the ARC Linkage project. As part of the obligations of the ARC Linkage project, habitat and site selection needed to satisfy the overall themes of the project rather than specifically to address my research questions. This led to some limitations on this PhD project that otherwise would not be present had the PhD project not been part of the larger ARC Linkage project.

In my PhD project I was interested in assessing the soil seed banks of different plant communities with the specific objectives of:

1) Describing the characteristics of soil seed banks and their relationship to the standing vegetation;
2) Assessing soil seed banks response to heating, in the presence of smoke;
3) Assessing soil seed bank response to burn severity;
4) Assessing soil seed bank response to time since fire;
5) Assessing seed response to post-fire soil temperatures.

Soil seed banks are difficult to study compared to canopy-held seed banks where estimates of seed bank size can be easily made by counting mature fruit or swollen woody follicles (e.g. *Banksia* species). Soil seed bank dynamics are influenced by seed input and loss, which varies on spatial and temporal scales (Parker *et al.* 1989). The resulting data sets may be highly variable, especially when sampling the soil seed bank at a community level. Plant functional groups have been used extensively to determine response to fire, based on a number of groupings including life form (Cornelissen *et al.* 2003) and sprouting ability (Bradstock and Kenny 2003; Pausas *et al.* 2004). I adopted this approach throughout this research to enable some generalised trends of soil seed banks across a number of different plant communities, as well as looking at individual species within each community studied.

Apart from the Introduction and Conclusion this thesis has five data chapters, each written in the style of a scientific paper with detailed analysis and discussion of literature relating to each chapter. As a result of this style, some of the introductory literature and methodology is repeated in each chapter where ideas and related experiments overlap; all cited references are located at the end of each chapter. A brief conclusion at the end of the thesis reiterates the main findings and any future directions of the research relating to soil seed banks and fire severity.

Outline of thesis

The focus of Chapter 1 is to assess the soil seed banks of five different plant communities in the Gibraltar Range and Washpool National Parks, New South Wales, Australia. The five communities range from those dominated by mesic elements (Wet Sclerophyll Forest, Rainforest), with low fire frequency to those with a strong sclerophyll component (Dry Sclerophyll Forest, Rocky Outcrop, Wet Health) and a relatively higher expected fire frequency. Here I wanted to examine the nature of the soil seed banks from these communities and their relationship to the corresponding standing vegetation. I also wanted to determine a sampling strategy for sampling the species richness and abundance in the soil seed banks of these communities which could be used throughout the remainder of the thesis and whether this differed between the communities.

The impact of heat on the soil seed banks of the five plant communities studied in Chapter 1 is examined in Chapter 2. I used multiple levels of heat in combination with one smoke treatment to determine the germination response of species from the soil seed bank of different plant communities and whether this had an impact of plant functional groups within each community. The temperature range used was to simulate different levels of soil heating experienced during fires of different intensities. I was interested to know if species in the more fire-prone communities, where fire is more frequent, are more likely to be tolerant of heat whereas species in the less frequently burnt mesic communities are less tolerant to heat.

In Chapter 3 I examine whether, almost six years after fire, burn severity had a detectable impact on the soil seed banks of the same five communities. In this study, burn severity refers to the loss of organic matter above ground during the passage of fire (Keeley 2009). Burn severity can affect the size of the soil seed bank due to the amount of soil heating that occurs during fires of different severities. Too much heating during more intense fires, or insufficient heating during less intense fires, is likely to affect the amount of seed remaining in the soil after fires. This is likely to impact on the soil seed banks of different communities in a variety of ways. Burn severity indices were developed using Landsat TM pre- and post-fire images to calculate the Normalised Burn Ratio (Kumar *et al.* 2008) and were developed as part of the larger ARC Linkage Project.

I assess the impact of time since fire on the soil seed banks of the five different communities in Chapter 4. The focus of this chapter is to determine if there is an immaturity and senescence risk of species in the soil seed bank of the five communities by assessing their respective soil seed banks in long unburnt vegetation and in vegetation burnt 6.25 and 4.5 years prior to sampling.

In Chapter 5 I examine if simulated post-fire soil temperatures would affect the germination or viability of seed of legume species found in the soil of a post-fire environment. Specifically I was interested in determining whether seed size played a role in germination success after seed had been exposed to heat, simulating elevated soil temperatures that may be experienced after fire. The implications of soil heating via increased solar radiation after a fire on the persistence of soil seed banks are also explored here.

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Chapter 1. Assessing soil seed banks of five different plant communities.

Introduction

Seed banks play a critical role in the persistence of plant populations in variable environments (Thompson 2000) and are an important source of seed following disturbances, such as flood (Leck 1989; Capon and Brock 2006; James *et al.* 2007; Porter *et al.* 2007), canopy-gap creation (Hopkins and Graham 1984; Garwood 1989) and fire (Keeley 1987; Archibold 1989; Parker and Kelly 1989; Ferrandis *et al.* 1996; Safford and Harrison 2004). In the context of fire, seed banks are a particularly important source of seed for species with different sprouting ability. Individual species within populations killed during a fire rely on seed stored in the canopy (Bradstock and Myerscough 1981; Zammit and Westoby 1987; Bradstock and O'Connell 1988) or the soil (Auld 1986; Parker and Kelly 1989) to regenerate and persist in the landscape following such disturbance events. Soil-stored seed banks enable the seeds of species which do not store seed on the parent plant to persist in the landscape, without relying on dispersal. Seeds germinate in the soil when seed dormancy has been broken and conditions are favourable.

Communities are likely to differ in their soil seed bank composition due to a number of factors. The composition of the soil seed bank reflects the regeneration strategies of the past and present components of any particular community (Roberts 1981). In some communities major differences are due to successional changes in vegetation (Hopkins and Graham 1987; Thompson 2000). Communities which experience frequent disturbances are characterised by long-lived seeds in their soil seed banks, whereas in more stable environments the seeds of species in the soil seed bank are more short-lived (Thompson *et al.* 1997; Bossuyt and Honnay 2008). The soil seed banks of Rainforest communities, for example, are dominated by successional and weed species (Table 1: Graham and Hopkins

1990), whereas soil seed banks of long unburnt fire-prone communities are dominated by species also found in the standing vegetation such as shrub species (Table 1: Enright *et al.* 1997; Enright and Kintrup 2001; Wills and Read 2002).

In many communities, disturbance is an important mechanism for determining species composition. In some communities, the similarity between the standing vegetation and the soil seed bank decreases with time since disturbance; in other communities similarity increases with time since disturbance (Hopfensperger 2007). A consistent trend across many communities is that pioneer species produce persistent seed banks. Similarity between the standing vegetation and the soil seed bank is affected by seed dispersal limitations, and whether seed germination cues are met (Hopfensperger 2007). For fire-prone communities, which experience frequent fire and thus more disturbances, similarity between the standing vegetation and the soil seed bank is predicted to be low. In contrast, mesic communities experience infrequent fire and thus less disturbance events, and similarity, therefore, is predicted to be higher than that measured in the fire-prone communities. This, however, assumes germination requirements are met and seed longevity rates are similar across communities.

In fire-prone communities, seeds of species stored in the soil can be triggered to germinate through the impact of factors such as heat (Auld and O'Connell 1991; Enright *et al.* 1997; Read *et al.* 2000), smoke (Brown 1993; Dixon *et al.* 1995; Keeley and Fotheringham 1998), their interaction (Kenny 2000; Thomas *et al.* 2003), and by the influence of other factors such as charred wood (Keeley and Fotheringham 2000) and ash (Enright *et al.* 1997). Burial and spatial distribution within the soil profile will be critical for the survival of seed during the passage of fire and the influence of germination cues (Auld 1987; Parker *et al.* 1989). In mesic communities, soil-stored seeds banks are less likely to be triggered to germinate by fire cues (Floyd 1966; 1976; Hopkins and Graham 1984; Tang *et al.* 2003); light and canopy gap creation are more important factors influencing germination from the soil seed bank (Garwood 1989). The dominant species stored in the soil seed banks of mesic communities appear to be pioneer or secondary successional tree or shrub species (Table 1), rather than primary canopy species, indicating primary species in these communities have a limited persistence in the seed bank. With fire, the species composition of the soil seed bank may be altered due to seed death from lethal soil temperatures or failure to germinate due to inappropriate germination cues.

Soil seed banks are more difficult to study than canopy-held seed banks where it is easier to estimate the seed bank size by counting the swollen woody follicles in an infructescence (e.g. *Banksia*). Soil seed bank dynamics are influenced by seed input and loss which varies on spatial and temporal scales (Parker *et al.* 1989). Processes, such as dispersal, predation, germination and burial, determine spatial patterns of seeds in the soil (Parker *et al.* 1989). The size and number of samples used to measure soil seed bank dynamics will be important as this will be affected by seed distribution and density of any particular species. Where this information is limited for particular species, Roberts (1981) proposed the general principle of using a large number of small samples, rather than small numbers of large samples. This is especially difficult when sampling the soil seed bank for community level comparisons rather than single species comparisons.

The soil seed banks of five contrasting plant communities were studied using pooled subsamples collected on replicate transects. The aims of this study were twofold. First, the nature of the soil seed banks of these communities and their relationships with the standing vegetation were explored by determining how the soil seed banks of five communities differed, and by discerning differences between the standing vegetation and soil seed bank in each of the five communities. The second aim was, determining the sampling strategy (number and size of sub-samples per transect) for sampling species richness and abundance in the soil seed bank of five contrasting communities. I asked specifically:

Q.1. How do the soil seed banks of communities differ in terms of the key variables of species richness, abundance and plant functional types?

Q.2. How do the soil seed bank and the standing vegetation of each community differ? Are soil seed bank/standing vegetation differences similar for all communities or are soil seed bank/standing vegetation patterns of variability different for different communities?

Q.3. Do estimates of species composition in communities differ depending on the size and number of samples?

Methods

Study area

The study was conducted in Gibraltar Range – Washpool National Parks, located on the Great Dividing Range in Eastern Australia (29°31’S, 152°18’E). The Parks occur in both the Northern Tablelands and North Coast botanical subdivisions of NSW, with area totalling approximately 73 000 hectares. The study area experiences warm wet summers, with average daily temperatures between 16–30°C, and cool dry winters with average daily temperatures of 5–20°C. The study area is dominated by shrubby eucalypt woodlands and forests with extensive areas of rocky outcrop and wet heath vegetation. The underlying geology in these areas is predominately granite, which forms shallow, low-nutrient soils. Rainforest and Wet Sclerophyll Forest are mainly confined to Washpool National Park, where the underlying geology is a mixture of sedimentary, acid volcanic and adamellite geology, which forms deep rich soils (Sheringham and Hunter 2002).

Seed bank sampling

Five broad communities were sampled: Dry Sclerophyll Forest, Rocky Outcrop, Wet Heath, Wet Sclerophyll Forest and Rainforest (Table 2). These broad community types were described for the study area by Sheringham and Hunter (2002), and Hunter and Sheringham (2008). These communities cover a gradient from nutrient poor to more nutrient rich soils. Two sites were sampled in each of the five communities. These had been burnt at a low to moderate severity during a wildfire in 2002 (Kumar *et al.* 2008). Sites in the same community were located at least 150 metres apart, but no further than ten kilometres from each other. Two contrasting strategies for soil sampling, based on the size of the replicate sample, were compared. Between mid-April–late-May 2007, five transects (10 x 1 metre) were randomly sampled at each site. Transects were at least 20 metres from each other in all communities, except in the Rocky Outcrop. The location of transects in this community were constrained by the availability of soil. In each transect two large (28 x 16 x 5cm: length x width x depth) and eight small (28 x 4 x 5cm: length x width x depth) soil samples were collected. The total volume of soil collected in both sampling strategies was equivalent. The different sampled sizes were randomly allocated at one metre intervals along each transect. Soil samples included litter. Samples of the same size were pooled to form a homogenous sample, each transect contributing two samples, one of each size sampling strategy. Samples were air dried at room temperature and then sieved (4mm aperture) to remove litter, roots and rocks. Seeds larger than 4mm were placed back into the soil sample. A sub-sample of soil was taken from each pooled sample and spread out on separate seedling trays to form a 1cm layer of soil (28 x 32 x 1cm: length x width x depth) over a layer of vermiculite/perlite mix (1:1) and placed in a glasshouse.

Table 1. Some examples of soil seed bank studies with different disturbance regimes.

Plant community and relevant references	Disturbance regime	Seeds/m^2	Seedling emergence (cm)	Predominant species	Similarity of soil seed bank with standing vegetation
Dry Sclerophyll Forest					
Vlahos and Bell (1986)	Frequent fire	377–1579	0–3	Annuals, subshrubs	Low
Enright *et al*. (1997)	Long unburnt (1966)	405–1080	0–5	Sedges, grasses and shrubs	N/A
Read *et al*. (2000)	Mine site rehabilitation	4607	0–7.5	Annual herbs, perennial grasses	N/A
Enright and Kintrup (2001)	Long unburnt (1983)	4227–12547	0–5	Shrubs, grasses, herbs	N/A
Grassy Woodland/grasslands					
Pierce and Cowling (1991)	Grazing/trampling	4273	0–5	Perennial grasses	50%
Thomas (1994)	Annual/biennial burning	336–2436	0–5	Perennial monocots and herbs, weeds	N/A
Morgan (1998)	Frequent fire	11390–23440	0–5	Annual, native and exotic monocots	Low
Hill and French (2003)	Frequent fire and grazing	725–1085	0–5	Perennial herbs, shrubs and grasses	Similar proportions of life forms
Rainforest					
Hopkins and Graham (1984)	Clearing, burning	244–3116	0–5	Secondary succession species	Low
Graham and Hopkins (1990)	Undisturbed	84–338	0–4	Secondary succession trees and shrubs	Low
Hopkins *et al*. (1990)	Grazing	434–4758		Pioneer and secondary tree and shrubs, vines and weeds	Low
Wet Sclerophyll Forest					
Wang(1997)	Clearing and burning	61–668	0–2	Forbs	Low
Stewart (1999)	Frequent fire	Not reported	0–5	Perennial herbs, shrubs, grasses	Low
Williams(2000)	Fire	Not reported	0–4	Herbs, grasses	N/A
Heathland					
Zammit and Zedler (1988)	Fire	8000–25000	0–5	Shrubs	N/A
Pierce and Cowling (1991)	Fire	1683–4518	0–5	Short- and medium-lived shrubs, graminoids	39-46%
Holmes and Cowling (1997)	Fire	1100–1900	0–10	Short-lived, and ephemeral species	Low
Wills and Read (2002)	Unburnt (1976)	485–4575	0-8	Shrubs	Low
Enright *et al*. (2007)	Fire	233–1435	0-5	Perennial species	Low

Table 2. Descriptions of the five plant communities sampled in Gibraltar Range – Washpool National Parks.

Community	Canopy layer	Mid-storey layer	Ground layer
Dry Sclerophyll Forest	Dominant species: *Eucalyptus olida, E. ligustrina* with *E. caliginosa, E. williamsiana*	Dominant species: *Leptospermum trinerium, Boronia microphylla, Bossiaea neo-anglica, B.scortechinii, Monotoca scoparia, Petrophile canescens*	Common species: *Caustis flexuosa, Dampiera stricta, Daviesia nova-anglica, Dillwynia phylicoides, Entolasia stricta, Hovea heterophylla, Patersonia glabata, Platysace ericoides*
Rocky Outcrop	Sparse scattering of *Eucalyptus ligustrina* and *E. williamsiana*	Dominant species: *Kunzea bracteolata* Common species: *Leptospermum nova-angliae, L. trinverium, Acacia venulosa, Allocasurina rigida, Boronia antheifolia, Callitris monticola, Cassytha glabella, Grevillea acerata, Leucopogon microphyllus, Mirbelia rubiifolia*.	Common species: *Caustis flexuosa, Dampiera stricta, Laxmannia gracilis, Patersonia glabata, Platysace ericoides*
Wet Heath			Dominant species: *Amphipogon strictus, Baeckea omissa, Baloskion fimbriatum, Blandfordia grandiflora, Empodisma minus, Hibbertia* sp. aff. *rufa, Lepidosperma limicola, Lepyrodia anarthria, Philothrix deusta*
Wet Sclerophyll Forest	Dominant species: *Eucalyptus campanulata*	Dominant species: *Goodia lotifolia, Gonocarpus oreophilus, Hibbertia scandens, Zieria* sp. aff. *smithii*	Dominant species: *Dianella caerulea, Glycine clandestina, Hibbertia dentata*
Rainforest	Dominant species: *Ackama paniculosa, Ceratopetalum apetalum, Schizomeria ovata*		Common species: *Aneilema acuminatum, Citriobatus pauciflorus, Eucalyptus campanulata, Hydrocotyle pedicellosa, Lobelia trigonocaulis, Palmeria scandens*

Source: (Sheringham and Hunter 2002; Hunter and Sheringham 2008).

Seedling emergence from the trays was monitored every week for 10–11 weeks, until no new species were recorded. Seedlings were individually pinned, with different coloured pins used at each census to avoid miscounting. Unidentified seedlings were replanted into larger plots for later identification. Initial sampling of the soil seed bank for the 10–11 week period were to detect readily germinable fraction of those species either killed by a fire cue or that did not respond to a fire cue. After 10 weeks in the glasshouse the soil samples were treated with a heat and smoke application to stimulate the germination of seeds of those species with a fire cue (Enright *et al.* 1997; Kenny 2000; Read *et al.* 2000). For these treatments, soil samples were air dried at room temperature and heated at 80°C for 15 minutes in a preheated oven. To minimise the loss of heat when the oven door was opened, the time to heat the samples did not commence until the oven thermostat indicated that the oven had returned to 80°C. Actual temperatures measured during the soil heating process were 52°C±2. The samples were then returned to the glasshouse and watered with one application of diluted (1:10) smoke water. Concentrated smoke water was prepared by drawing smoke from burning a mix of fresh and dried eucalypt litter through two litres of deionised water for 30 minutes using a water smoke apparatus. The application of the heat and smoke treatments were randomly allocated to batches of samples (both large and small) from the same transect of each community type to ensure independence of replication (Morrison and Morris 2000).

The average minimum and maximum temperatures in the glasshouse for the duration of the experiment were 13°C and 30°C, respectively. Seedling trays were randomly placed in the glasshouse and watered to keep the soil moist. Monitoring of seedling emergence continued every week for a further 10 weeks.

Vegetation sampling

Species present in the standing vegetation were recorded along each of the 10 x 1 metre transects at each site during September 2007. Species presence/absence was noted for species rooted in the transect area, as well as those species with canopy cover over the transect. All species recorded in the soil seed bank and standing vegetation were grouped on their known response to fire, either resprouters or obligate seeders (NSWFFRD 2002, T. Auld pers. comm. 2008; Clarke *et al.* 2009). Species were also grouped on their life form (grasses, graminoids, forbs, shrubs, trees and twiners) and their life history (perennial, annual, or variable strategy) following descriptions in Harden (1990; 1991; 1992; 1993). For those species where the known fire response was variable, the dominant response was used. Species classified as small trees were grouped into the shrub category, as in most cases they contributed to the mid-storey stratum. Nomenclature follows Harden (1990; 1991; 1992; 1993) and http://plantnet.rbgsyd.nsw.com.au/.

Statistical analyses

Soil seed bank characteristics and the sampling strategy

In order to test differences between the two soil sampling methods, as well as soil seed bank characteristics among communities, the factors communities, sample size, and sites (nested within communities) were assessed using a mixed model Analysis of Variance (ANOVA). The variables tested were: total species richness; total species abundance; species richness and abundance of species emerging from the soil seed bank based on their sprouting ability (reprouters versus obligate seeders). In addition, the proportion of species with different sprouting ability emerging from the soil seed bank were also compared among communities, sample size and sites nested within communities, using a mixed model ANOVA.

Post-hoc Scheffe's test was used to test for differences between levels of a significant factor. All univariate statistical analyses were performed using DataDesk® 6.1 (Velleman 1997). Data were tested for normality using box plots where the response variable was plotted by community, and In(y +1) transformed to satisfy assumptions of normality where appropriate. All analyses using proportional data were square root and arcsine transformed to improve the underlying distribution (Zar 1984).

Comparison between the soil seed bank and the standing vegetation

A mixed model ANOVA was used to compare the soil seed bank and the standing vegetation components of each community using the factors communities, stratum (soil seed bank versus standing vegetation) and sites (nested within communities). The variables tested were: total species richness, and richness of species based on their sprouting ability (resprouters versus obligate seeders). Stratum was a two-level factor and refers to the presences of species in either the soil seed bank and/or standing vegetation. The soil seed bank data were pooled at the sample size level (transects) to enable direct comparisons with the standing vegetation data, where transects were the replicates. Data were tested for normality using box plots where the response variable was plotted by community. Analyses were performed using DataDesk® 6.1 (Velleman 1997). Post-hoc Scheffe's tests were performed to determine where differences occurred between levels of a significant factor.

A two-way crossed PERMANOVA tested whether differences in species composition occurred between the soil seed bank and standing vegetation for each community using a Bray-Curtis similarity matrix. Pairwise tests were used to determine where differences occurred. Non-metric multi-dimensional scaling (nMDS) of the data was used to display the similarity of the 50 transects for the standing vegetation and the soil seed bank. The similarity matrix for the nMDS was calculated using the Bray-Curtis coefficient and was constructed in two dimensions. A similarity percentage (SIMPER) analysis, which produces an average similarity for set groups (soil seed bank and standing vegetation), based on the Bray-Curtis index of similarity, was used to determine the species that contributed most strongly to similarities among samples within the soil seed bank and the standing vegetation, and to identify species that contributed to differences between the soil seed bank and the standing vegetation. Analyses were performed on presence/absence data using PRIMER v6 (Clarke and Gorley 2006) and PERMANOVA+ (Anderson et al. 2008).

Results

Soil seed bank characteristics

The most seedling emergents were recorded from the soil seed bank of the Rainforest (RF) community followed by Wet Heath (WH), Rocky Outcrop (RO), Wet Sclerophyll Forest (WSF) and Dry Sclerophyll Forest (DSF) communities (Table 3). Soil seed bank densities of the Rainforest (834/m^2) and Wet Heath communities (739/m^2) were more than twice those of other communities, with Dry Sclerophyll Forest having the lowest density (145/m^2). A total of

125 species representing 47 plant families were recorded across all communities. The Asteraceae, Fabaceae and Cyperaceae were the most speciose families (17, 13 and seven species, respectively).

The soil seed banks of all five communities recorded a greater number of perennials than any other life history categories (Table 4). Forb, tree and twiner species were more abundant in the soil seed bank of the Rainforest community than the other communities. Shrub and graminoid species were more abundant in the soil seed bank of the Wet Heath community compared to the other communities. Grass species did not dominate any one particular community and were generally low in abundance (Table 4). Most soil seed banks were characterised by an abundance of forb and shrub emergents, with the exception of the Rainforest community, which was characterised by forb and tree emergents (Table 4).

Table 3. Mean, total abundance and seed density of seedling emergents from the soil seed bank of five contrasting communities[†]

Plant communities	Mean abundance (± s.e.)	Total abundance	Seeds/m^2
Dry Sclerophyll Forest	25.9 (± 3.29)	259	145
Rocky Outcrop	56.5 (± 23.42)	565	315
Wet Heath	132.4 (± 47.77)	1324	739
Wet Sclerophyll Forest	30.7 (± 8.02)	307	171
Rainforest	149.4 (± 47.01)	1494	834

[†] (n = 10), soil seed bank data pooled at the sample size level (transects)

Table 4. Characteristics of the soil seed bank: total seedlings in each functional group recorded from the soil seed banks of five communities[†]

	[‡]DSF	RO	WH	WSF	RF
Grasses	9	9	3	9	2
Gramionds	17	22	24	11	11
Forbs	71	340	119	107	499
Shrubs	162	192	1176	143	91
Trees	0	0	0	25	860
Twiners	0	2	2	12	31
Resprouters	201	420	847	120	1313
Obligate seeders	58	145	477	187	181
Shrub – Resprouters	148	101	728	29	27
Shrub – Obligate seeders	14	91	448	114	64
Annual	22	21	6	20	29
Variable	17	14	10	29	21
Perennial	220	530	1308	258	1444

[†] (n = 10), soil seed bank data pooled at the sample size level (transects)

[‡] DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Species richness and abundance were not significantly different among communities (Fig 1a, b; Table 5). The shrub component of the soil seed banks were dominated by resprouting species in the fire-prone communities (Table 4: DSF, RO, WH), compared to the mesic communities (Table 4: WSF, RF) where obligate seeding shrub species dominated. Resprouters were more abundant than obligate seeders in the soil seed banks of the fire-prone communities (Table 4: DSF, RO, WH) and one mesic community (Table 4: RF), however, there was no significant difference for the average abundance among communities (Table 6a, b). There were significant differences in species richness among communities for obligate seeders, however, the Scheffe's post-hoc test did not yield a significant result at $P = 0.050$. The percentage of resprouter and obligate seeder species were found to be significantly different for community type (Fig 2; Table 6c), with differences occurring between the Wet Sclerophyll Forest and Wet Heath communities. There were proportionally more ($P = 0.038$) resprouter and less obligate seeding species sampled in the soil seed banks of the Wet Heath (R: 87 ± 8; OS: 30 ± 5) than in the Wet Sclerophyll Forest (R: 36 ± 5; OS 80 ± 9). There were also significant differences in the percentage of individuals based on sprouting ability (Table 6d), however, the Scheffe’s post-hoc test were non-significant ($P = 0.05$).

Table 5. *F*-statistic and *p*-value of the three-way ANOVA for (a) species richness, (b) abundance of individuals emerging from the soil seed bank of five communities.

Factor	d.f.	(a) Species richness		(b) Seedling abundance	
		F	*P*	*F*	*P*
Community	4	3.810	0.087	1.591	0.308
Sample size	1	0.579	0.481	0.029	0.871
Community x Sample size	4	0.583	0.689	0.210	0.922
Site(community)	5	**3.759**	**0.004**	**3.607**	**0.005**
Site(community) x Sample size	5	0.718	0.611	0.891	0.491

Significant sources of variation are in bold

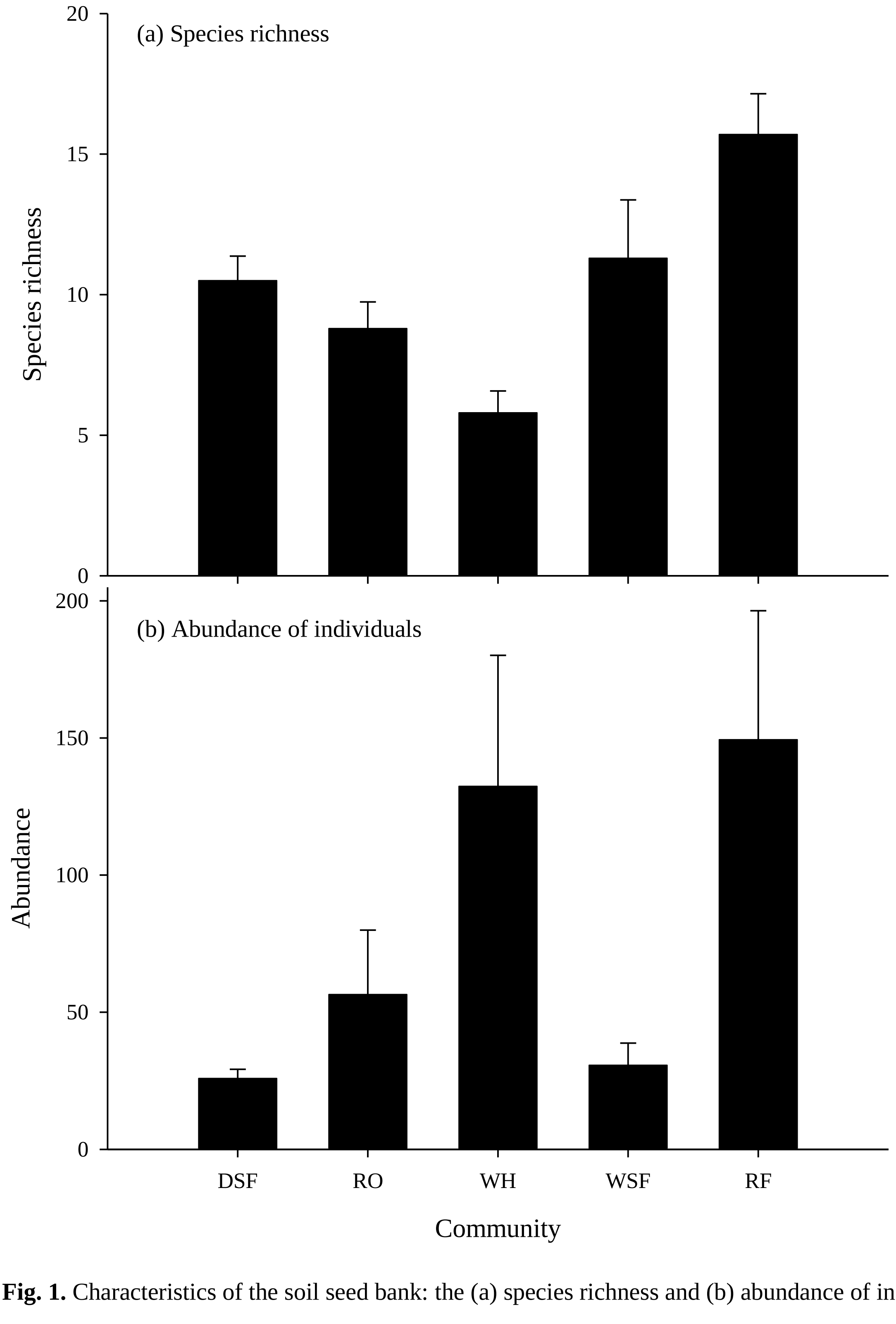

Fig. 1. Characteristics of the soil seed bank: the (a) species richness and (b) abundance of individuals (n = 10, + s.e.) emerging from the soil seed bank of five communities. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

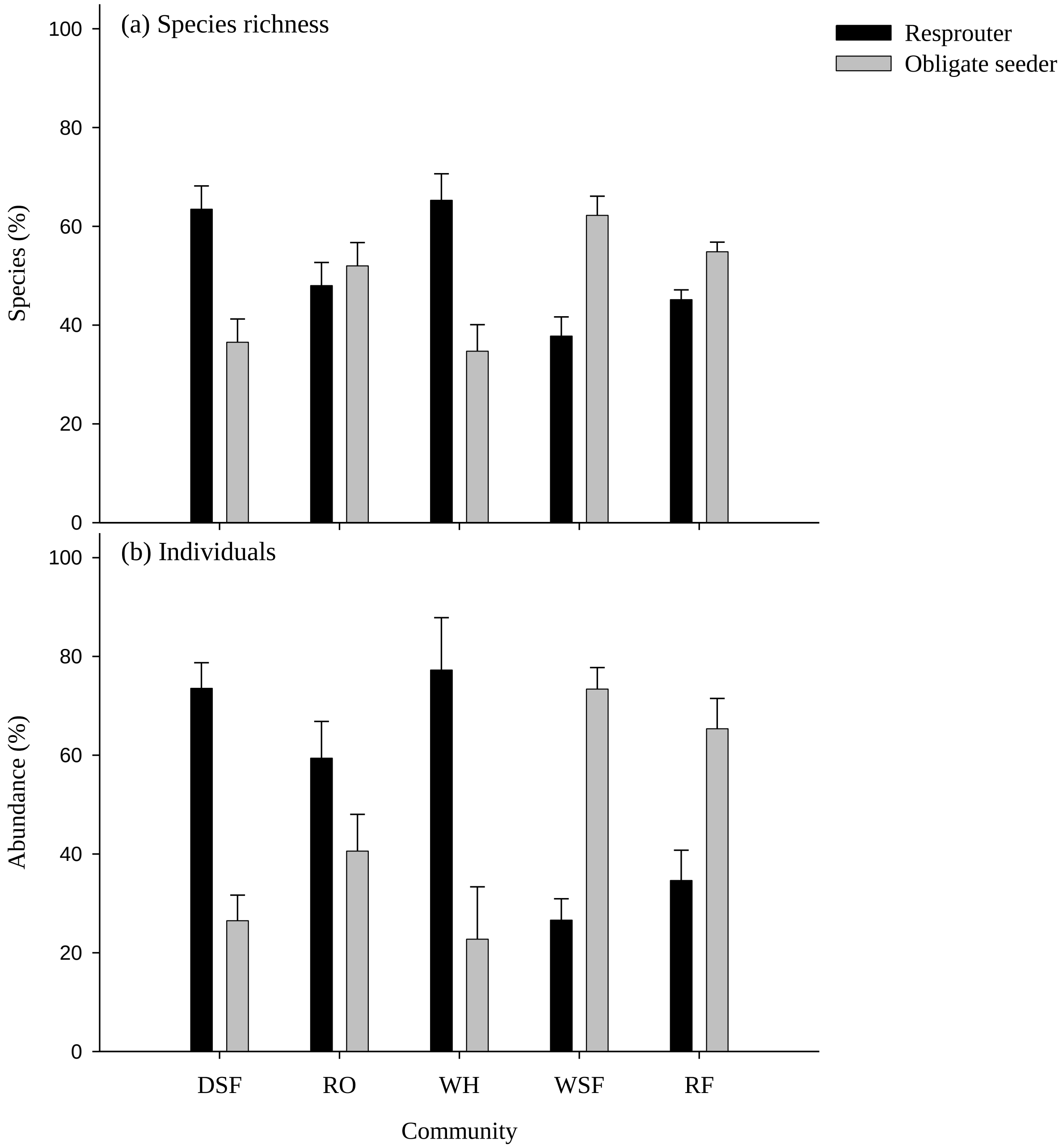

Fig. 2. Characteristics of the soil seed bank: the percentage of (a) species and the (b) abundance of individuals (+ s.e.) emerging from the soil seed bank of five communities based on their sprouting ability. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Table 6. *F*-statistic and *p*-value of the three-way ANOVA for (a) species richness, (b) abundance of individuals, (c) percentage of species and (d) percentage of individuals emerging from the soil seed bank of five communities based on fire response.

Factor	d.f.	Resprouters		Obligate seeders	
		F	*P*	*F*	*P*
		(a) Species richness			
Community	4	2.751	0.148	**6.113**	**0.037**
Sample size	1	0.139	0.725	0.667	0.451
Community x Sample size	4	0.426	0.786	0.217	0.918
Site(community)	5	**2.779**	**0.023**	**4.275**	**0.002**
Site(community) x Sample size	5	1.740	0.135	0.588	0.709
		(b) Abundance			
		F	*P*	*F*	*P*
Community	4	2.691	0.153	5.128	0.051
Sample size	1	1.174	0.328	0.034	0.862
Community x Sample size	4	1.011	0.481	0.150	0.955
Site(community)	5	**3.029**	**0.015**	**2.791**	**0.023**
Site(community) x Sample size	5	0.332	0.892	0.373	0.866
		(c) Percentage of species			
		F	*P*	*F*	*P*
Community	4	**9.219**	**0.016**	**9.219**	**0.016**
Sample size	1	0.220	0.659	0.220	0.659
Community x Sample size	4	1.032	0.473	1.032	0.473
Site(community)	5	1.492	0.202	1.492	0.219
Site(community) x Sample size	5	1.562	0.181	1.562	0.181
		(d) Percentage of individuals			
		F	*P*	*F*	*P*
Community	4	**6.639**	**0.031**	**6.636**	**0.031**
Sample size	1	0.162	0.704	0.149	0.716
Community x Sample size	4	1.068	0.460	1.063	0.462
Site(community)	5	**2.476**	**0.039**	**2.484**	**0.038**
Site(community) x Sample size	5	0.562	0.729	0.565	0.727

Significant sources of variation are in bold

Comparison between the soil seed bank and the standing vegetation

A total of 249 species were sampled from the soil seed bank and standing vegetation across the five plant communities, with 85 species common to all. Overall 40 species were unique to the soil seed bank and 124 unique to the standing vegetation. The soil seed bank of the Rainforest community was the most speciose, followed by the soil seed banks of the Wet Sclerophyll Forest, Dry Sclerophyll Forest, Rocky Outcrop and Wet Heath communities (Fig. 3; Table 7). The trend was different for the standing vegetation, where the Dry Sclerophyll Forest community was the most speciose, followed by the Rocky Outcrop, Wet Sclerophyll Forest, Wet Heath and Rainforest communities (Fig. 3; Table 7). There was a significant interaction between community and stratum (i.e. soil seed bank versus standing vegetation) with more species sampled in the standing vegetation of the Dry Sclerophyll Forest (Scheffe's $P = 0.005$) and the Rocky Outcrop (Scheffe's $P = 0.036$) communities than in the soil seed bank of each community (Fig. 3; Table 8).

Perennial species dominated the soil seed bank and standing vegetation of all five communities. The soil seed bank and standing vegetation of the Rocky Outcrop and Dry Sclerophyll Forest communities were dominated by shrub species, compared to any other life form. More forb species were sampled in the soil seed bank and standing vegetation of the Wet Sclerophyll Forest community than any other life form. Graminoid and shrub species dominated the standing vegetation of the Wet Heath, whereas forb species dominated the soil seed bank of this community. Forb species also dominated the soil seed bank of the Rainforest community; however, the standing vegetation of this community was dominated by shrub and tree species.

Resprouting species dominated the soil seed bank and standing vegetation of all communities, except for the soil seed bank of the Wet Heath community, where equal numbers of obligate seeders and resprouting species were detected (Table 7). There was a significant interaction detected between community type and stratum for resprouting species, with significantly more detected in the standing vegetation of the fire-prone communities (DSF Scheffe's $P = 0.002$, RO Scheffe's $P = 0.041$), than in the soil seed banks of these communities (Fig. 4; Table 8). A significant interaction between community type and stratum was also detected for obligate seeding species, with more species in the soil seed bank than the standing vegetation of the Rainforest community (Scheffe's $P = 0.024$; Fig. 4).

Table 7. Plant life form (grasses, graminoids, forbs, shrubs, trees and twiners), sprouting ability (resprouters and obligate seeders) and life history characteristics based on total number of species detected in the soil seed bank and standing vegetation of five communities.[†]

	DSF[‡]		RO		WH		WSF		RF	
	S*	V	S	V	S	V	S	V	S	V
Total species richness	35	68	32	65	20	33	47	57	53	55
Grasses	2	5	1	3	1	2	2	4	1	0
Gramionds	4	7	8	11	5	11	5	4	4	1
Forbs	10	11	7	6	9	8	19	19	24	12
Shrubs	19	37	15	42	4	11	11	13	9	17
Trees	0	6	0	2	0	0	5	5	8	15
Twiners	0	2	1	1	1	1	5	12	7	10
Resprouters	22	55	19	44	10	28	25	41	30	44
Obligate seeders	13	13	13	21	10	5	22	16	23	11
Shrubs – Resprouter	14	26	8	23	3	7	3	6	4	14
Shrubs – Obligate seeder	5	11	7	19	1	4	8	7	5	3
Annual	4	0	2	0	4	0	4	1	6	1
Variable	1	0	1	0	2	0	3	3	3	1
Perennial	30	68	29	65	14	33	40	53	44	53

[†] (n = 10), soil seed bank data pooled at the sample size level (transects)

[‡] DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest

*S = soil seed bank, V = standing vegetation

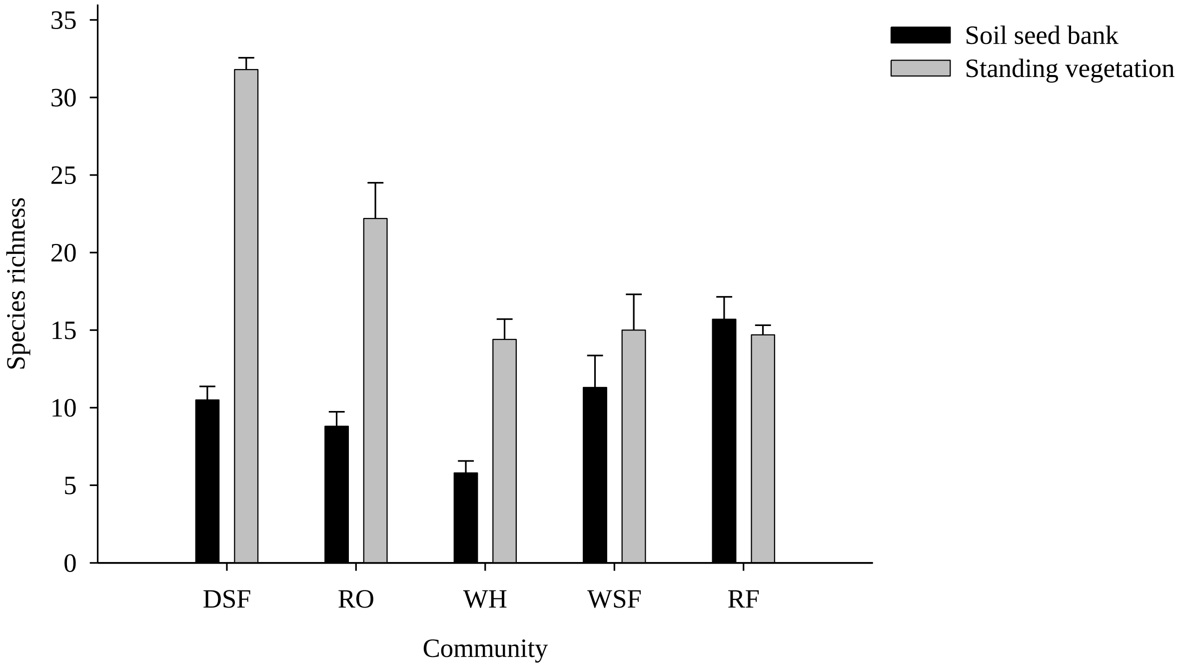

Fig. 3. Mean species richness (n = 10, + s.e.) detected in the soil seed bank and the standing vegetation of five communities. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Table 8. *F*-statistic and *p*-value of the three-way ANOVA for the comparison of (a) richness of seeder and sprouter species, (b) resprouting and (c) obligate seeding species between the soil seed bank and the standing vegetation of five communities.

Factor	d.f.				
		(a) Total species richness			
		F	*P*		
Community	4	3.217	0.116		
Stratum*	1	**58.036**	**0.001**		
Community x Stratum*	4	**10.241**	**0.013**		
Site(community)	5	**6.356**	**<0.001**		
Site(community) x Stratum*	5	2.275	0.055		
		(b) Resprouters		(c) Obligate seeders	
		F	*P*	*F*	*P*
Community	4	4.921	0.055	5.157	0.051
Stratum*	1	**104.87**	**0.002**	2.409	0.181
Community x Stratum*	4	**9.021**	**0.017**	**9.468**	**0.015**
Site(community)	5	**8.430**	**<0.001**	**4.212**	**0.002**
Site(community) x Stratum*	5	**3.214**	**0.011**	1.501	0.199

Significant sources of variation are in bold, *refers to lifecycle stage, i.e. soil seed bank or standing vegetation.

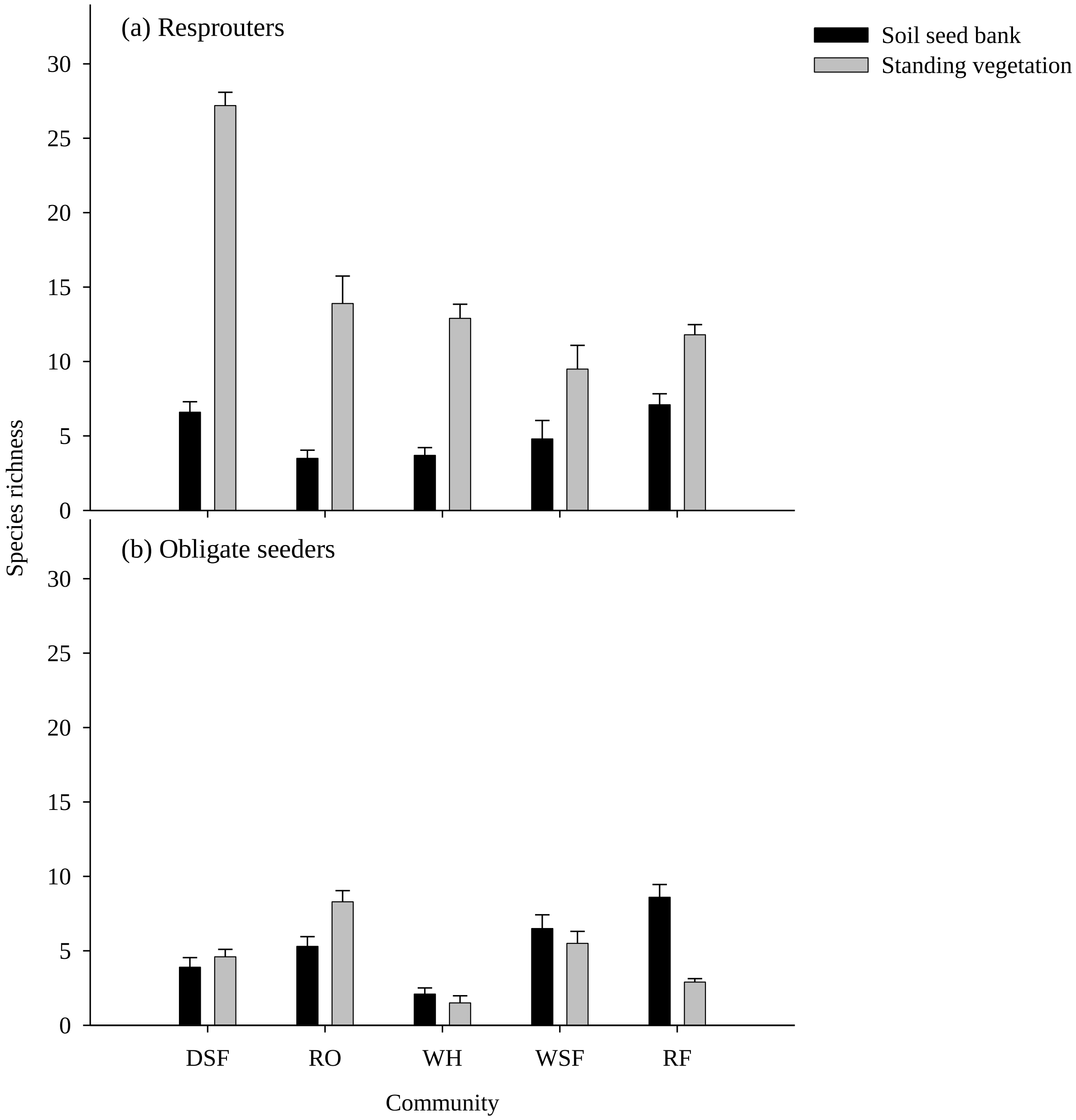

Fig. 4. Mean species richness (n = 10, + s.e.) of (a) resprouters and (b) obligate seeders recorded from the soil seed bank and the standing vegetation of five communities. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Samples clustered separately based on community type. Within all five communities standing vegetation clustered separately from the soil seed bank (Fig. 5). A two-way crossed PERMANOVA found there was a significant interaction between the factors community and stratum (Table 9). Pairwise tests found there were significant differences between and within all the communities based on the similarity of the species composition of the standing vegetation and the soil seed bank (Fig. 5; Table 9).

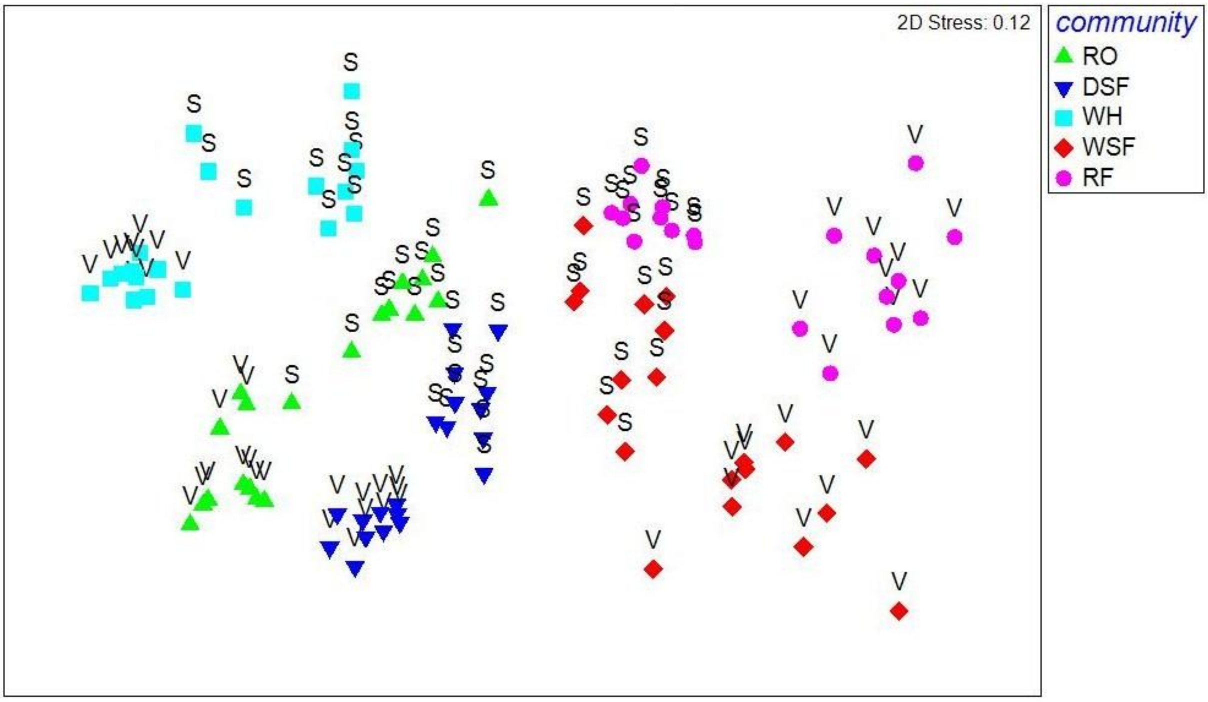

Fig. 5. Ordination of samples (nMDS) in two dimensions recorded from the standing vegetation and the soil seed bank of five communities based on presence/absence data. Resemblance is based on Bray-Curtis similarity index. Stress = 0.12. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest. S = soil seed bank, V = standing vegetation.

Few species contributed up to 50% of the average Bray-Curtis similarity for the soil seed bank and standing vegetation for each community (Table 10), with similarity higher in the standing vegetation than the soil seed bank in every community except the Rainforest (Table 10). Seven species contributed up to 50% of the similarity in the Rocky Outcrop standing vegetation, with *Cassytha glabella* the main contributor, whereas only three species, *Laxmannia gracilis*, *Kunzea bracteolata* and *Gamochaeta spicata*, accounted for the similarity within the soil seed bank of the Rocky Outcrop community (Table 10). In the Dry Sclerophyll Forest community, eleven species contributed up to 50% of the similarity in the standing vegetation, with *Boronia microphylla, Entolasia stricta*, *Hovea heterophylla, Petrophile canescens, Platysace ericoides* contributing the greatest to the overall similarity. Four species, *Gamochaeta spicata, Gamochaeta calviceps, Leptospermum trinervium* and *Boronia microphylla*, were the main contributors to the similarity within the soil seed bank (Table 10). Only four species characterised the composition of the standing vegetation in the Wet Heath community. These included: *Baloskion fimbriatum, Lepidosperma limicola, Lepyrodia anarthria* with *Baeckea omissa* characteristic of both the standing vegetation and the soil seed bank (Table 10). The standing vegetation of the Wet Sclerophyll Forest community contained five characteristic species, with *Goodia lotifolia* and *Geitonoplesium cymosum* contributing the most to the similarity within this group. In the soil seed bank of the Wet Sclerophyll Forest community, three species contributed up to 50% of the similarity, with *Gamochaeta spicata* contributing most to the overall similarity (Table 10). In the Rainforest community, six species characterised the standing vegetation and four species characterised the soil seed bank, with *Ackama paniculosa* contributing the greatest to the overall similarity in both the standing vegetation and the soil seed bank (Table 10).

The average dissimilarity (using SIMPER analysis) between the soil seed bank and the standing vegetation for the five communities ranged from 77.35 to 85.78, indicating similarity was quite low (Table 11). The Wet Sclerophyll Forest community was the least similar (85.78), followed by the Rainforest community (83.93), then the Rocky Outcrop (81.10), Wet Heath (77.76) and Dry Sclerophyll Forest (77.35) communities.

Table 9. PERMANOVA identifying difference in species composition between the soil seed bank and standing vegetation of five communities.

Factors		d.f.	Pseudo-F	*P*
Community		4	23.203	**0.0001**
Stratum		1	23.63	**0.0001**
Community x Stratum		4	6.4918	**0.0001**
Pairwise				
Community x Stratum			T statistic	
Within standing vegetation				
	RO[†] *vs.* DSF		4.386	**0.0002**
	RO *vs.* WH		5.679	**0.0001**
	RO *vs.* WSF		3.887	**0.0001**
	RO *vs.* RF		4.040	**0.0001**
	DSF *vs.* WH		6.807	**0.0001**
	DSF *vs.* WSF		4.424	**0.0001**
	DSF *vs.* RF		4.628	**0.0001**
	WH *vs* WSF		4.650	**0.0002**
	WH *vs.* RF		4.829	**0.0002**
	WSF *vs.* RF		2.698	**0.0001**
Within soil seed bank				
	RO *vs.* DSF		2.727	**0.0001**
	RO *vs.* WH		3.612	**0.0001**
	RO *vs.* WSF		2.758	**0.0002**
	RO *vs.* RF		3.718	**0.0001**
	DSF *vs.* WH		3.647	**0.0001**
	DSF *vs.* WSF		2.810	**0.0001**
	DSF *vs.* RF		3.808	**0.0001**
	WH *vs.* WSF		3.314	**0.0001**
	WH *vs.* RF		4.340	**0.0001**
	WSF *vs.* RF		2.295	**0.0002**
Standing vegetation vs. soil seed bank				
	DSF		3.680	**0.0001**
	RO		3.152	**0.0001**
	WH		3.898	**0.0001**
	WSF		2.327	**0.0001**
	RF		3.147	**0.0001**

Significant values in bold. [†]DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Eighteen species contributed to the dissimilarity between the standing vegetation and the soil seed bank in the Rocky Outcrop community. Differences could be attributed to the absence of six species: *Grevillea acerata, Allocasuarina rigida, Dampiera stricta, Callitris monticola Lepidosperma laterale* and *Acacia suaveolens* from the soil seed bank; and three species: *Gamochaeta spicata*, *Conyza sumatrensis* and *Gamochaeta calviceps* from the standing vegetation (Table 11). In the Dry Sclerophyll Forest community, twenty-three species contributed to the dissimilarity between the standing vegetation and soil seed bank, with thirteen species: *Petrophile canescens*, *Platysace ericoides, Dampiera stricta, Goodenia hederacea* subsp. *hederacea, Lomandra filiformis, Leptospermum minutifolium, Eucalyptus ligustrina, Persoonia media, Schizaea bifida, Haemodorum planifolium, Hakea laevipes* subsp. *graniticola, Dianella caerulea* and *Banksia marginata* absent from the soil seed bank, and two species: *G. spicata* and *G. calviceps* absent from the standing vegetation (Table 11). Nine species contributed to the dissimilarity in the Wet Heath community, with *Baloskion fimbriatum, Lepidosperma limicola, Lepyrodia anarthria, Ptilothrix deusta, Amphipogon strictus, Blandfordia grandiflora* and *Xyris operculata* absent from the soil seed bank only (Table 11). Twenty species contributed to the dissimilarity in the Wet Sclerophyll Forest community, with six species: *Solanum densevestitum, Hibbertia scandens* var. *glabra, Eucalyptus obliqua, Acacia irrorata* subsp. *irrorata, Calochlaena dubia* and *Eucalyptus campanulata* absent from the soil seed bank, and three species: *G. spicata*, *C. sumatrensis* and *Oxalis exilis* absent from the standing vegetation (Table 11). In the Rainforest community, twenty-one species contributed to the difference between the standing vegetation and the soil seed bank, with nine species: *Blechnum cartilagineum, C. dubia, Palmeria scandens, Anopterrus macleayanus, Ceratopetalum apetalum, Acmena smithii, Doryphora sassafras, Rubus nebulosus* and *E. campanulata* absent from the soil seed bank; four species: *Solanum aviculare, G. spicata, Berberidopsis bechlei* and *C. sumatrensis* were absent from the standing vegetation (Table 11).

Sampling strategy

The number and size of samples did not significantly affect the species richness and abundance of seedlings germinating from the soil seed bank among the five communities (Fig 6; Table 5). Sampling strategy also did not significantly affect the richness, abundance and proportion of species emerging from the soil seed banks of the five communities within each fire response category (Table 6).

Variation within samples were quite large, especially for the abundance of individuals emerging from the soil seed banks of the Rocky Outcrop, Wet Heath and Rainforest communities (Figs. 1, 6). Site was found to be significant for different variables, indicating the patchiness of the soil seed bank across sites which may have contributed to non-significant results (Tables 5, 6, 8).

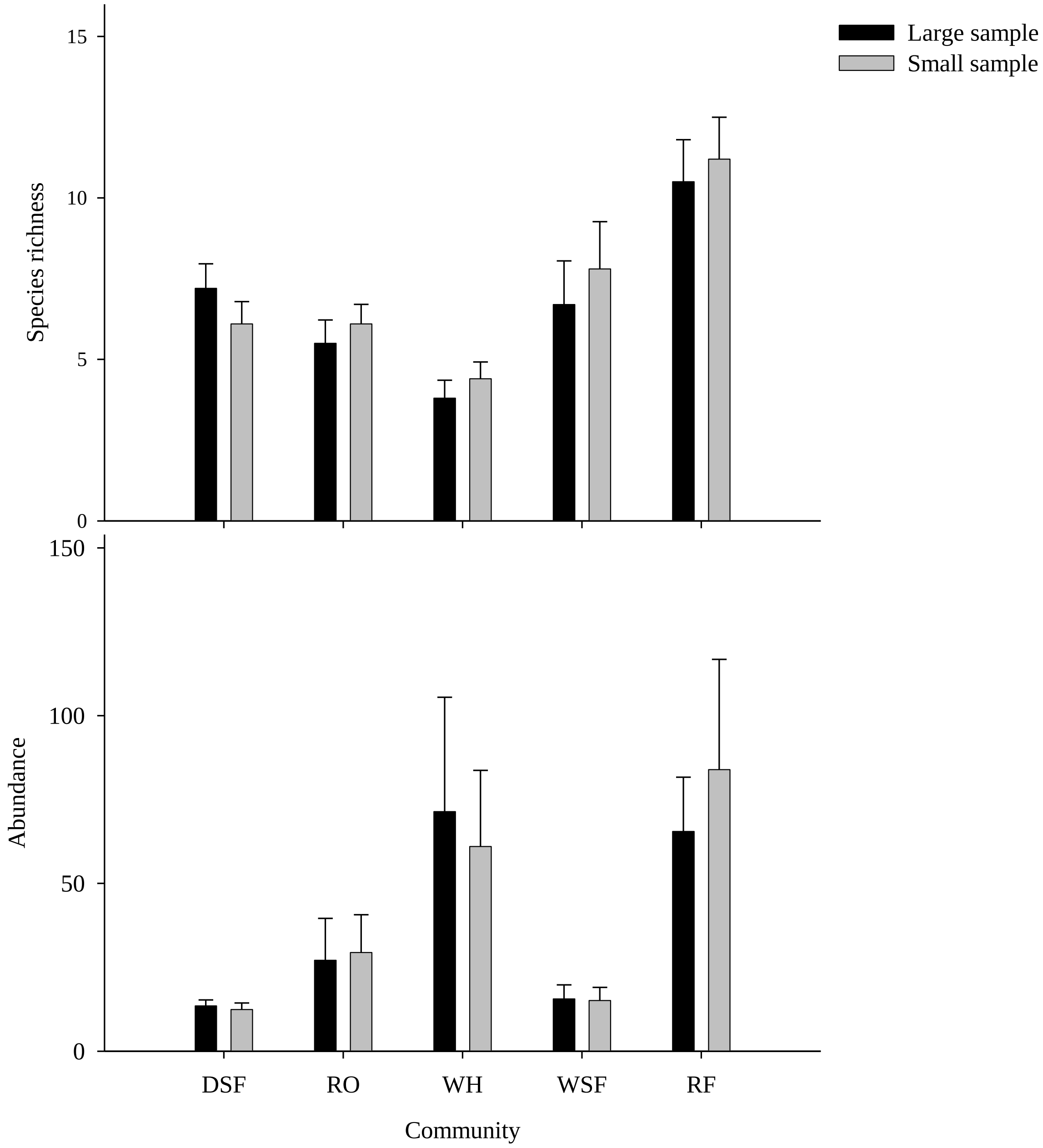

Fig. 6. Sampling the soil seed bank: the (a) mean species richness (+ s.e.) and the (b) abundance of individuals (+ s.e.) measured in the large and small pooled samples from the soil seed bank of five communities. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Table 10. Species contributing up to 50% of the average Bray-Curtis similarity in the soil seed bank and standing vegetation of five communities.

Community	Fire response[†]	Species	Average abundance	Contribution (%)	Cumulative percentage
Dry Sclerophyll Forest					
	Standing vegetation	Average similarity = 60.48			
	R	*Boronia microphylla*	1	5.21	5.21
	R	*Entolasia stricta*	1	5.21	10.42
	R	*Hovea heterophylla*	1	5.21	15.63
	R	*Petrophile canescens*	1	5.21	20.84
	R	*Platysace ericoides*	1	5.21	26.06
	R	*Dampiera stricta*	0.9	4.23	30.28
	R	*Monotoca scoparia*	0.9	4.17	34.46
	OS	*Dillwynia phylicoides*	0.9	4.14	38.6
	R	*Bossiaea scortechinii*	0.9	4.14	42.74
	R	*Austrostipa rudis*	0.9	4.10	46.84
	R	*Aotus subglauca* var. *subglauca*	0.8	3.24	50.07
	Soil seed bank	Average similarity = 40.31			
	OS	*Gamochaeta spicata*	0.8	14.79	14.79
	OS	*Gamochaeta calviceps*	0.7	11.69	26.48
	R	*Leptospermum trinervium*	0.7	11.01	37.49
	R	*Boronia microphylla*	0.7	10.67	48.16
Rocky Outcrop					
	Standing vegetation	Average similarity = 46.26			
	OS	*Cassytha glabella*	1	10.24	10.24
	R	*Leptospermum novae-angliae*	0.9	8.42	18.66
	R	*Grevillea acerata*	0.9	7.57	26.22
	OS	*Allocasuarina rigida*	0.8	6.20	32.43
	R	*Dampiera stricta*	0.8	6.15	38.58
	OS	*Acacia venulosa*	0.8	6.05	44.63
	OS	*Callitris monticola*	0.8	5.98	50.61
	Soil Seed bank	Average similarity = 38.31			
	R	*Laxmannia gracilis*	0.8	18.01	18.01
	R	*Kunzea bracteolata*	0.8	17.30	35.31
	OS	*Gamochaeta spicata*	0.7	15.49	50.8

Table 10. Species contributing to the Bray-Curtis similarity cont.

Community	Fire response	Species	Average abundance	Contribution (%)	Cumulative percentage
Wet Heath					
	Standing vegetation	Average similarity = 64.71			
	R	*Baeckea omissa*	1	11.14	11.14
	R	*Baloskion fimbriatum*	1	11.14	22.27
	R	*Lepidosperma limicola*	1	11.14	33.41
	R	*Lepyrodia anarthria*	1	11.14	44.55
	Soil seed bank	Average similarity = 41.31			
	R	*Baeckea omissa*	1	45.10	45.10
Wet Sclerophyll Forest					
	Standing vegetation	Average similarity = 31.56			
	OS	*Goodia lotifolia*	0.9	17.05	17.05
	R	*Geitonoplesium cymosum*	0.7	11.37	28.42
	OS	*Gonocarpus oreophilus*	0.5	7.43	35.85
	OS	*Solanum densevestitum*	0.5	7.43	43.29
	R	*Hibbertia scandens* var. *glabra*	0.6	6.48	49.76
	Soil seed bank	Average similarity = 28.75			
	OS	*Gamochaeta spicata*	0.8	24.37	24.37
	OS	*Goodia lotifolia*	0.6	11.15	35.53
	R	*Rubus rosifolius*	0.6	10.96	46.49
Rainforest					
	Standing vegetation	Average similarity = 32.95			
	R	*Ackama paniculosa*	0.8	13.02	13.02
	R	*Blechnum cartilagineum*	0.6	7.05	20.06
	R	*Calochlaena dubia*	0.6	6.96	27.02
	R	*Palmeria scandens*	0.6	6.79	33.81
	R	*Hydrocotyle pedicellosa*	0.6	6.47	40.29
	R	*Lobelia trigonocaulis*	0.6	6.47	46.76
	Soil seed bank	Average similarity = 48.50			
	R	*Ackama paniculosa*	1	13.66	13.66
	OS	*Solanum aviculare*	1	13.66	27.32
	OS	*Gamochaeta spicata*	0.9	11.34	38.66
	R	*Acacia melanoxylon*	0.9	11.07	49.73

[†] Fire response refers to R = Resprouter, OS = Obligate seeder (NSWFFRD 2002; Clarke *et al.* 2009)

Table 11. Species contributing up to 50% of the average Bray-Curtis dissimilarity in the soil seed bank and standing vegetation of five communities.

Community	Fire response[†]	Species	Standing vegetation Average abundance	Soil seed bank Average abundance	Contribution (%)	Cumulative percentage
Dry Sclerophyll Forest		Average dissimilarity =77.35				
	R	*Petrophile canescens*	1	0	3.08	3.08
	R	*Platysace ericoides*	1	0	3.08	6.15
	R	*Hovea heterophylla*	1	0.1	2.81	8.96
	R	*Dampiera stricta*	0.9	0	2.80	11.76
	R	*Monotoca scoparia*	0.9	0.1	2.55	14.31
	R	*Goodenia hederacea* subsp. *hederacea*	0.8	0	2.46	16.77
	OS	*Gamochaeta spicata*	0	0.8	2.45	19.22
	R	*Lomandra filiformis*	0.8	0	2.41	21.63
	R	*Austrostipa rudis*	0.9	0.2	2.24	23.87
	OS	*Gamochaeta calviceps*	0	0.7	2.17	26.04
	R	*Leptospermum minutifolium*	0.7	0	2.16	28.20
	R	*Eucalyptus ligustrina*	0.7	0	2.15	30.35
	OS	*Persoonia media*	0.7	0	2.15	32.51
	R	*Melichrus procumbens*	0.7	0.1	2.05	34.55
	R	*Boronia algida*	0.7	0.1	2.01	36.56
	R	*Schizaea bifida*	0.6	0	1.85	38.42
	R	*Haemodorum planifolium*	0.6	0	1.85	40.27
	R	*Hakea laevipes* subsp. *graniticola*	0.6	0	1.83	42.10
	R	*Dianella caerulea*	0.6	0	1.81	43.90
	OS	*Banksia marginata*	0.6	0	1.80	45.70
	R	*Goodenia bellidifolia* subsp. *argentea*	0.4	0.6	1.60	47.30
	OS	*Dillwynia phylicoides*	0.9	0.5	1.58	48.88
	R	*Entolasia stricta*	1	0.5	1.57	50.46
Rocky Outcrop		Average dissimilarity = 81.10				
	OS	*Cassytha glabella*	1	0.1	3.91	3.91
	R	*Grevillea acerata*	0.9	0	3.60	7.51
	OS	*Allocasuarina rigida*	0.8	0	3.36	10.87
	R	*Dampiera stricta*	0.8	0	3.36	14.22

Table. 11. Species contributing to the Bray-Curtis dissimilarity cont.

Community	Fire response	Species	Standing vegetation Average abundance	Soil seed bank Average abundance	Contribution (%)	Cumulative percentage
	R	*Leptospermum novae-angliae*	0.9	0.2	3.30	17.52
	OS	*Callitris monticola*	0.8	0	3.24	20.76
	OS	*Gamochaeta spicata*	0	0.7	3.04	23.81
	OS	*Boronia anethifolia*	0.6	0.1	2.77	26.58
	OS	*Conyza sumatrensis*	0	0.6	2.64	29.22
	OS	*Acacia venulosa*	0.8	0.3	2.63	31.85
	R	*Patersonia glabrata*	0.7	0.1	2.42	34.27
	OS	*Gamochaeta calviceps*	0	0.6	2.42	36.69
	R	*Kunzea bracteolata*	0.3	0.8	2.37	39.06
	R	*Leptospermum trinervium*	0.7	0.4	2.24	41.30
	R	*Lepidosperma laterale*	0.5	0	2.20	43.50
	OS	*Leucopogon microphyllus* var. *pilibundus*	0.7	0.4	2.20	45.70
	OS	*Acacia suaveolens*	0.6	0	2.11	47.81
	R	*Caustis flexuosa*	0.6	0.1	2.10	49.91
Wet Heath		Average dissimilarity = 77.76				
	R	*Baloskion fimbriatum*	1	0	6.75	6.75
	R	*Lepidosperma limicola*	1	0	6.75	13.51
	R	*Lepyrodia anarthria*	1	0	6.75	20.26
	R	*Ptilothrix deusta*	1	0	6.75	27.02
	R	*Amphipogon strictus*	0.9	0	6.21	33.23
	R	*Blandfordia grandiflora*	0.9	0	6.21	39.44
	R	*Xyris operculata*	0.6	0	4.39	43.83
	R	*Drosera spatulata*	0.5	0.7	3.57	47.33
	R	*Empodisma minus*	0.8	0.5	3.44	50.78
Wet Sclerophyll Forest		Average dissimilarity = 85.78				
	OS	*Gamochaeta spicata*	0	0.8	4.29	4.29
	R	*Geitonoplesium cymosum*	0.7	0.1	3.51	7.79
	OS	*Solanum densevestitum*	0.5	0	3.11	10.91
	R	*Rubus rosifolius*	0.1	0.6	2.92	13.83
	R	*Hibbertia scandens* var. *glabra*	0.6	0	2.74	16.57
	R	*Hydrocotyle peduncularis*	0.3	0.6	2.63	19.20
	OS	*Conyza sumatrensis*	0	0.5	2.61	21.81
	OS	*Gonocarpus oreophilus*	0.5	0.5	2.59	24.40

Table. 11. Species contributing to the Bray-Curtis dissimilarity cont.

Community	Fire response	Species	Standing vegetation Average abundance	Soil seed bank Average abundance	Contribution (%)	Cumulative percentage
	R	*Eucalyptus obliqua*	0.4	0	2.49	26.89
	OS	*Viola hederacea*	0.6	0.3	2.48	29.37
	OS	*Goodia lotifolia*	0.9	0.6	2.27	31.64
	OS	*Acacia irrorata* subsp. *irrorata*	0.5	0	2.05	33.69
	R	*Calochlaena dubia*	0.5	0	2.05	35.74
	R	*Eucalyptus campanulata*	0.5	0	2.05	37.79
	R	*Oxalis exilis*	0	0.5	2.05	39.83
	OS	*Zieria* sp. aff. *smithii*	0.5	0.2	2.04	41.88
	R	*Glycine clandestina*	0.4	0.2	1.96	43.83
	R	*Billardiera scandens*	0.4	0.1	1.87	45.70
	OS	*Ozothamnus rufescens*	0.2	0.3	1.86	47.56
	OS	*Astrotricha latifolia*	0.4	0.1	1.78	49.35
Rainforest		Average dissimilarity = 83.93				
	OS	*Solanum aviculare*	0	1	4.03	4.03
	OS	*Gamochaeta spicata*	0	0.9	3.69	7.72
	OS	*Berberidopsis bechlei*	0	0.9	3.48	11.20
	R	*Acacia melanoxylon*	0.1	0.9	3.34	14.54
	R	*Rubus rosifolius*	0.1	0.8	2.84	17.38
	R	*Blechnum cartilagineum*	0.6	0	2.44	19.82
	R	*Calochlaena dubia*	0.6	0	2.42	22.24
	R	*Palmeria scandens*	0.6	0	2.39	24.63
	OS	*Conyza sumatrensis*	0	0.6	2.38	27.02
	R	*Callicoma serratifolia*	0.2	0.5	2.15	29.17
	OS	*Anopterus macleayanus*	0.5	0	2.09	31.26
	R	*Ceratopetalum apetalum*	0.5	0	2.09	33.36
	R	*Acmena smithii*	0.5	0	2.06	35.42
	R	*Doryphora sassafras*	0.5	0	2.03	37.45
	R	*Aneilema acuminata*	0.5	0.5	2.00	39.44
	R	*Lobelia trigonocaulis*	0.6	0.6	1.98	41.42
	R	*Hydrocotyle pedicellosa*	0.6	0.6	1.97	43.40
	R	*Rubus nebulosus*	0.5	0	1.95	45.35
	R	*Schizomeria ovata*	0.5	0.1	1.94	49.23
	R	*Eucalyptus campanulata*	0.5	0	1.95	47.30
	OS	*Solanum vicinum*	0.3	0.3	1.73	50.96

[†] Fire response refers to R = Resprouter, OS = Obligate seeder (NSWFFRD 2002; Clarke *et al.* 2009)

Discussion

Soil seed bank characteristics

The estimated seed density of the soil seed bank ranged from 145–834 seeds/m^2, with the soil seed banks of the Rainforest and Wet Heath communities having the greatest seed density and the Dry Sclerophyll Forest community having the least (Table 3): this is a similar trend to other studies (Table 1). The high seed density in the Wet Heath community can be attributed to four species (*Baeckea omissa*, *Epacris obtusifolia*, *Drosera spatulata* and *E. gunni*), with their combined abundance representing 95% of the total abundance in the soil seed bank, whereas graminoid species represented only 2% of the total abundance, despite their characterising the standing vegetation of this community (Table 10). The high seed density in the Rainforest community can be attributed to three species (*Ackama paniculata*, *Hydrocotyle pedicellosa* and *Callicoma serratifolia*) whose combined abundance represented 75% of the total abundance in the soil seed bank. The mean and total abundance of seedlings had a very similar trend, with more seedlings recorded in the Rainforest and the least number recorded from the Dry Sclerophyll Forest community (Fig. 1; Table 3). Although not a significant trend (Table 5), it is not clear whether this pattern is a reflection of the abundance of seed in the different communities, or an artefact of the way samples were processed. In the fire-prone communities, for example, seed of some species require complex dormancy breaking cues to promote seed germination (Auld and O'Connell 1991; Keeley and Fotheringham 1998; Thomas *et al.* 2003; Ooi 2007; Thomas *et al.* 2007), whereas the seed of species in closed canopy communities, similar to the Rainforest community studied here, respond to increased light on the forest floor when gaps in the canopy are created (Garwood 1989). When samples were initially placed in the glasshouse without any fire-related stimulation, such as heat or smoke, the seed in the Rainforest samples are more likely to have germinated due to the greater availability of light. This is in contrast to the seed of species in the Dry Sclerophyll Forest community, which may require fire-related germination cues to break seed dormancy or promote germination (Enright and Kintrup 2001; Thomas *et al.* 2003; Ooi 2007). In some communities where fire is rare, seed of some species are known to be killed by fire; the addition of fire cues such as heat and smoke to soil samples may not have promoted germination of specific species (Hopkins and Graham 1984; Tang *et al.* 2003). An alternative to using the emergence technique to assess the soil seed bank would be to use a flotation method, where soil is suspended in a saturated salt solution and organic particles are filtered from the soil (Malone 1967; Brown 1992; De Villiers *et al.* 1994; Ishikawa-Goto and Tsuyuzaki 2004). The major advantage of this technique is that it does not rely on breaking seed dormancy or specific germination requirements to estimate the size of the seed bank. A major disadvantage of this technique, however, is that accuracy may be low for small or cryptically coloured species, and the presence of unviable seed overestimates the size of the seed bank. It is also extremely laborious and probably impractical for large-scale studies, such as this one which involved sampling over 200 species across five different plant communities (Brown 1992; De Villiers *et al.* 1994; Ishikawa-Goto and Tsuyuzaki 2004). It is likely the use of the emergence technique with fire-related cues may have favoured some species but not others, however, applying the heat and smoke treatments 10 weeks after the initial glasshouse treatment would have enabled those species usually inhibited by fire-cues to germinate.

Soil seed banks among the communities also differed in species richness and abundance of some, but not all, functional groups. Perennial species were the dominant life history category in the soil seed bank across all communities (Table 4). Generally, graminoids, forbs and shrubs were the most abundant and speciose life forms within the fire-prone communities (Table 4: DSF, RO, WH), whereas forbs, shrubs and trees were the most abundant and speciose life forms within the mesic communities (Table 4: WSF, RF). Tree and twiner species were more speciose and abundant in the Rainforest community than in the fire-prone communities (DSF, RO, WH). This result is not unexpected as tree species are generally not a typical component of either the Rocky Outcrop or Wet Heath communities, and eucalypt species, which dominate the canopy of the Dry Sclerophyll Forest community, lack a soil-stored seed bank (Wang 1997). Twiner species tended to dominate the mesic (Table 4: WSF, RF), but not the fire-prone communities (Table 4: DSF, RO, WH). Differences in shrub abundance were recorded between the communities, with the Rainforest community exhibiting the least number of individual shrubs compared to the other communities (Table 4). These patterns of abundance of life history and life form are similar to those of other studies that have measured soil seed banks at a community level, the soil seed banks of communities generally reflecting the life form trends of the standing vegetation (Zammit and Zedler 1988; Thomas 1994; Enright and Kintrup 2001; Hill and French 2003; Hewitt 2007).

Resprouters were more abundant than obligate seeders across all communities, except the Wet Sclerophyll Forest (Table 4). Resprouting shrubs were most abundant in the fire-prone communities (Table 4: DSF, RO, WH) and the least abundant in the mesic communities (Table 4: WSF, RF), indicating communities that experience fire on a more regular basis are not only dominated by resprouting shrubs, but also have a soil seed bank containing these shrub species. When overall proportions of resprouters and obligate seeders were examined among communities, there were clear trends between communities based on fire frequency. Resprouters were more abundant in the fire-prone communities (Fig. 2b: DSF, RO, WH) and obligate seeders more abundant in the mesic communities (Fig. 2b: WSF, RF). This result is exemplified by the two communities where significant differences were detected – the percentage of resprouting species were greater in the Wet Heath community, whereas the proportion of obligate seeders were greater in the Wet Sclerophyll Forest community (Fig. 2; Table 6). This indicates fire frequency influences the abundance of individuals with different sprouting abilities in the soil seed bank based.

Comparison between the soil seed bank and the standing vegetation

There were clear differences between number and composition of species recorded in the standing vegetation and the soil seed bank of all communities (Figs. 3–5; Tables 8, 11). The main reasons for this disparity were: first, more species sampled in the standing vegetation than in the soil seed bank, with over half of all the species sampled in this study unique to the standing vegetation; and second, the number of species with different sprouting abilities recorded in both the standing vegetation and the soil seed banks (Fig. 4; Table 8).

More species were recorded in the standing vegetation than the soil seed bank of all communities except the Rainforest community, with both the Dry Sclerophyll Forest and Rocky Outcrop communities showing a significant trend (Fig. 3; Table 8). Differences occurred in the composition of species sampled in the soil seed bank and standing vegetation of each community (Fig. 5; Table 9). There are two main factors contributing to this pattern. First, few species occurred in more than one community, as indicated by distinct clustered patterns of samples from each community (Fig. 5). The exception to this were the soil seed banks of the mesic communities (WSF, RF), where clustering of samples suggests there are some species common to both. Second, very few species contributed to the similarity within the standing vegetation and the soil seed bank of each community. Average dissimilarity ranged from 77.35 to 85.78, indicating similarity within and between the standing vegetation and the soil seed bank is quite low (Table 10). This is also clearly indicated by the clustering of strata for each community (Fig.5). The high dissimilarity can also be attributed to the large number of species unique to either the standing vegetation or the soil seed bank of each community (Table 11). This, combined with the fact that there was a significant site effect within communities (Tables 5, 6 and 8), may indicate a high level of spatial variability across the study area.

Generally, more species with an annual life history were present in the soil seed bank, but absent or in low numbers in the standing vegetation (Table 7). Three short-lived species

(*Conyza sumatrensis*, *Gamochaeta calviceps, G. spicata*) were missing from the standing vegetation of the Dry Sclerophyll Forest, Rocky Outcrop, and Wet Sclerophyll Forest communities. Although little is known about their seed storage mechanisms, it is probable these species have persistent soil seed banks. Their absence from the standing vegetation and presence in the seed bank is not unexpected due to their annual life history strategy. Of those species contributing to the dissimilarity between the standing vegetation and the soil seed bank in the Wet Heath community, no species were absent from the standing vegetation, whereas in the Rainforest community four species were missing from the standing vegetation but present in the soil seed bank. Two species (*C. sumatrensis*, *G. spicata*) have already been discussed above; the remaining two (*Berberidopsis beckleri*, *Solanum aviculare*) are native obligate seeding species, probably with persistent seed banks. Their lack of detection in the standing vegetation could be attributed to short-lived plants in the case of one species (*Solanum aviculare*) and possibly the cryptic nature due to its habit (canopy vine) of *Berberidopsis beckleri*.

In the fire-prone communities (DSF, RO, WH), similarity in composition between the standing vegetation and the soil seed bank was quite low, ranging from 77.35 to 81.10 (Table 11). The major contributing factor was the absence of species from the soil seed banks of these communities. For some species the nature of their seed storage is an important determining factor for their absence from the seed bank. For example, *Allocasurina rigida, Banksia marginata,* and *Callitris monticola* are known to have canopy-held seed banks. Missing canopy-held species from the soil seed bank is not unexpected since the release of canopy-held seed may be timed for an immediately post-fire environment (Bradstock and Myerscough 1981; Lamont *et al.* 1991). In the fire-prone communities, six species with known canopy-held seed storage were missing from the soil seed bank (Table 11). In contrast, other species missing from the seed bank are known to have a persistent soil-stored seed bank. Missing species with a persistent soil seed bank may be indicative of uneven distribution of the seed of some species in the soil. For example, *Acacia suaveolens* is known to be ant dispersed with seeds clustered in ant nests (Auld 1986). If ant nest distributions are at larger scales than soil sampling, sampling may miss ant nests and so the seeds. Sampling suggest this species is absent from the soil seed bank, when its absence may be an artefact of sampling; given *Acacia suaveolens* has a primary juvenile period of three years, this is a likely conclusion (Clarke *et al.* 2009). Other reasons for this pattern could be the lack of response to germination cues, i.e. inappropriate cues and/or failure to break seed dormancy even though seeds may be present in the soil. I suspect this is the case for ten species – these species were recorded in experiments reported in Chapter 2, where soil sampled at the same time was used to determine the response of seeds of species in the soil seed bank to heating. This work indicated some species in the fire-prone communities require higher temperatures, not achieved in this work, to break seed dormancy. Some species (*Dianella caerulea*, *Schizaea bifida*) have a transient seed bank and detection is unlikely due to the transient nature of seed in the soil. For one species (*Haemodorum planifolium*) nothing is known of its seed storage mechanism.

In the Wet Heath community, similarity within and between the standing vegetation and the soil seed bank was quite low, with very few species contributing to both (Tables 10, 11). This dissimilarity was mainly due to resprouting graminoid species and of the seven species absent from the soil seed bank, five (*Baloskion fimbriatum*, *Lepidosperma limicola*, *Lepyrodia anarthria*, *Ptilothrix duesta* and *Xyris opercularia*), have a known persistent soil storage mechanism. Their absence from the soil seed bank may be a result of a trade-off between the allocation of resources to clonal growth rather than seed production, despite their known seed storage.

The mesic communities had the highest dissimilarity between the standing vegetation and soil seed bank compared to the fire-prone communities (Table 11: WSF, RF). Very few species contributed to the similarity within both the standing vegetation and the soil seed bank of each community (Table 10), suggesting each group was quite spatially variable. This can be seen in the ordination analysis where the mesic communities lack the tight clustering that is seen in the other communities (Fig. 5). As with the fire-prone communities, the other contributing factor of the high dissimilarity between the standing vegetation and the soil seed bank in the mesic communities was the absence of species from the seed bank (Table 11). Seed storage (*Eucalyptus* species), the transient nature of seeds (*Acmena smithii, Calochlaena dubia*) and possible lack of appropriate dormancy breaking cues (*Acacia irrorata* subsp. *irrorata*) are all possible explanations for the absence of some species, whereas little is known about the type of seed banks of the five remaining species absent from the seed bank. Primary canopy species may have a limited persistence in the soil seed bank due to reduced seed viability over time, lack of seed dormancy, or the presence of a 'seedling' bank which enables these species to take advantage of favourable condition to regenerate (Hopkins and Graham 1987).

Communities subjected to fewer disturbance events have low similarity between standing vegetation and soil seed banks (Thompson and Grime 1979; Bossuyt and Honnay 2008). This pattern of dissimilarity is reinforced in the present study, where across all communities there was little similarity between the two strata. The trend here seemed to be the mesic communities (WSF, RF) having the greatest dissimilarity; historically these communities are subjected to disturbance (in this context fire) less frequently than the fire-prone communities (DSF, WH). Interestingly, the Rocky Outcrop community appeared to be an exception to the trend: although this community was dominated by sclerophyll (i.e. fire-prone) vegetation (as with DSF and WH) the high dissimilarity between the standing vegetation and the soil seed bank suggests that Rocky Outcrop is also subject to fewer disturbance events. It may act as a refuge from fire on occasion because of low fuel continuity.

Sampling strategy

The two sampling strategies used in this study, to determine species richness and abundance of seed, ensures the size and number of samples needed is the same for all communities. Due to the heterogeneous nature of soil seed banks, associated with seed rain and dispersal and thus seed distribution and seed density within the soil, the collection of many smaller soil samples, rather than fewer larger samples, has been widely reported as the most accurate method to sample soil seed banks for number of species and seed densities (Roberts 1981; Simpson *et al.* 1989). Although more species were sampled in the smaller sample unit (small pooled samples) across all communities, except Dry Sclerophyll Forest (Fig. 6), the results were not significantly different (Table 5). This suggests this range of sample sizes does not influence the number of species or the abundance of seeds sampled in the five plant communities for this particular study area.

For species with different sprouting abilities (resprouters versus obligate seeders), there was no effect of sample unit for the number and abundance of species sampled in each fire response category (Table 6). The proportions of species in the fire response categories also were not significant (Table 6); this then does not support the generally held view that using many smaller samples rather than fewer large samples, more of the soil seed bank is sampled (Roberts 1981).

Some studies have found precision (sampling variance) was improved by collecting larger numbers of smaller samples. However, these studies have focused on target species, native (Bigwood and Inouye 1988) or exotic (Benoit *et al.* 1989; Dessaint *et al.* 1996), to improve their sampling methodology. The difficulty with sampling the soil seed bank at a community level and across five compositionally different communities (Fig. 5) is finding a sampling method that does not bias any one species because of particular seed traits. The general trend for seed banks in Mediterranean communities is that small-seeded species have large seed banks whereas large-seeded species have small seed banks; the possible exception is members of the Fabaceae family, in particular *Acacia* species, in Australia (Parker and Kelly 1989). The other difficulty in sampling the soil seed bank at a community level lies in distinguishing whether species have a transient or persistent soil-stored seed bank. Persistent seed banks are generally characterised by very small, light-weight seeds, especially weedy ephemeral species (Harper 1977; Thompson and Grime 1979), although in Australia many taxa have been suggested to have persistent soil seed banks (Auld *et al.* 2000).

The aim of this work was not to determine primarily how to sample the whole seed bank; this aim would be impractical due to the nature of the seeds and the seed characteristics of different communities, and to time and space limitations. Using different sized pooled samples did not have an effect on the number or abundance of species recorded from the soil seed bank across five contrasting communities. Nor did the size of the sampling unit influence the number or abundance of species with different sprouting abilities detected in the soil seed bank of the five communities. The number of samples used in any soil seed bank study depends on the precision required in the study, the practical constraints of available greenhouse space and the time taken to process the samples (Dessaint *et al.* 1996). Given that overall there were no differences between sampling units, it was therefore more practical to use the larger sized samples in further seed bank work in the broader study as larger samples require less time to collect and process compared with smaller sized ones.

This study examined the most effective way to sample the soil seed bank, described the characteristics of the soil seed bank, and compared the soil seed bank and standing vegetation of five contrasting communities. This study therefore established the sampling methodology for the other seed bank studies within the thesis. As this thesis examines soil seed bank fire responses of a diverse range of plant communities within a landscape scale context, getting the sampling correct is crucial. It is also important to the thesis as a whole to initially describe the general characteristics of the soil seed bank of each community and to make direct comparisons between the standing vegetation and the soil seed bank. The establishment of ideas on within and between community patterns of variation between the standing vegetation and the soil seed bank need to be set in place before direct relationships of the impacts of fire severity can be explored.

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Chapter 2. The effect of heating on the soil seed banks of five different plant communities.

Introduction

The persistence of plant species in disturbance-prone habitats is often dependent upon soil-stored seed banks. Soil seed banks buffer plant populations against environmental variability and are important in reducing the extinction risk of species (Parker *et al.* 1989; Thompson 2000). Habitats subjected to frequent disturbance regimes are characterised by species which generally produce long-lived seeds in the soil seed bank, while in contrast, habitats that are considered stable, with minimal environmental variability, are generally represented by species with seeds that are short-lived in the soil seed bank (Thompson *et al.* 1997; Bossuyt and Honnay 2008a). In fire-prone habitats, soil seed banks are important for the regeneration of species killed by fire as these species rely on seed in the soil to re-establish new individuals following such events (Auld 1986a; Parker and Kelly 1989; Måren and Vandvik 2009).

Fire can trigger the germination of species from the soil seed bank through the influence of factors such as heat (Auld and O'Connell 1991; Hanley *et al.* 2001; Gashaw and Michelsen 2002; Delgado *et al.* 2008) and smoke (Brown 1993; Dixon *et al.* 1995; Keeley and Fotheringham 1998). In natural populations of plants, both heat and smoke will interact during or after the passage of fire; this is highlighted in the finding of significant interactions between heat and smoke (Kenny 2000; Thomas *et al.* 2003; Clarke and French 2005; Thomas *et al.* 2007).

The impacts of fire-cues on the regeneration of species from the soil seed bank have been studied in detail for specific species (Auld and O'Connell 1991; Herranz *et al.* 1998; Kenny 2000; Morris 2000; Thomas *et al.* 2003; 2007; Delgado *et al.* 2008) and for many plant communities (Keeley 1991; Izhaki *et al.* 2000; Read *et al.* 2000; Wills and Read 2002; Tang *et al.* 2003; Penman *et al.* 2008). In many plant communities a number of species respond to the heating effects of fire or other germination-promoting fire-cues such as smoke, charred wood and ash (Brown 1993; Dixon *et al.* 1995; Thanos and Rundel 1995; Keeley and Fotheringham 1998). For other species, germination occurs in the absence of fire-related cues (Keeley 1991; Overbeck *et al.* 2006) or the role of fire cues in promoting germination is poorly known (Ooi 2007).

Different plant communities are likely to respond differently to heating during a fire. Species in fire-prone communities which experience regular fire events would be expected to respond positively to heat, smoke or a combination of these two factors (Enright *et al.* 1997; Read *et al.* 2000; Thomas *et al.* 2003; 2007). In contrast, most species in more mesic communities, where fire is rarer, would be expected to respond negatively to fire cues. Seeds in these communities may be killed or fail to germinate in response to fire (Hopkins and Graham 1984; Tang *et al.* 2003).

Temperatures measured during fires in the soil of mesic communities are lower than sclerophyllous communities; fuel in these communities burns less fiercely than in the more fire-prone communities (Floyd 1966; 1976). The frequency and severity of fires could be expected to influence species composition, leading to increases in the more fire-tolerant species. Fire would also result in losses due to high soil temperatures killing seed of less heat-tolerant species (Hopkins and Graham 1984). In the context of this study, high intensity fires are those that produce a lot of heat in the soil profile due to the large consumption of fuel, generate high soil temperatures, and heat the soil to greater depths, whereas low intensity fires consume little fuel, generate low soil temperatures, and heat a smaller area of the soil profile (Keeley 2009). It is likely fires of different intensities heat soil at different temperatures (Beadle 1940) and this may result in changes in composition due to the influence of heat on the seeds of different species in the soil, especially obligate seeders. It is expected the soil temperatures experienced during a high intensity fire within the top layer of soil would kill the seeds of most species, whilst the temperatures experienced during low intensity fires fail to stimulate germination of heat-cued seeds (Raison *et al.* 1986; Bradstock and Auld 1995; Penman and Towerton 2008). Repeated low intensity fires may result in low recruitment of heat-cued species. High intensity fires heat the soil to temperatures that are sufficient to germinate heat-cued seeds, but may deplete the soil seed bank in a single fire event. High soil temperatures above 80°C can also kill both heat- and non-heat-cued seeds in the soil seed bank, changing the composition to those species responding favourably to heat.

The impact of multiple levels of heat at single durations on species regenerating from the soil seed bank is less well known (but see Floyd 1966; Auld 1986b; Auld and O'Connell 1991; Enright and Kintrup 2001; Hill and French 2003; Thomas *et al.* 2003; Clarke and French 2005; Bossuyt and Honnay 2008b). Many studies on fire-related germination response from the soil seed bank have concentrated on the role of one level of heat, either as a single effect or in combination with smoke (Enright *et al.* 1997; Read *et al.* 2000; Wills and Read 2002; Penman *et al.* 2008). While this approach is valid, it does not consider the effect of heat at different temperatures and durations. Such variation is more representative of temperatures experienced during a fire event, where there will be spatial patchiness in soil heating. Similarly there is likely to be spatial variation in the concentrations of other fire-related germination cues such as smoke. The concentration of smoke will vary temporally and spatially during a fire, however this is yet to be explored in the fire literature (but see Thomas *et al.* 2007). It could be argued smoke naturally occurs together with heat in the context of fire, and any study of the variation in heating should include smoke. Therefore, this study combines the interaction of four levels of heat with one smoke treatment to determine the germination response of species from the soil seed bank of different plant communities. The application of smoke is not made as an independent factor, but rather as a background treatment across all levels of heating.

The following questions were asked for five different plant communities ranging from those dominated by mesic elements (Wet Sclerophyll Forest (WSF), Rainforest (RF)), with low fire frequency (experienced 1–2 fires) to those with a strong sclerophyll component (Dry Sclerophyll Forest (DSF), Rocky Outcrop (RO), Wet Heath (WH)) and a relatively higher expected fire frequency (experienced 2–4 fires):

Q.1. Does heating affect the key variables of species richness, abundance and species composition of the soil seed banks?

Q.2. How do different plant functional types respond to heating?

Methods

Study area

The study was conducted in Gibraltar Range – Washpool National Parks, located on the Great Dividing Range in Eastern Australia (29°31'S, 152°18'E). The Parks occur in both the Northern Tablelands and North Coast botanical subdivisions of NSW with the area totalling approximately 73 000 hectares. The area experiences warm wet summers with average daily temperatures between 16–30°C, and cool dry winters with average daily temperatures of 5–20°C. The study area is dominated by shrubby eucalypt woodlands and forests with extensive areas of rocky outcrop and wet heath vegetation. The underlying geology in these areas is predominately granite, which forms shallow, low-nutrient soils. Rainforest and Wet Sclerophyll Forest are mainly confined to Washpool National Park, where the underlying geology is a mixture of sedimentary, acid volcanic and adamellite geology, which forms deep rich soils (Sheringham and Hunter 2002). The five broad communities sampled in this study have been described in detail in Chapter 1.

Soil heating

Soil used in this study was surplus soil from the study described in Chapter 1. The design for this experiment is the same as the study described in Chapter 1: five communities, two sites for each community and five transects at each site. The large and small soil samples from the Chapter 1 study were pooled to form a homogenous sample. Each pooled sample was then split into four equal parts using a soil splitter and randomly allocated one of four heat treatments (40, 80, 120°C and control (no heat)). Soil samples were spread in aluminium trays to a thickness of approximately 1–1.5cm on a base of vermiculite:perlite mix. Temperatures experienced in the soil samples were monitored by Thermax® temperature strips placed in the sample just above the surface. The actual temperatures measured for the three heat treatments during the soil heating process were 35(± 1), 52(± 2) and 85(± 7)°C and are referred to throughout the text as low, moderate and high respectively. The control samples were kept at room temperature which was generally 19°C. Soil samples were heated in batches of five (one sample from each community), in the same oven at different times to ensure independence of replication (Morrison and Morris 2000). For each temperature treatment the oven was preheated to the appropriate temperature and samples heated for 15 minutes. To minimise the effect of heat loss when the oven door was open, the time to heat the samples did not commence until the oven thermostat indicated that the oven had returned to the set temperature. After heating, each soil sample was watered with one application of diluted (1:10) smoke water (Tieu *et al.* 1999; Clarke *et al.* 2000; Clarke and French 2005). Concentrated smoke water was prepared by drawing smoke from burning a mix of fresh and dried eucalypt litter through four litres of deionised water for 30 minutes using a water smoke apparatus. Smoke was made up in five batches and pooled.

Seedling emergence from the trays was monitored every week for 10 weeks from November 2007–January 2008. The average minimum and maximum temperatures in the glasshouse for the duration of the experiment were 14 and 32°C, respectively. Seedling trays were placed randomly in a glasshouse and watered to keep the soil moist. Seedlings were individually pinned, with different coloured pins used for each weekly census to avoid miscounting. Unidentifiable seedlings were replanted into larger plots for further growth and later identification.

Species were classified on their known response to fire (NSW Flora Fire Response Database V1.3a). Where fire responses varied, species were placed in the majority response category. For seven species with an unknown fire response (5% of total species), response was inferred from the known responses of other species in the respective genus, with the most sensitive response adopted (in this case obligate seeder) if a particular genus had variable responses. Comparisons were also made for the most abundant species emerging from the soil seed bank based on life form and the families dominating each plant community.

Statistical analysis

A three-way general linear model was used to compare differences between heat treatments and communities. The factors tested in the model were: community, heat and site (nested in community). As there was a significant interaction between community and heat, a series of two-way general linear models were also used to compare differences between heat treatments within each community. The factors for each two-way model were heat and site. The variables assessed in both models were: total species richness, and abundance of seedlings emerging from the soil seed bank. Post-hoc Scheffe's test was used to test for differences between levels of a significant factor. All univariate statistical analyses were performed using DataDesk® 6.1 (Velleman 1997). Data were tested for normality using box plots, where the response variable was plotted by heat treatment for each community, and 4th root transformed to satisfy assumptions of normality where appropriate (Zar 1984).

Differences in species composition between heat treatments and communities were compared using a two-way PERMANOVA, tested using a Bray-Curtis similarity matrix. Pairwise tests were used to determine where differences occurred. A similarity percentage

(SIMPER) analysis was used to determine the average similarity for each heat treatment, based on the Bray-Curtis index of similarity, for species emerging from the soil seed bank of each community which contributed most strongly to similarities among samples within each heat treatment. Analyses were performed on abundance data using PRIMER v6 (Clarke and Gorley 2006) and PERMANOVA+ (Anderson *et al.* 2008) and were transformed where appropriate to improve the underlying distribution.

To determine if germination of species within each community was associated with a particular heat treatment, χ^2 analysis was used. The null expectation was no difference in abundance among treatments. This analysis used species that recorded ten or more individuals germinating from the soil seed bank of each community, as χ^2 analyses are not valid when expected values are low (Zar 1984).

To determine if the proportion of obligate seeders and resprouting species in each community was affected by heat, a log-likelihood ratio analysis was used. The log-likelihood ratio analysis statistic (G test) has an approximate distribution of χ^2 (Zar 1984).

Results

The number of species detected in the soil seed bank varied between communities, as did family composition, although some families were the most speciose in more than one community (Table 1).

Response to heat: Species richness and abundance

There was a significant interaction between heat treatments and communities. Heating the soil resulted in a variable response of species emerging from the soil seed banks of the different communities (Fig. 1a; Table 2). At the highest heat level there was a significant increase in species richness (Fig. 1a, Table 2: DSF, RO) and abundance (Fig. 1b, Table 2: RO, WH) in the more fire-prone communities. Site was found to be significant for different variables within each community, except Wet Sclerophyll Forest, indicating the patchiness of soil seed banks across sites (Table 2) and although there appears to be a high reduction in the Rainforest abundance (Fig. 1b), the significant site effect for this community contributes to the non-significant effect of heat. Variation was also smaller in the high treatment due to the decline in abundance of one species, *Hydrocotyle pedicellosa*, which had a significant response to heat (see Table 5).

Table 1. The major family groupings for the total number of species detected in the soil seed bank of five communities.

Community	Number of species	Number of families	Major family groups
Dry Sclerophyll Forest	41	22	Asteraceae, Ericaceae, Fabaceae
Rocky Outcrop	45	26	Asteraceae, Ericaceae, Fabaceae
Wet Heath	21	16	Asteraceae, Ericaceae, Cyperaceae
Wet Sclerophyll Forest	59	32	Asteraceae, Poaceae, Fabaceae, Solanaceae
Rainforest	53	35	Asteraceae, Solanaceae

Table 2. *F*-statistic and *p*-value of the General Linear Model for (a) species richness and (b) abundance of individuals emerging from the soil seed bank of five communities.

			(a) Species richness		(b) Abundance	
	Factor	d.f.	F	*P*	F	*P*
3-way GLM	Community	4	1.1946	0.4154	3.8736	0.0850
	Heat	3	**3.4471**	**0.0438**	**7.3702**	**0.0029**
	Community x Heat	12	**3.5902**	**0.0110**	**7.3287**	**0.0003**
	Site (Community)	5	**15.862**	**<0.0001**	**9.601**	**<0.0001**
	Heat x Site (community)	15	0.7201	0.7517	0.1555	0.9999
2-way GLM						
Dry Sclerophyll Forest	Heat	3	**13.9210**	**0.0289**	9.1332	0.0510
	Site	1	0.4384	0.5127	**11.5130**	**0.0019**
Rocky Outcrop	Heat	3	**31.9470**	**0.0089**	**25.2260**	**0.0125**
	Site	1	**30.1180**	**<0.0001**	2.7133	0.1093
Wet Heath	Heat	3	1	0.5	**11.7260**	**0.0365**
	Site	1	**24.8470**	**<0.0001**	3.5198	0.0698
Wet Sclerophyll Forest	Heat	3	1	0.5	1.2515	0.4290
	Site	1	0.4928	0.4878	0.0609	0.8065
Rainforest	Heat	3	1.4324	0.3874	1.5087	0.3718
	Site	1	**41.4400**	**<0.0001**	**26.8410**	**<0.0001**

Significant values in bold

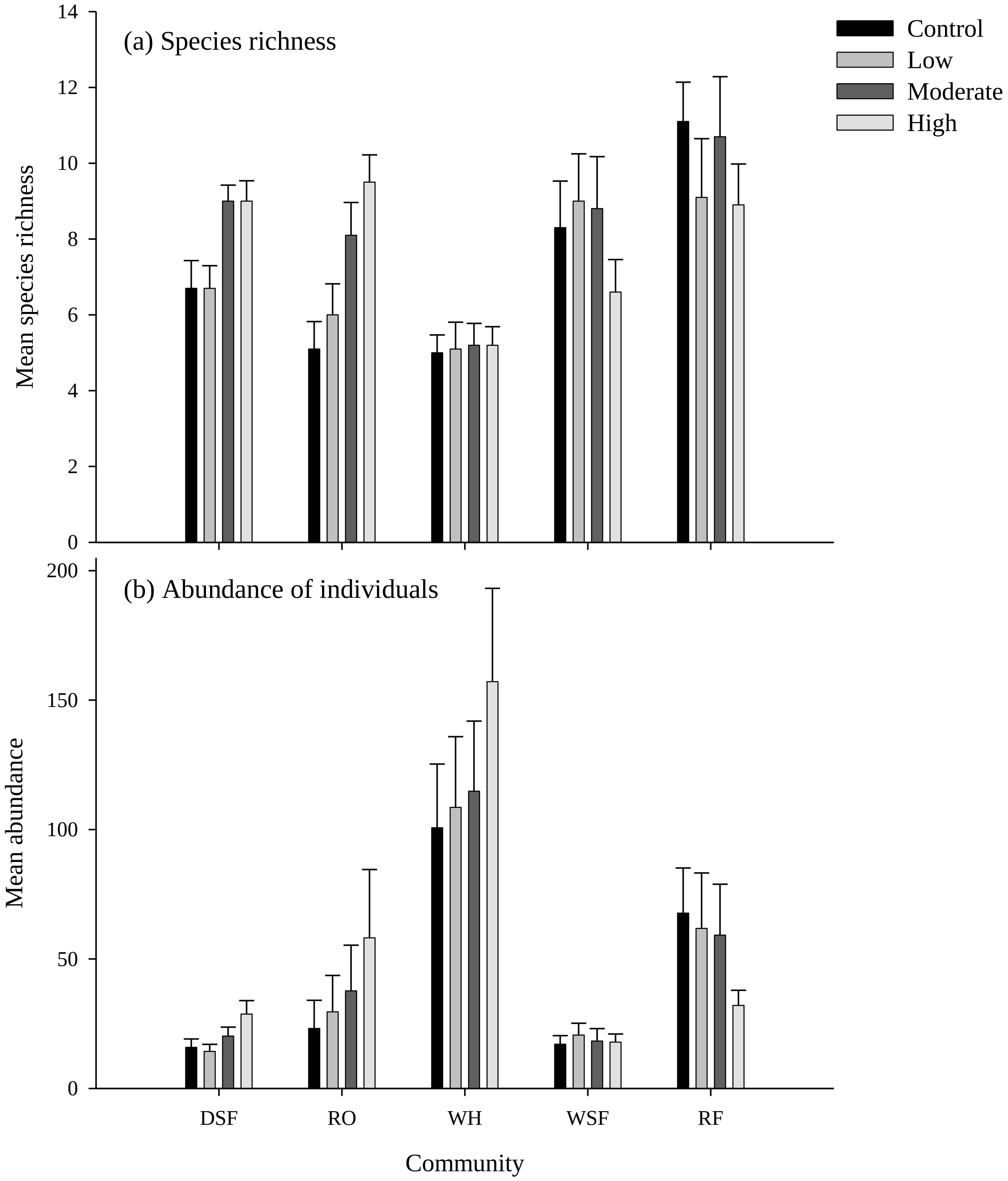
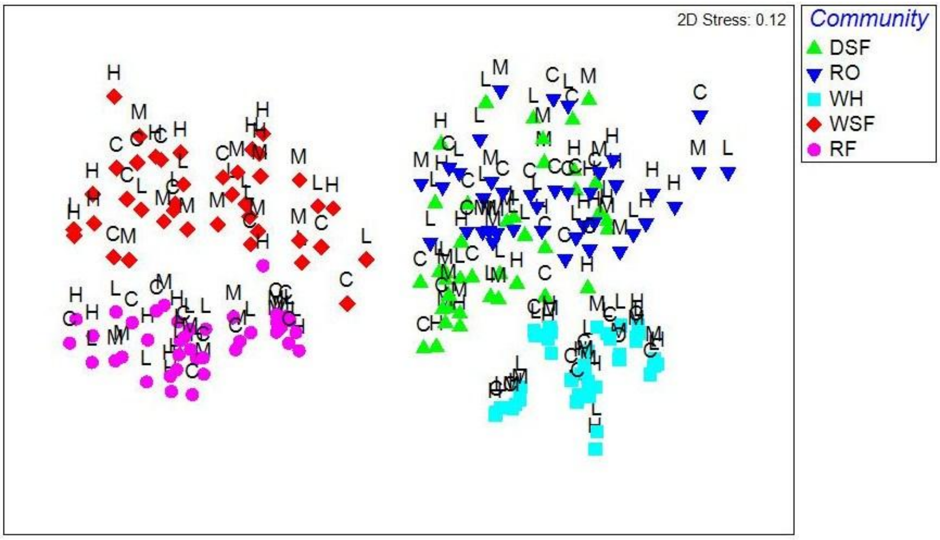


Fig. 1. Effect of heat on the emergence of (a) species (± s.e.) and the (b) abundance of individuals (+ s.e.) from the soil seed bank of five communities. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Response to heat: Species composition

Soil seed bank samples clustered separately based on the similarity within community type rather than as an effect of heating (Fig. 2a). A two-way PERMANOVA used to test the effect of heating on the species composition, found a significant difference between communities but no difference between heat treatments or an interaction between community and heat treatments (Table 3). Average similarity values (using SIMPER analysis) within heat treatments for each community ranged from 21% (in the Wet Sclerophyll Forest) to 43% (in the Wet Heath), indicating similarity within the heat treatments were quite low across all communities (Table 4). Few species contributed to this similarity, indicating species composition was not affected by heat, whereas individual species responded differently to heat which may have contributed to this low similarity within heat treatments (See Response to heat: Individual species). Soil seed bank samples clustered separately for sites within communities (Fig. 2b. e.g. Rainforest), indicating species composition of each site was quite different within each community. This suggests the significant site effect identified in the GLM analysis (see Table 2) can be attributed to the large spatial variability within each community.

(a) The effect of heat on species composition across different communities



(b) An example of site effects on heat treatments in the Rainforest community

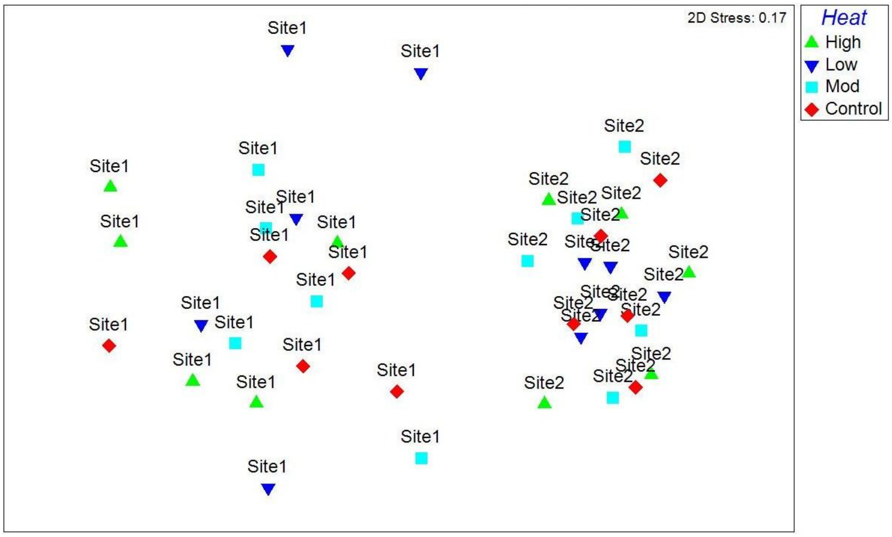


Fig. 2. Ordination of samples (nMDS) in two dimensions for the (a) abundances of species recorded from the soil seed bank of each community within each heat treatment (Heat treatments are: C = Control, L = Low, M = Medium, H = High); and for the (b) abundances of species recorded within each heat treatment from the soil seed bank of the Rainforest community. Resemblance is based on Bray-Curtis similarity index. Stress = (a) 0.12 and (b) 0.17. Communities referred to as: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Table 3. PERMANOVA identifying the effect of heating on the species composition of the soil seed banks of each community.

Factor	d.f.	Pseudo F	P (perm)
Community	4	30.717	**0.0001**
Heat	3	0.980	0.5334
Community x Heat	12	0.736	0.9996
Pairwise test		T statistic	P (perm)
DSF[†] *vs.* RO		3.760	**0.0001**
DSF *vs.* WH		5.608	**0.0001**
DSF *vs.* WSF		5.164	**0.0001**
DSF *vs.* RF		6.143	**0.0001**
RO *vs.* WH		5.451	**0.0001**
RO *vs.* WSF		5.007	**0.0001**
RO *vs.* RF		5.980	**0.0001**
WH *vs.* WSF		6.164	**0.0001**
WH *vs.* RF		7.230	**0.0001**
WSF *vs.* RF		4.830	**0.0001**

Significant values in bold. [†]DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Table 4. Average similarity indices (calculated from average Bray-Curtis similarity index) for each heat treatment among the five communities.

Community	Control	Low	Moderate	High
Dry Sclerophyll Forest	29.44	28.39	27.49	26.04
Rocky Outcrop	27.96	22.67	24.03	33.80
Wet Heath	42.23	42.23	41.82	43.48
Wet Sclerophyll Forest	21.39	23.02	23.08	25.53
Rainforest	35.99	35.17	36.75	32.07

Response to heat: Individual species

A number of species germinated in large enough numbers (>10 individuals) among heat treatments to be able to make some generalisation about how heat affects individual species across different communities. Responses varied from positive to negative effects of heating or no heat effect (Table 5). In the latter case, for example, a number of species representing a variety of families and life forms showed no preference for germinating under any particular heat treatment across all communities (Table 5, $P > 0.05$ e.g. *Berberidopsis beckleri, Galium binifolium, Leptospermum trinervium*).

Heat had either a negative or positive effect on key family groups and lifeforms for the most abundant species emerging from the soil seed bank (Fig. 3; Table 5). Tree and twiner species had a negative response to heat, with proportionally more individuals emerging in the control and low temperature treatments compared to the highest heat treatment. In contrast, shrub, grass, and graminoid species had proportionally more individuals emerging in the higher temperature treatments, indicating these life forms have a positive response to heat (Fig. 3a; Table 5). In the Fabaceae, there was a positive response to heat with proportionally more individuals emerging in the two higher heat treatments compared to the lower temperature and control treatments (Fig. 3b). All seven Fabaceae species emerging from the soil seed bank across all communities showed this significant response to heat (Table 5). Species in the Ericaceae also had a similar trend with proportionally more individuals emerging in the highest heat treatment (Fig. 3b). This pattern could be attributed to two species (*Epacris gunni*, *E. obtusifolia*), which had a significant response to heat with more individuals germinating in the higher heat treatment than at lower temperatures whereas the fleshly fruited species (*Leucopogon* and *Monotoca*) did not have a significant response to heat. Species from the Asteraceae germinated across all heat treatments, indicating heat did not affect their emergence from the soil seed bank. Species did not germinate in large enough abundances to indicate a distinct pattern, with only two species (*Conyza sumatrensis*, *Ozothamnus rufescens*) showing a significant negative response to heat (Fig. 3b; Table 5).

Five species sampled in more than one community responded differently to heat. Two species (*Caustis flexuosa*, *Rhytidosporum procumbens*) were sampled from the soil seed banks of both the Dry Sclerophyll Forest and Rocky Outcrop communities and three species (*Hedycarya angustifolia*, *Rubus rosifolius*, *Solanum aviculare*) were sampled from the soil seed banks of the Wet Sclerophyll Forest and Rainforest communities. In most cases the trend in the response to heat was similar although likely non-significant results for these species could be attributed to the difference in abundance between the communities or the absence of the species from a particular heat treatment (Table 5). Caution should be taken when making generalisations about responses to heat especially in the cases where a species is recorded in more than one community.

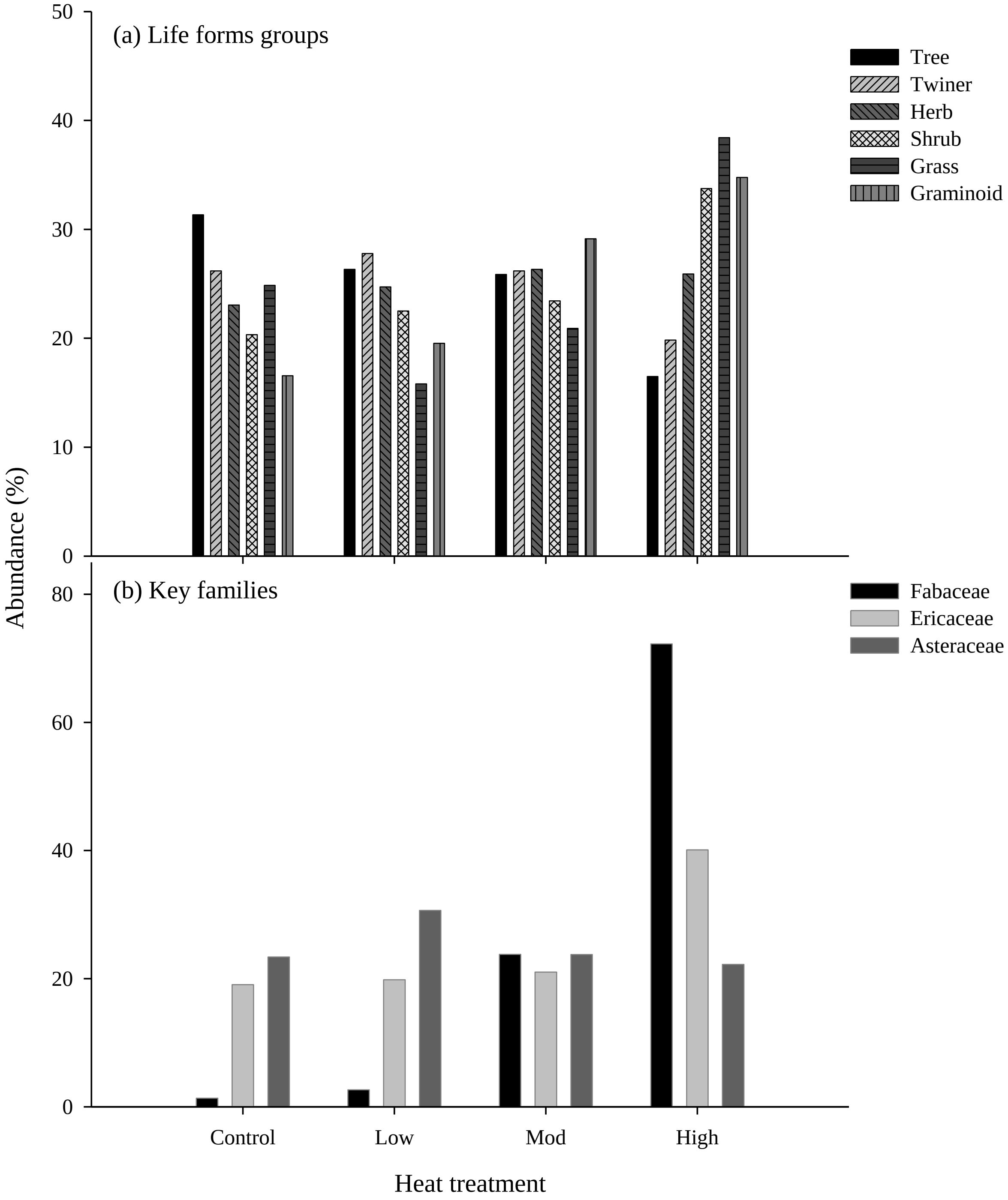


Fig 3. Responses to heating across all communities for (a) Life form groups and (b) Key family groupings of the most abundant species. Percentage abundance is based on groupings among treatments.

Table 5. Species germinated (and their abundances) from the soil seed bank of five communities based on the four heat treatments. Species had more than 10 seedlings germinating across all heat treatments. All heat treatments received a single application of smoke.

Species	Fire response[†]	Seed dormancy type[‡]	High	Mod	Low	Control	χ^2 probability
Dry Sclerophyll Forest							
Aotus subglauca subsp. *glauca*	R	PY	13	5	0	0	**<0.0001**
Austrostipa rudis	R	PD	4	3	3	5	0.8653
Boronia microphylla	R	PD	5	3	4	0	0.1978
Bossiaea scortechinii	R	PY	8	5	0	0	**0.0024**
Caustis flexuosa	R	PD	11	7	3	5	0.1457
**Conyza parva*	OS	PD	4	7	8	3	0.3778
Entolasia stricta	R	PD	26	11	10	17	**0.0175**
Epacris gunnii	R	PD	97	49	38	49	**0.0001**
**Euchiton involucratus*	OS	PD	4	2	5	3	0.6988
Euchiton sphaericus	unknown	PD	5	2	4	2	0.5565
**Gamochaeta spicata*	OS	PD	1	4	3	4	0.5724
Goodenia bellidifolia	R	PD	13	23	6	7	**0.0018**
Leptospermum trinervium	R	ND	19	11	15	14	0.5279
Leucopogon microphyllus var. *pilibundus*	OS	MPD	3	8	2	3	0.1386
Monotoca scoparia	R	MPD	7	6	5	2	0.4234
Platysace ericoides	R	PD	16	13	12	15	0.8698
Prostanthera sp. aff. *howelliae*	R	PD	4	1	5	1	0.2004
Rhytidosporum procumbens	R	MPD	7	4	4	5	0.7530
Stackhousia vimenera	OS	PD?	7	1	0	2	**0.0088**
Trachymene incisa	R	MD	8	13	9	10	0.7055
Rocky Outcrop							
Amperea xiphoclada var. *xiphoclada*	R	unknown	9	4	3	3	0.1570
Boronia anethifolia	OS	PD	3	4	4	2	0.8383
Cassytha glabella	OS	PD	9	9	0	0	**0.0004**
Caustis flexuosa	R	PD	7	2	1	1	**0.0292**
**Conyza parva*	OS	PD	2	7	3	5	0.3245
Drosera spatulata	R	PD	231	162	143	115	**<0.0001**
Entolasia stricta	R	PD	34	17	11	15	**0.0011**
**Euchiton involucratus*	OS	PD	3	4	6	4	0.7728
Gonocarpus teucriodes	OS	unknown	8	6	6	5	0.8590
Kunzea bracteolata	R	ND	28	41	40	28	0.2055

Table 5. cont.

Species	Fire response	Seed dormancy type	High	Mod	Low	Control	χ^2 probability
Laxmannia gracilis	OS	unknown	78	28	26	26	**<0.0001**
Lepidosperma gunnii	R	unknown	16	5	4	1	**0.0001**
Leucopogon microphyllus var. *pilibundus*	OS	MPD	17	7	10	9	0.1524
Leucopogon sp. aff. *apressus*	OS	MPD	4	6	5	0	0.1366
Mirbelia confertifolia	R	PY	7	3	0	0	**0.0042**
Mirbelia rubiifolia	OS	PY	65	26	0	0	**<0.0001**
Platysace ericoides	R		21	8	16	10	0.0545
Rhytidosporum procumbens	R	MPD	2	7	2	0	**0.0210**
Wet Heath							
Actinotus gibbsonii	OS	PD	2	4	0	4	0.2213
Baeckea omissa	R	PD	681	547	557	509	**<0.0001**
Boronia polygalifolia	R	PD	2	5	5	3	0.6149
Caesia sp.	R	PD/ND?	1	7	1	2	**0.0292**
Drosera spatulata	R	PD	275	239	203	194	**0.0004**
Empodisma minus	R	PD?	66	62	45	38	**0.0168**
Epacris gunnii	R	MPD	162	71	67	44	**<0.0001**
Epacris obtusifolia	OS	MPD	335	181	182	190	**<0.0001**
Gonocarpus micranthus	OS	unknown	7	7	4	4	0.6511
Hibbertia sp. aff. *rufa*	R	PY+PD	27	17	12	11	**0.0223**
Wet Sclerophyll Forest							
Acacia irrorata	OS	PY	8	3	0	0	**0.0014**
Asterolasia correifolia	OS	PD	3	7	3	3	0.3916
Berberidopsis beckleri	OS	unknown	3	6	11	7	0.1829
**Conyza sumatrensis*	OS	PD	1	3	3	13	**0.0005**
Galium binifolium	OS	PD	5	9	6	7	0.7300
**Gamochaeta spicata*	OS	PD	9	6	6	3	0.3916
Gonocarpus oreophilus	OS	unknown	21	33	29	26	0.4207
Goodia lotifolia	OS	PY	45	2	5	2	**<0.0001**
Hedycarya angustifolia	R	MPD	1	3	2	5	0.3644
Helichrysum elatum	OS	PD	9	7	10	5	0.5927
Hydrocotyle peduncularis	R	unknown	5	11	10	8	0.4806
Notodanthonia longifolia?	R?	PD	4	6	4	7	0.7325
Oxalis exilis	R	PD/ND	4	3	13	7	**0.0292**
Ozothamnus rufescens	OS	unknown	13	11	21	6	**0.0272**
Poranthera microphylla	OS	unknown	9	6	8	6	0.8179
Rubus rosifolius	R	PD	1	11	7	15	**0.0056**
Solanum aviculare	OS	PD	0	14	15	10	**0.0023**

Table 5. cont.

Species	Fire response	Seed dormancy type	High	Mod	Low	Control	χ^2 probability
Viola hederacea	OS	PD	11	8	6	8	0.6718
Rainforest							
Acacia irrorata	OS	PY	11	3	1	0	**0.0001**
Acacia melanoxylon	R	PY	7	7	0	1	**0.0097**
Ackama paniculata	OS	unknown	121	243	258	279	**<0.0001**
Aneilema acuminatum	R	unknown	10	25	15	27	**0.0167**
Berberidopsis beckleri	OS	unknown	10	16	23	20	0.1390
Callicoma serratifolia	R	unknown	29	22	26	41	0.0780
Cissus hypoglauca	R	unknown	3	2	1	6	0.1978
Cyperus disjunctus	R	PD	2	6	2	2	0.2614
**Euchiton involucratus*	OS	PD	1	3	4	5	0.4415
Gahnia melanocarpa	R	unknown	2	5	2	2	0.4835
**Gamochaeta spicata*	OS	PD	2	4	3	2	0.8012
Hedycarya angustifolia	R	MPD	2	3	0	11	**0.0005**
Hydrocotyle pedicellosa	R	unknown	2	125	152	123	**<0.0001**
Hydrocotyle peduncularis	R	unknown	1	3	3	4	0.6308
Lobelia trigonocaulis	R	ND/PD	37	45	54	52	0.2853
Lophostemon confertus	R	unknown	2	0	2	7	**0.0210**
Rorippa dictyosperma	OS	PD	17	12	14	15	0.8262
Rubus moluccanus var. *trilobus*	R	PD	1	5	1	6	0.0943
Rubus rosifolius	R	PD	1	5	5	6	0.3245
Solanum aviculare	OS	PD	17	17	20	18	0.9536
Solanum nobile	OS	PD	1	6	2	4	0.2088
Solanum opacum	OS	PD	2	2	4	4	0.7212
Solanum vicinum	OS	PD	4	2	7	9	0.1528
Viola hederacea	OS	PD	3	2	2	4	0.8012
Zieria sp. aff. *smithii*	OS	PD	8	4	2	2	0.1116

Significant values in bold

*exotic species

†Fire response refers to R= Resprouter, OS = Obligate seeder (NSWFFRD 2002; Clarke *et al.* 2009)

‡Seed dormancy type refers to PY = physical dormancy, PD = physiological dormancy, MD = morphological dormancy, MPD = morphophysiological dormancy. Seed dormancy type has been inferred from families/genera reported in the literature (Baskin and Baskin 1998; Merritt *et al.* 2007; Ooi 2007; Auld and Ooi 2008; Martyn *et al.* 2009)

Response to heat: Fire response categories

Resprouting species were proportionally greater in the standing vegetation of all communities than obligate seeding species (Table 6). Without heating (control treatment) there were relatively more resprouting species detected in the fire-prone communities, in contrast to less resprouting species detected in the mesic communities. Heating the soil seed bank of the fire-prone communities did not change this pattern (Table 6: DSF, RO, WH). In the mesic communities, obligate seeding species were greater in the soil seed bank than the standing vegetation (Table 6: WSF, RF). Heating the soil seed bank of the Wet Sclerophyll Forest community resulted in significantly high proportions of obligate seeding species emerging in the highest temperature treatment. This was in response to a decline in the number of species from 19 in the control treatment to 15 in the high heat treatment indicating heat kills resprouting species in the soil seed bank of this community (Fig. 4; Table 6). The pattern for total abundance was comparable to that found in species richness (Table 6).

Table 6. Proportion of (a) species richness and (b) abundance based on fire response sampled from the different heat treatments within each community. The fire response of the species in the standing vegetation is shown here as a baseline comparison.*

	Fire response[†]	Control	Low	Moderate	High	χ^2 probability	Standing vegetation fire response
(a) Species richness							
Rocky Outcrop						0.6682	
	OS	42	39	45	37		37
	R	58	61	55	63		63
Dry Sclerophyll Forest						0.7879	
	OS	24	25	20	23		15
	R	76	75	80	77		85
Wet Heath						0.5749	
	OS	16	12	17	20		9
	R	84	88	83	80		91
Wet Sclerophyll Forest						**0.0039**	
	OS	58	65	64	81		38
	R	42	35	36	19		62
Rainforest						0.5737	
	OS	55	63	54	56		19
	R	45	37	46	44		81
(b) Abundance							
Rocky Outcrop						0.5145	
	OS	27	33	37	33		
	R	73	67	63	67		
Dry Sclerophyll Forest						0.8528	
	OS	17	20	16	16		
	R	83	80	84	84		
Wet Heath						0.7581	
	OS	18	16	13	17		
	R	82	84	87	83		
Wet Sclerophyll Forest						**0.0056**	
	OS	67	72	71	87		
	R	33	28	29	13		
Rainforest						0.6481	
	OS	56	65	61	60		
	R	44	35	39	40		

*Proportions were calculated as means for heat treatments and the standing vegetation fire response.

[†] R = Resprouter, OS = Obligate seeder. Significant values in bold.

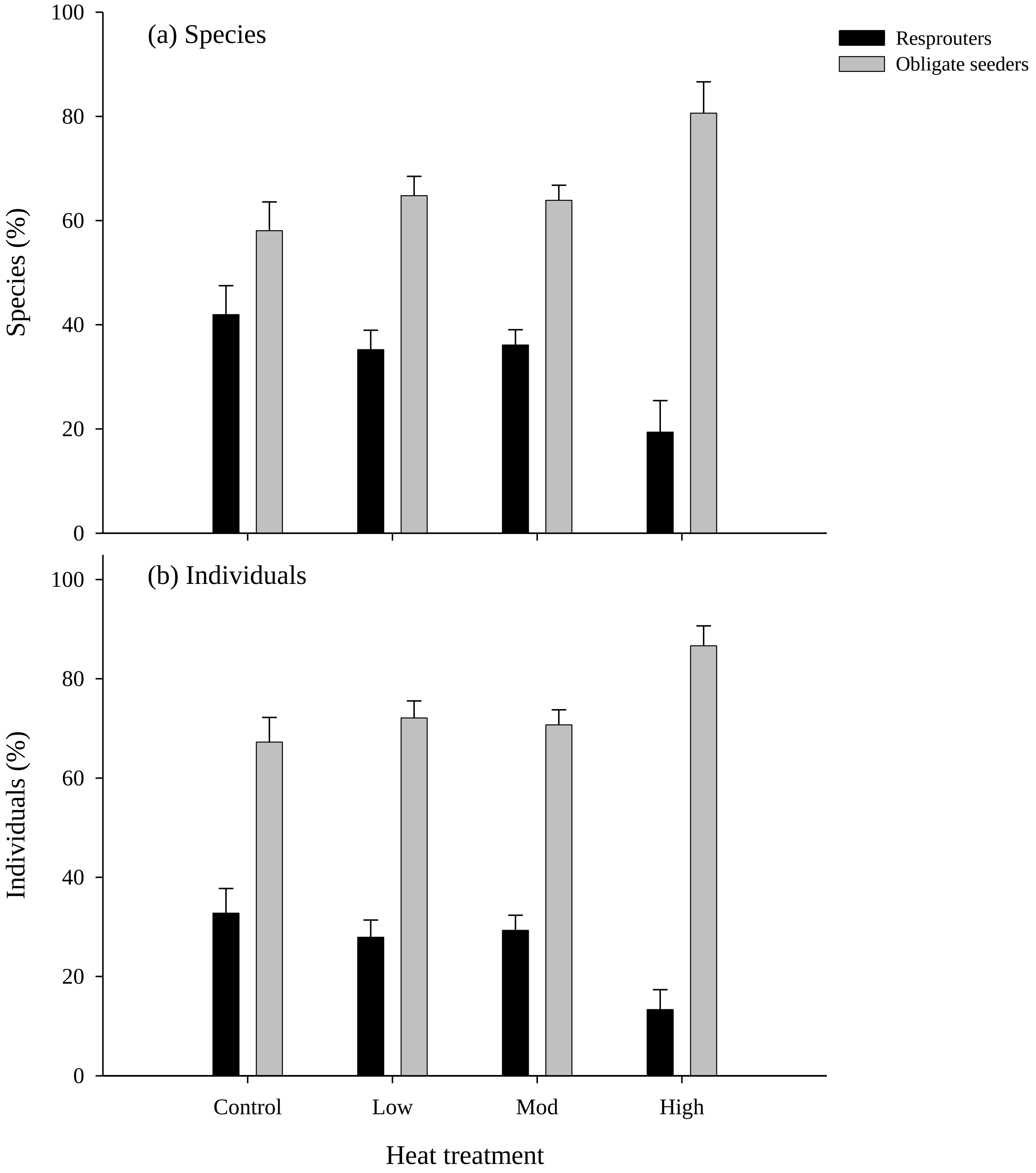


Fig. 4. Effect of heat: Percentage of (a) species (± s.e.) and (b) individuals (± s.e.) emerging from the soil seed bank of the Wet Sclerophyll Forest community based on their sprouting ability.

Discussion

Soil heating affects the germination of seeds within the soil seed banks of different communities in a variety of ways. Generally, heating promoted the germination of species in the more fire-prone communities to a greater degree than the mesic communities. During a fire event that heated soil to high temperatures, more germination from the soil seed bank would occur in the fire-prone communities, whereas seed from some species in the mesic communities would be killed by the high temperatures. For those species relying on regeneration from seed in the soil to persist in the landscape, this may result in a decline of their soil-stored seed bank. Should a second fire occur prior to significant replenishment of the soil seed bank, species composition within these communities is likely to be altered. Population sizes of obligate seeder species would decline and sprouting species would dominate if fire return intervals were insufficient for species to accumulate a seed bank (Keeley and Zedler 1978), especially where a high severity fire, which resulted in heating of soil to significant depths, had exhausted the soil seed bank either by seed death or seed germination (see Fig. 3. 11B, Whelan 1995). Alternatively, soil which remained unheated or did not reach sufficient temperatures to stimulate germination (Penman and Towerton 2008) would buffer any species loss from the landscape after a subsequent fire event as the seed would remain dormant (Auld 1987).

In this study, heating did not affect the broad species composition of any community, with samples clustering strongly based on community type, but individual species within each community, as well as the proportion of species with different fire response strategies in one community (WSF), had a positive or negative response to heat. This result could be attributed to three reasons. First, the presence of a smoke treatment across all heat levels in this study may have negated the effect of heat on particular species within each community. Smoke treatments have been shown to reverse the negative effects of heat, particularly at high temperatures, for some species (Thomas *et al.* 2007). The abundance of individuals recorded in the high temperature treatments may be an indication that this is also the case for the communities studied here. However, as smoke naturally occurs with soil heating in fires, this may represent the ‘real world scenario’. Second, this trend could also be attributed to the low similarity among heat treatments across the communities (Table 4). Few species typified the heat treatments and this could be attributed to the large proportion of species with few individuals. Across all communities there was a total of 128 species (58% of total) recorded with less than ten individuals, indicating the soil seed bank was spatially patchy regardless of community or heat treatment. These species were either poorly represented in the soil seed bank due to the nature of their distribution in the soil profile or germination cues used in the study failed to stimulate sufficient seeds to germinate. It is likely a number of species germinate regardless of heat treatment in each community, e.g. through the promoting effects of smoke, as a large suite of species were found in all treatments (Table 5). Third, the significant site effect recorded for most communities (Fig. 2b; Table 2) would lend support to the conclusion the soil seed bank was quite variable within each community. Differences between sites were likely to influence the effect of heat on the species composition of each community. Increased replication at the site level may reduce this effect, however this was beyond the logistical constraints for this project. Alternatively, a reduction in the number of transects sampled at each site and increasing the number of sites sampled may have reduced the between site variation but not the within site variation.

Heat can have an impact on different life forms in a number of plant communities, with shrubs (generally legumes) responding positively to heat (Read *et al.* 2000; Enright and Kintrup 2001; Hill and French 2003) while taxa such as grasses and graminoids respond negatively to heat (Warcup 1980; Overbeck *et al.* 2006) or germination patterns are inconsistent within taxa (Clarke and French 2005). In this study, species classified into different life forms also had a variable response to heat across all communities (Fig. 3a; Table 5). Generally, tree and twiner species with a soil seed bank responded negatively to heat, whereas shrub, grass and graminoid species had a positive response to high soil temperatures. Herbaceous species germinated regardless of heat treatments.

Heat is known to increase germination of hard-seeded species with physical dormancy (Auld and O'Connell 1991; Bell 1999; Hanley *et al.* 2001). In this study seven Fabaceae species responded to heat regardless of the community from where they were sampled (Fig 3b; Table 5). Although some species germinated at a range of temperatures (e.g. *Goodia lotifolia*), the majority of germination occurred at the highest temperatures, indicating hard-seeded species are likely to be depleted from the soil seed bank of all communities following a fire event which has heated the soil to moderate temperatures, but also that fires that produce this amount of heating are needed to maintain these species. Ericaceae species also had a similar response to heat. Both *Epacris* species (*E. gunnii* and *E. obtusifolia*) recorded greater germination in the high heat treatment and this agrees with other studies (Enright and Kintrup 2001; Penman *et al.* 2008) that have reported a positive response to heat and smoke for *Epacris* species. However, recent work has shown a mixed response to heat and smoke for other *Epacris* species (Thomas *et al.* 2007).

In most studies there is a component of the soil seed bank unaffected by heat. In these studies a suite of species germinate in great abundances regardless of treatment, and exhibit a neutral response to heat (Enright *et al.* 1997; Hill and French 2003; Penman *et al.* 2008). In this study, fifty-five of the most abundant species sampled from the soil seed bank of five communities did not respond significantly to heat in the presence of smoke (Table 5). These species are likely to have a seed dormancy mechanism unrelated to the interaction between heat and smoke. For these taxa, however, it is not clear whether the breaking of seed dormancy is unrelated to fire due to the absence of an independent control. Also increased light and moisture conditions experienced during a germination experiment in a glasshouse would be favourable for those species which were non-dormant. Seed dormancy types for these species, inferred from the literature, indicate they predominately have physiological dormancy. The results here indicate the effect of heat and smoke may be more complex than simply breaking seed dormancy and these factors may also promote or inhibit seed germination for these species.

When sprouting ability (resprouter versus seeder) was assessed, a number of responses across the community were also found. Resprouting species dominated the standing vegetation of all communities and the soil seed banks of the fire-prone communities, while obligate seeding species dominated the soil seed banks of the mesic communities (Table 6). Obligate seeders are generally thought to have larger seed banks and it would be expected that they dominate the soil seed bank (Carpenter and Recher 1979; Bellairs and Bell 1990; Hansen *et al.* 1991; Hansen *et al.* 1992). However, in some studies comparing seeder and resprouter congeneric pairs where plants are matched for size, they store the same number of seeds (Zammit and Westoby 1987). For species with a soil seed bank, plant age seems to play an important role in comparisons of seed output between seeder and resprouter congeneric pairs (Hansen *et al.* 1991). As I have sampled almost five years post-fire, those species with a long primary juvenile period are likely to be absent from the soil seed bank due to insufficient time to allow for the accumulation of seed in the soil. The lower numbers of obligate seeding species in the soil seed banks of the fire-prone communities (Table 6) indicate this could be the case in the present study. Only one community, the Wet Sclerophyll Forest, recorded significant differences in the proportion of species based on their sprouting ability across the different heat treatments, with high temperatures increasing the proportion and abundance of obligate seeding species recorded in that community (Fig 4; Table 6). Over time, this may result in changes in species composition with obligate seeding species dominating the landscape where fires of higher severity have occurred. Given that the most abundant species in this community were long-lived obligate seeders (Table 5), it is likely changes in species composition will be long-term, assuming the fire return interval is long enough to enable seed bank accumulation (see Keeley and Zedler 1978).

The difficulty in heating soil in laboratory conditions is obtaining uniform heat throughout the sample for a particular duration. The soil temperatures experienced during this study were generally less than the set oven temperature. Soil has an insulating effect on seeds present in the soil, which may result in seeds not being heated at the set temperature for each treatment in a uniform manner. Seeds at the top of the soil sample may have experienced a different temperature than those in the middle or bottom of the sample. This may contribute to differences between studies where seeds of species have been heated (e.g. Thomas *et al.* 2003; Clarke and French 2005; Thomas *et al.* 2007) compared with studies which has heated soil containing seed (e.g. Hill and French 2003; Penman *et al.* 2008). However, this insulating effect would have been standardised across all treatments for all communities and the temperatures measured in the samples were in three distinct ranges.

The impact of fire severity on communities is likely to increase the presence and abundance of species that respond positively to fire in fire-prone communities and decrease those species that respond negatively to fire in mesic communities. This study demonstrated that some species respond to heat with enhanced germination at high temperatures and some species have limited germination at high temperatures. Species within communities that were considered more fire-prone, generally, had a positive response to heat, whereas species in the more mesic, less fire-prone communities responded negatively to heat, with the exception of Fabaceae species which responded the same across all communities. Variable responses to different levels of soil heating enables a degree of buffering against the impact of subsequent fire events on the soil seed banks of different communities, thus enabling species to persist in the landscape.

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Chapter 3. The effect of burn severity on the soil seed banks of five different plant communities.

Introduction

Seed banks are important for the regeneration of species following disturbance (Parker *et al.* 1989). Species often rely on seed stored in canopy (Bradstock and Myerscough 1981; Zammit and Westoby 1987) and soil seed banks (Auld 1986a; Parker and Kelly 1989) to persist in disturbance-prone landscapes; this is particularly important for species killed during a disturbance event such as fire. Resprouting species have the ability to regenerate vegetatively as well as from seed stores following a disturbance, whereas obligate seeding species are killed by fire and rely solely on seed to regenerate (Gill 1981). In this case seed banks, particularly soil-stored seed banks, buffer plant populations against environmental variability and reduce the risk of extinction allowing species to persist in the landscape (Parker *et al.* 1989; Keith 1996; Thompson 2000).

Many species with soil seed banks require fire to trigger germination through factors associated with fire, such as heat (Keeley 1987; Auld and O'Connell 1991), smoke (Brown 1993; Keeley and Fotheringham 1998) and the interaction between these two factors (Kenny 2000; Thomas *et al.* 2003; 2007). In different plant communities, responses to fire cues may vary. Species in more fire-prone communities generally having a positive response to different fire cues (Enright *et al.* 1997; Hanley *et al.* 2001), whereas species in mesic communities, which experience infrequent fire, may have a negative response to different fire cues (Hopkins and Graham 1984). In the context of burn severity, fire-driven local extinction of species in communities relying on soil seed banks may be a result of the death of seeds due to high soil temperatures during more intense fires or the failure of seeds to germinate as a result of insufficient soil heating during less intense fires (Keith 1996).

Fire severity can vary depending on a number of factors including intensity, duration of the fire, fuel loads, fuel and vegetation type, topography, climatic conditions, soil texture and moisture, soil organic content, time since last fire and area burnt (Whelan 1995; Neary *et al.* 1999). Essentially fires that consume a large amount of fuel can result in greater heat penetration of the soil profile, greater soil temperatures and longer residence times during the passage of fire (Bradstock and Auld 1995; Neary *et al.* 1999). Soil temperatures experienced at depths of 0–4 cm are as a result of the consumption of fine fuels (< 6mm thickness) on the ground (Bradstock and Auld 1995). This is particularly important for species with a soil-stored seed bank, as heating of soil may promote or inhibit the germination of seeds in the soil and affect the regeneration of species following fires of different severities. Soil heating can vary with three main effects being observed. First, as fuel consumption increases so do the temperatures measured in the soil. Second, heat penetration down the soil profile is greater with increasing fuel consumption. Third, duration of heating will have the greatest influence on the recovery after fire due to the length of exposure to lethal temperatures and the death of seeds or exposure to temperatures that may promote germination (Floyd 1966; 1976; Auld and O'Connell 1991; Neary *et al.* 1999). In this study, burn severity refers to the loss of organic matter above ground during the passage of fire (Keeley 2009). This loss has been estimated post-fire, using remote sensing techniques to calculate the normalised burn ratio and map burn severity classes for the study area. Understanding the relationship between organic matter loss and the response of soil seed banks is important to determine whether burn severity affects regeneration of plant communities in a post-fire environment.

During a fire, seeds in the soil seed bank are either killed, stimulated to germinate by fire or remain dormant and viable following fire (Auld 1987; see Fig. 3.11, Whelan 1995). The potential seedling recruitment from the soil seed bank will vary depending on the intensity of fire and as this increases we are likely to see decreases in the size of any residual soil seed bank post-fire (Auld 1987). The amount of heat penetrating the soil profile affects germination. High and moderate intensity fires should promote more germination than fires of lower intensity, especially for those species where seed dormancy is broken by heat (Auld 1986b; Auld and O'Connell 1991; Knox and Clarke 2006). As burn severity increases so does soil heating; a depletion of the soil seed bank is expected due either to the death of seeds from lethal temperatures during the passage of fire or as a result of the germination of seed due to heat reaching greater depths in the soil profile or a combination of both. In areas in the landscape that have experienced a low severity burn, or remain unburnt, the soil seed bank would remain much larger, with seeds remaining viable and dormant due to limited soil heating and the lack of cues breaking dormancy or promoting seed germination. The role of smoke in this context however, is a little unclear, as the effect of smoke may not vary with burn severity or soil depth in the way heat does. Smoke may act independently of burn severity and, because smoke occurs naturally in all fires, it could be predicted that species with germination promoted by smoke would be depleted from the soil seed bank to the same degree independent of burn severity. Equally, this becomes more complex for species where seed dormancy is broken by the interaction of heat and smoke cues (Auld and Denham 2006).

A variable response would also be expected for species with different modes of regeneration (resprouter versus obligate seeder) following fire. Obligate seeders have a larger seed bank compared to resprouting species (Carpenter and Recher 1979; Bellairs and Bell 1990; Hansen *et al.* 1991; Benwell 1998). As burn severity increases, however, the size of the seed bank would be expected to decline and there would be a decrease in obligate seeders detected in the soil seed bank. This could vary across communities. In fire-prone communities, species are more likely to be tolerant of heat (Auld and O'Connell 1991; Enright *et al.* 1997; Hanley *et al.* 2001); mesic communities, which experience fire less frequently, would be expected to have species less tolerant to heat (Hopkins and Graham 1984).

These expectations were tested by assessing the impact of burn severity on the soil seed banks of five different plant communities, ranging from those dominated by mesic elements (Wet Sclerophyll Forest, Rainforest) to those with strong sclerophyllous components (Dry Sclerophyll Forest, Rocky Outcrop, Wet Heath) six years following fire. I specifically asked:

Q.1. How does burn severity affect the key variables of species richness, abundance and species composition of the soil seed bank among communities?

Q.2. How do different germination responses and plant functional types respond to burn severity among communities?

Methods

Study area

The study was conducted using soil collected from Gibraltar Range – Washpool National Parks, located on the Great Dividing Range in Eastern Australia (29°31’S, 152°18’E). The study area and the characteristics of the five communities sampled in this study have been described in detail in Chapter 1.

Seed bank sampling

Five broad communities were sampled: Rocky Outcrops, Dry Sclerophyll Forest, Wet Heath, Wet Sclerophyll Forest, Rainforest (see Chapter 1 for a full description) in areas identified as experiencing different levels of burn severity. Burn severity indices were developed using Landsat TM pre- and post-fire images to calculate the Normalised Burn Ratio (Kumar *et al.* 2008). Three burn severity classes were identified for each community: low, high and unburnt. Two sites within each severity class were sampled for each community, except the Wet Heath community, where a paucity of unburnt sites within the study area prevented sampling in a balanced design for this community. The unburnt Wet Heath site was quite large and sites were placed diagonally opposite each other to ensure the maximum spatial variability. At each site, five randomly selected transects (10 x 1 metre) were sampled. Along each transect two random samples were collected using the large core size (28 x 16 x 5cm: length x width x depth) (determined as an adequate sampling technique in Chapter 1 – Sampling the soil seed bank).

Soil samples were collected in January 2009, 6.25 years after the fire event in October 2002. The soil samples were air dried at room temperature, then sieved (4mm aperture) to remove litter and rocks and finally spread out on a seedling tray to form a one centimetre layer of soil (28 x 32 x 1cm: length x width x depth) over the top of a layer of vermiculite/perlite mix (1:1). Seeds larger than 4mm were placed back into the soil sample. Seedling emergence was monitored every week for 10 weeks, until no new species were recorded. Seedlings were individually pinned, with different coloured pins used for each census to avoid miscounting. Unidentified seedlings were replanted in larger plots for later identification. After 10 weeks in the glasshouse, the soil samples were air dried at room temperature and treated with a heat and smoke application to stimulate the germination of seeds of those species with a fire cue (Enright *et al.* 1997; Kenny 2000; Read *et al.* 2000). Air dried samples were heated at 80°C for 15 minutes in a preheated oven. To minimise the loss of heat when the oven door was opened, the time to heat the samples did not commence until the oven thermostat indicated that the oven had returned to 80°C. After heating the samples were returned to the glasshouse and watered with one application of diluted (1:10) smoke water, which was prepared by drawing smoke from burning a mix of fresh and dried eucalypt litter through two litres of deionised water for 30 minutes using a water smoke apparatus. To ensure independence of replication (Morrison and Morris 2000), the application of the heat and smoke treatments were randomly allocated to batches of samples from the same transect for each community type. There were five batches of six trays of soil. The average minimum and maximum temperatures in the glasshouse for the duration of the experiment were 14°C and 29°C, respectively. Seedling trays were randomly placed in the glasshouse and watered to keep the soil moist. Monitoring of seedling emergence continued every week for a further 10 weeks. Any unidentified seedlings were replanted into larger plots for later identification.

Species recorded in the soil seed bank were grouped on their known response to fire (Hunter 1998; NSWFFRD 2002; Sheringham and Hunter 2002; Clarke *et al.* 2009) and their germination cue (Floyd 1976; Warcup 1980; Hopkins and Graham 1984; Auld and O'Connell 1991; Dixon *et al.* 1995; Keith 1996; Enright *et al.* 1997; Keith 1997; Bell 1999; Read *et al.* 2000; Enright and Kintrup 2001; Wills and Read 2002; Hill and French 2003; Tang *et al.* 2003; Thomas *et al.* 2003; Clarke and French 2005; Ooi *et al.* 2006; Thomas *et al.* 2007; Penman and Towerton 2008). The most abundant species from each community were compared on life form patterns, their germination response, and the dominant families sampled in each burn severity class. Unidentified species were not included in these comparisons.

Statistical analysis

A three-way general linear model was used to compare differences between fire severity and communities. The factors tested in the model were: community, severity and site (nested in communities). The variables assessed in the model were total species richness and abundance of seedlings emerging from the soil seed bank of each community. Post-hoc Scheffe’s test was used to test for differences between levels of a significant factor. All univariate statistical analyses were performed using DataDesk® 6.1 (Velleman 1997). Data were tested for normality using box plots where the response variable was plotted for each community, and transformed to satisfy assumptions of normality where appropriate (Zar 1984).

Differences in species composition between severity treatments and communities were compared using a two-way PERMANOVA tested using a Bray-Curtis similarity matrix. Pairwise tests were used to determine where differences occurred. A similarity percentage (SIMPER) analysis was used to determine the average similarity for each severity treatment, based on the Bray-Curtis index of similarity, for species emerging from the soil seed bank of each community, which contributed most strongly to similarities among samples within each severity treatment. Analyses were performed on abundance data using PRIMER v6 (Clarke and Gorley 2006) and PERMANOVA+ (Anderson *et al.* 2008) and were transformed where appropriate to improve underlying distribution.

To determine if germination of species within each community was associated with a particular burn severity treatment, χ^2 analysis was used. The null expectation was no difference in abundance among treatments. Species used in this analysis recorded ten or more individuals germinating from the soil seed bank of each community, as χ^2 analyses are not valid when expected values are low (Zar 1984).

To determine if the proportion of obligate seeders and resprouting species in each community was affected by fire severity, a log-likelihood ratio analysis was used. The log-likelihood ratio analysis statistic (G test) has an approximate distribution of χ^2 (Zar 1984).

Results

Response to severity: Species richness and abundance

Burn severity had a significant effect on species germinating from the soil seed bank of all communities, with more species germinating in the unburnt treatment compared with the low and high severity treatments (Fig. 1; Table 1). Generally, within each community, the number of species emerging from the soil seed bank was similar across severity treatments (Fig. 1). Between communities, the number of individuals emerging from the soil seed bank varied, with significantly more individuals sampled in the Rainforest community compared with the Dry Sclerophyll Forest community (Fig. 1b; Table 1). Fire severity appeared to affect the different plant communities in different ways (Fig. 1), but there was no significant interaction between community and fire severity for both variables (Table 1) and there was considerable variation in the data for some samples (e.g. see Fig. 1b, abundance of individuals in rainforest) There was a significant site effect and the interaction between site and severity was also significant, indicating the patchiness of the soil seed bank across sites within each community and fire severity contributed to the non-significant effect of fire severity within each community (Table 1).

Table 1. *F*-statistic and *p*-value of the General Linear Model for (a) species richness and (b) abundance of individuals emerging from the soil seed bank of five communities.

Factor	d.f.	(a) Species richness		(b) Abundance	
		F	*P*	F	*P*
Community	4	3.822	0.087	**6.664**	**0.031**
Severity	2	**7.121**	**0.012**	3.439	0.074
Community x Severity	8	0.673	0.707	0.273	0.961
Site (Community)	5	**8.456**	**<0.0001**	**4.136**	**0.002**
Site (Community) x Severity	10	**4.121**	**<0.0001**	**5.263**	**<0.0001**

Significant values in bold

Response to severity: Species composition

Soil seed bank samples clustered according to vegetation community type and within vegetation community in relation to burn severity (Fig. 2). A two-way PERMANOVA, used to test the effect of burn severity on the species composition, found a significant interaction between community and fire severity, with pairwise comparisons indicating significant differences between severities among all communities (except RO: low versus high, Table 2). Average similarity values (using SIMPER analysis) within severity treatments for each community ranged from 19 (in the low severity treatment of WSF) to 55% (in the high severity treatment of WH), indicating similarity within severity treatments was quite variable (Table 3).

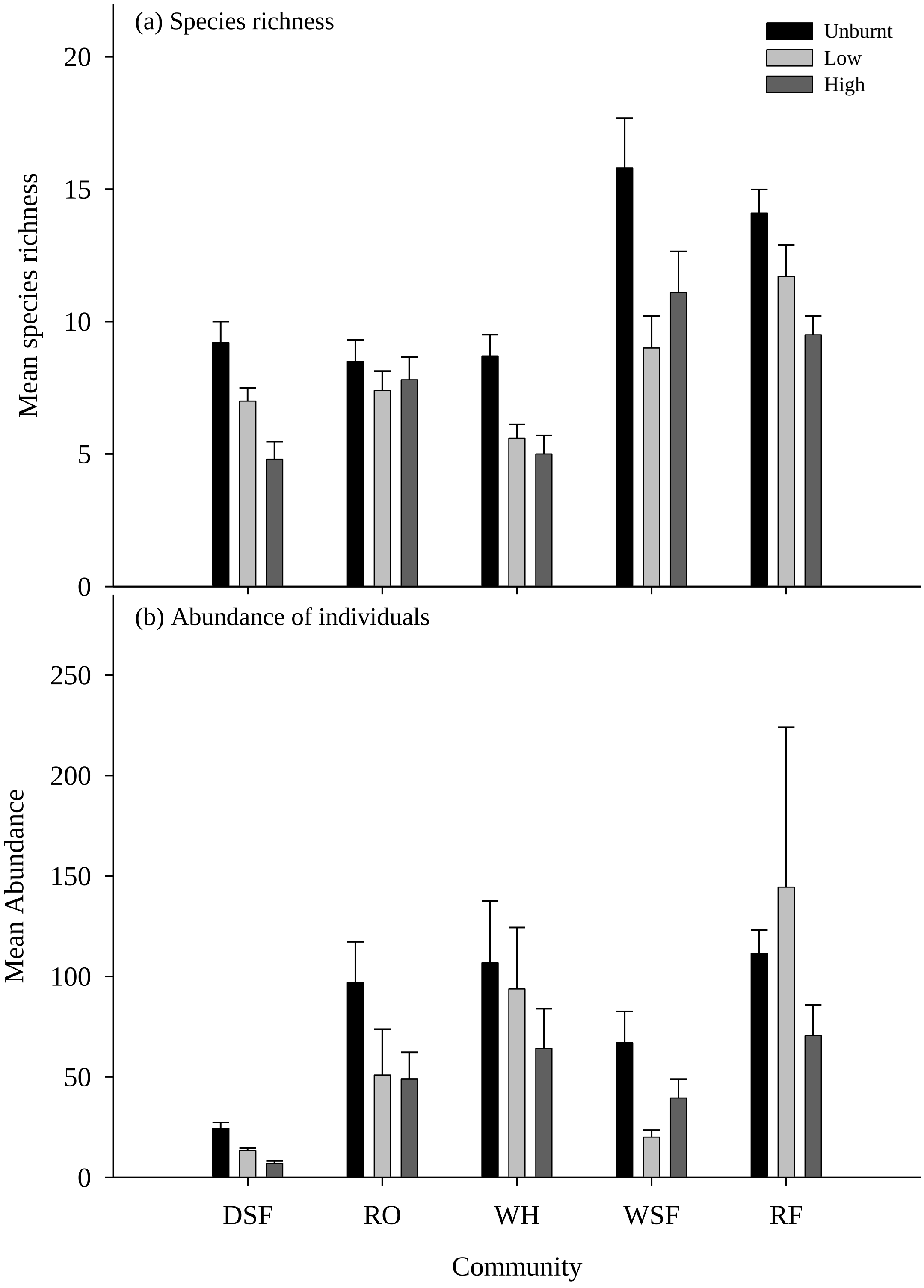

Fig. 1. Effect of burn severity on the emergence of (a) species (+ s.e.) and the (b) abundance of individuals (+ s.e.) from the soil seed bank of five communities. Communities referred to as DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

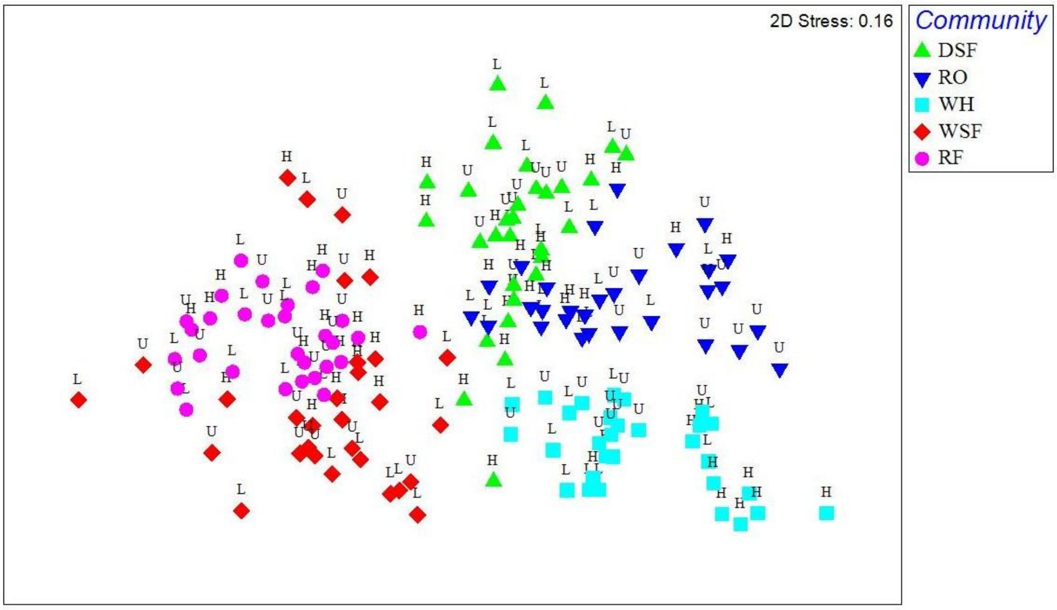

Fig. 2. Ordination of samples (nMDS) in two dimensions for the abundance of species recorded from the soil seed bank of each community within each severity treatment. Resemblance is based on Bray-Curtis similarity index. Stress = 0.16. Communities referred to: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest. Burn severities referred to as: U = unburnt, L = Low severity, H = High severity.

Table 2. PERMANOVA identifying the effect of burn severity on the species composition of the soil seed banks of five communities.

Factor		d.f.	Pseudo F	P (Perm)
Community		4	25.315	**0.0001**
Severity		2	3.247	**0.0001**
Community x Severity		8	4.035	**0.0001**
Pairwise			T statisitc	P (Perm)
DSF[†]	Low *vs.* High		1.661	**0.005**
	Low *vs.* Unburnt		1.580	**0.0077**
	High *vs.* Unburnt		2.305	**0.0001**
RO	Low *vs.* High		1.352	0.080
	Low *vs.* Unburnt		1.916	**0.0010**
	High *vs.* Unburnt		2.041	**0.0017**
WH	Low *vs.* High		2.482	**0.0003**
	Low *vs.* Unburnt		2.296	**0.0001**
	High *vs.* Unburnt		2.944	**0.0001**
WSF	Low *vs.* High		1.692	**0.0004**
	Low *vs.* Unburnt		1.867	**0.0004**
	High *vs.* Unburnt		1.967	**0.0002**
RF	Low *vs.* High		2.162	**0.0001**
	Low *vs.* Unburnt		1.820	**0.0027**
	High *vs.* Unburnt		1.818	**0.0052**

Significant values in bold. [†]DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest

Table 3. Average similarity indices (calculated from average Bray-Curtis similarity index) for the soil seed banks of five communities within each burn severity category.

Community	Unburnt	Low	High
Dry Sclerophyll Forest	40.17	24.33	24.96
Rocky Outcrop	37.98	33.65	31.85
Wet Heath	47.75	38.37	55.94
Wet Sclerophyll Forest	29.32	19.61	30.73
Rainforest	43.77	39.83	40.15

Response to severity: Individual species

The most abundant species sampled from the soil seed banks of the different communities had a varied response to burn severity. Responses varied from positive to negative or no effect (Table 4). A number of species representing a variety of families and life forms showed no preference for germinating under any particular severity treatment across all communities (Table 4, $P > 0.05$). These species fell into two categories: the first were mostly exotic, obligate seeding species from the Asteraceae family (e.g. *Conyza sumatrensis*, *Euchiton involucratus*, *Gamochaeta spicata*) which have an ephemeral seed bank and wind dispersed seeds. The second group were mostly native species with no clear trends in terms of fire responses, life forms or seed germination response.

Burn severity had either a positive or negative effect on key family groups and life forms for the most abundant species emerging from the soil seed bank (Fig. 3; Table 4). Tree, grass, graminoid and twiner species had a negative response to fire, with proportionally more individuals emerging in the unburnt treatment compared to the low and high severity treatments. In contrast, the herbaceous species responded positively to low burn severity with proportionally more individuals in the low severity treatment only, whereas there was no distinct pattern for shrub species. Severity had a negative impact on three families with proportionally more individuals emerging in the low and unburnt treatments compared to the high severity treatment. This pattern could be attributed to one species in each family (Apiaceae: *Hydrocotyle pedicellosa*, Cunoniaceae: *Ackama paniculata* and Myrtaceae: *Baeckea omissa*; Table 4). Species from the Asteraceae family germinated across all treatments, indicating burn severity did not affect their emergence from the soil seed bank, although individual species did showed a positive or negative trend to severity. Species from the Ericaceae family also showed a varied response to burn severity, with overall more individuals germinating in the unburnt treatment. This pattern could be attributed largely to

Epacris obtusifolia which dominated the soil seed bank of the Wet Heath community (Table 4).

Burn severity had a positive or negative effect on species when classified on their germination cue (Fig. 4). Species which where classified into the combined heat and smoke response group were the least abundant in the high severity treatment. Species which had a smoke only response were most abundant in the low severity treatment, whereas species which had a heat only seed germination response were most abundant in the unburnt and high severity treatments. Those species that did measure a response to heat or smoke were the most abundant in the unburnt treatment (Fig. 4).

Sixteen species sampled in more than one community responded differently to burn severity across communities. In most cases the difference was between the unburnt treatment in one community versus a severity treatment in the other community in which the species was sampled (e.g. *Epacris gunni*, RO and WH; Table 4), indicating responses varied between communities.

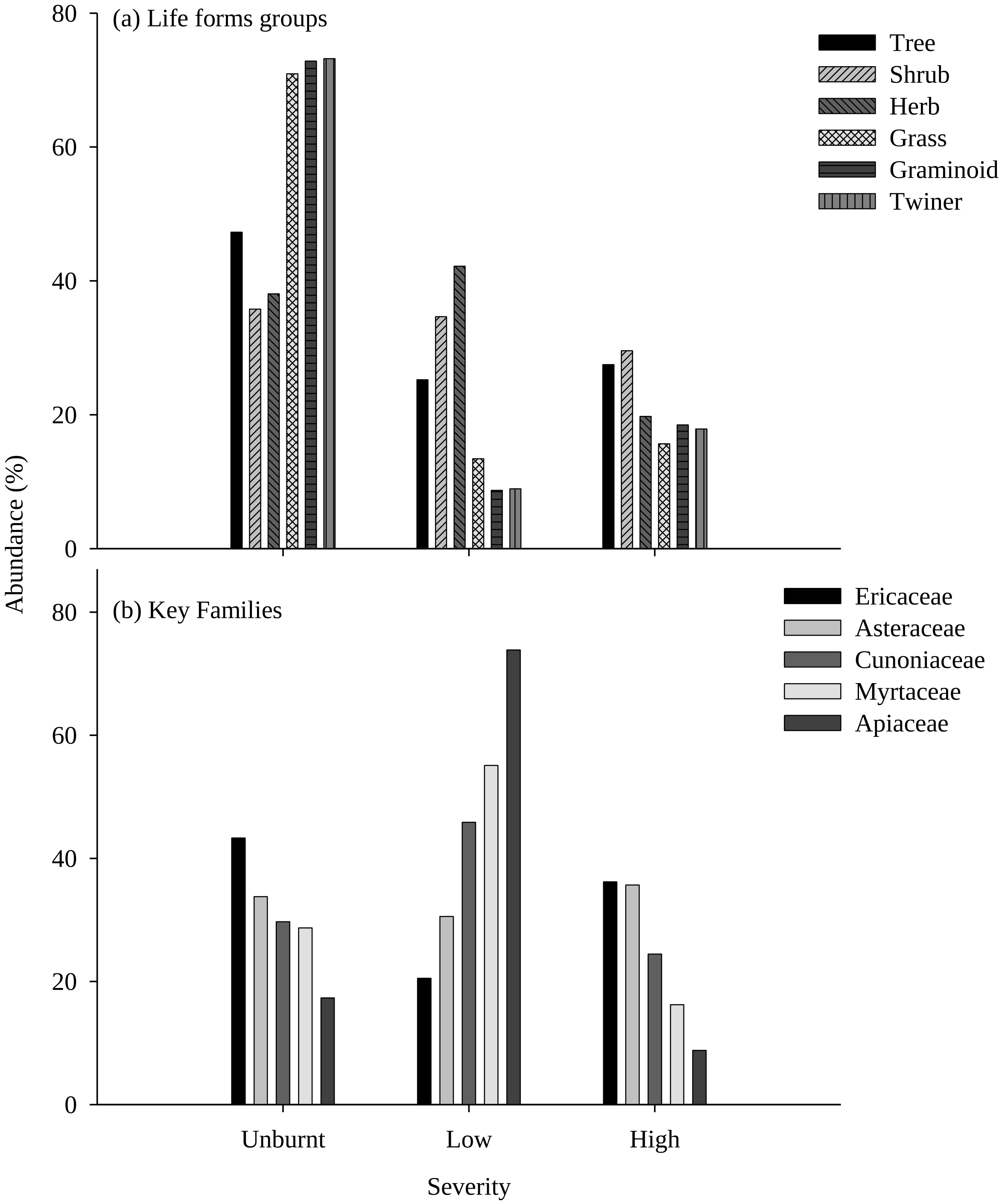

Fig. 3. Responses to different burn severities of (a) Life form groups and (b) Key family groupings of the most abundant species. Percentage abundance is based on groupings among treatments.

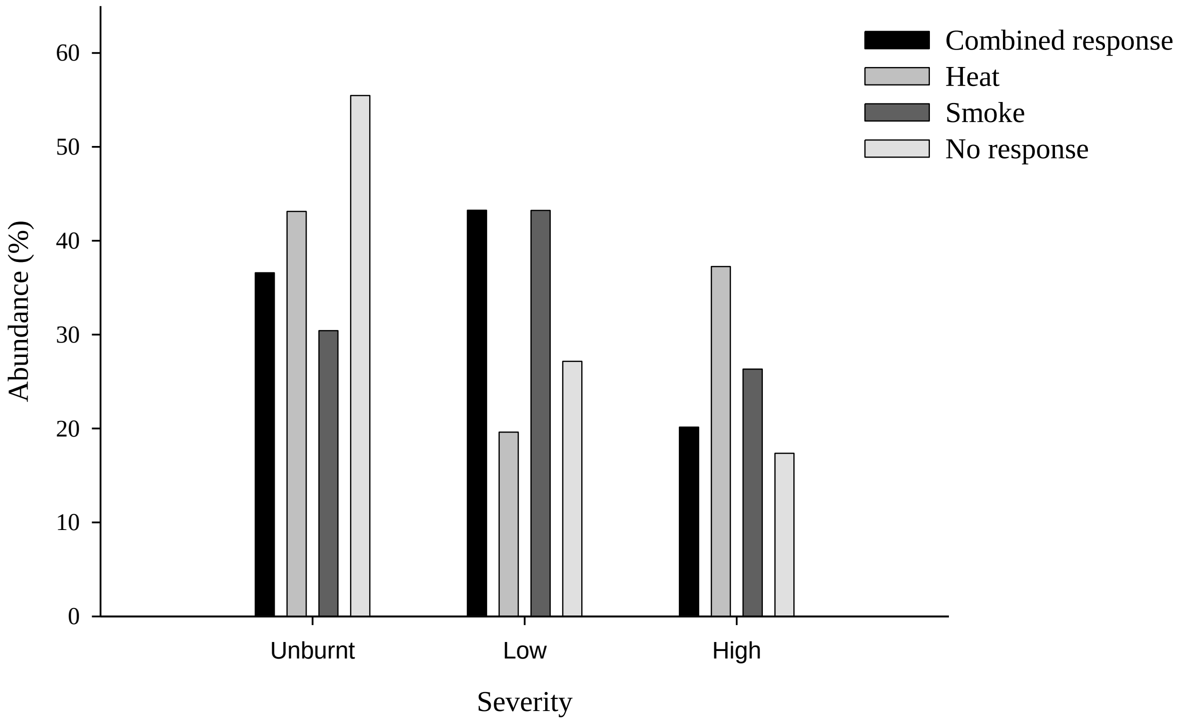

Fig. 4. Responses to burn severity of the most abundant species based on their seed germination response. Combined heat/smoke category has not distinguished between whether the combined response is unitive or obligatory, independent and additive or synergistic.

Table 4. Species germinated (and their abundance) from the soil seed banks of five communities across burn severity treatments. To be included, species had to have more than 10 seedlings germinate across all severity treatments.

Species	Fire response†	Germination cue‡	Unburnt	Low	High	χ^2 probability
Dry Sclerophyll Forest						
Austrostipa rudis	R	S	8	3	0	**0.0116**
**Conyza sumatrensis*	OS	N?	12	5	11	0.2153
Dillwynia phylicoides	OS	H	14	9	0	**0.0014**
Entolasia stricta	R	N	60	14	7	**<0.0001**
**Euchiton involucratus*	OS	H	3	5	3	0.6951
Gamochaeta calviceps	OS	N?	4	9	4	0.2297
**Gamochaeta spicata*	OS	N?	6	5	9	0.5220
Goodenia bellidifolia	R	C	0	12	0	**0.0000**
Goodenia hederacea	R	C	13	0	0	**<0.0001**
Leptospermum trinervium	R	C	34	20	4	**<0.0001**
Leucopogon microphyllus	OS	C	0	3	9	**0.0052**
Platysace ericoides	R	C	16	9	3	**0.0107**
Prostanthera saxicola var. *major*	OS	H	33	0	0	**<0.0001**
Rocky Outcrop						
Boronia anethifoia	OS	C	3	6	15	**0.0076**
Calytrix tetragonia	OS	N	12	0	3	**0.0004**
**Conyza sumatrensis*	OS	N?	1	9	5	**0.0407**
Drosera spatulata	R	N	265	148	0	**<0.0001**
Entolasia stricta	R	N	20	22	40	**0.0118**
Epacris gunni	R	C	177	0	0	**<0.0001**
**Euchiton involucratus*	OS	H	2	4	6	0.3678
**Gamochaeta spicata*	OS	N?	4	10	11	0.1790
Gonocarpus micranthus subsp *micranthus*	OS	H	20	0	0	**<0.0001**
Goodenia bellidifolia	R	C	15	0	0	**<0.0001**
Graminoid sp. 20	unknown	unknown	24	0	18	**<0.0001**
Graminoid sp. 23	unknown	unknown	16	1	0	**<0.0001**
Kunzea bracteolata	R	C	104	39	117	**<0.0001**
Laxmannia gracilis	OS	C	215	179	186	0.1519
Lepidosperma gunnii	R	H	14	2	13	**0.0101**
Leptospermum trinervium	R	C	1	4	20	**<0.0001**
Leucopogon microphyllus	OS	C	1	29	0	**<0.0001**
Micromyrtus sessilis	R	C	30	0	0	**<0.0001**
Mirbelia rubiifolia	OS	H	16	18	7	0.0810

Table 4. Species germinated (and their abundance) from the soil seed banks of five communities across burn severity treatments cont.

Species	Fire response[†]	Germination cue[‡]	Unburnt	Low	High	χ^2 probability
Monotoca scoparia	R	H	11	2	1	**0.0015**
Stylidium laricifolium?	OS	C	0	0	13	**<0.0001**
Wet Heath						
Baeckea omissa	R	C	21	440	4	**<0.0001**
Caesia parvifolia var. *minor*	R?	S	12	0	1	**<0.0001**
Drosera spatulata	R	N	379	189	83	**<0.0001**
Empodisma minus	R	unknown	80	12	8	**<0.0001**
Entolasia stricta	R	N	63	3	2	**<0.0001**
Epacris gunni	R	C	0	34	1	**<0.0001**
Epacris obtusifolia	OS	S	364	194	451	**<0.0001**
**Gamochaeta spicata*	OS	N?	11	4	2	**0.0194**
Gonocarpus micranthus subsp. *micranthus*	OS	H	70	25	80	**<0.0001**
Graminoid sp.11	unknown	unknown	14	0	1	**<0.0001**
Laxmannia gracilis	OS	C	0	20	2	**<0.0001**
Wet Sclerophyll Forest						
Ackama paniculata	OS	unknown	83	0	39	**<0.0001**
Archirhodomytrus beckleri	R	unknown	60	0	0	**<0.0001**
Berbridopsis bechlei	OS	unknown	24	6	2	**<0.0001**
Billardiera scandens	R	C	8	0	4	**0.0183**
Callitriche muelleri	unknown	unknown	18	0	0	**<0.0001**
**Conyza sumatrensis*	OS	N?	2	1	8	**0.020**
Cyperus disjunctus	R	H	38	0	0	**<0.0001**
Galium binifolium	OS	S	6	1	20	**<0.0001**
**Gamochaeta spicata*	OS	N?	10	11	13	0.8139
Geranium solanderi	R	H	15	0	2	**<0.0001**
Gonocarpus humilis	OS	C	2	0	10	**0.0009**
Gonocarpus oreophilus	OS	C	0	35	2	**<0.0001**
Hedycarya angustifolia	R	unknown	10	3	1	**0.0083**
Helichrysum elatum	OS	N	4	3	28	**<0.0001**
Hydrocotyle pedicellosa	R	S	0	3	58	**<0.0001**
Hydrocotyle peduncularis	R	S	49	10	17	**<0.0001**
Juncus usitatus	R	C	15	9	1	**0.0026**
Lobelia trigonocaulis	R	unknown	76	0	1	**<0.0001**
Olearia nernstii	OS	N	23	11	0	**<0.0001**
Oplismenus imbecillis	R	S	71	0	0	**<0.0001**

Table 4. Species germinated (and their abundance) from the soil seed banks of five communities across burn severity treatments cont.

Species	Fire response†	Germination cue‡	Unburnt	Low	High	χ² probability
Oxalis exilis	R	S	3	6	1	0.1495
Ozothamnus rufescens	OS	H	2	22	2	**<0.0001**
Rubus rosifolius	R	N	6	7	10	0.5682
Senecio minimus	OS	N	14	0	0	**<0.0001**
**Sigesbeckia orientalis* subsp. *orientalis*	OS	S	7	2	1	**0.0450**
Solanum aviculare	OS	S	4	3	15	**0.0023**
Solanum vicinum	OS	S	1	0	19	**<0.0001**
Veronica notabilis	OS	unknown	0	3	8	**0.0116**
Viola hederacea	OS	N	30	6	19	**0.0003**
Zieria sp. aff. *smithii*	OS	H	13	15	71	**<0.0001**
Rainforest						
Acacia melanoxylon	R	H	38	5	1	**<0.0001**
Ackama paniculata	OS	unknown	137	394	95	**<0.0001**
Ameilema acuminatum	R	unknown	0	20	0	**<0.0001**
Berberidopsis beckleri	OS	unknown	31	10	34	**0.0010**
Callicoma serratifolia	R	H	52	41	98	**<0.0001**
Cissus hypoglauca	R	unknown	54	5	2	**<0.0001**
Clematis aristata	R	C	55	0	0	**<0.0001**
**Conyza sumatrensis*	OS	N	5	3	7	0.4493
Galium binifolium	OS	S	1	21	0	**<0.0001**
Gamochaeta calviceps	OS	N	4	5	7	0.6456
**Gamochaeta spicata*	OS	N	8	5	6	0.6918
Graminoid sp. 25	unknown	unknown	0	0	10	**<0.0001**
Hydrocotyle laxifolia	R	S	0	26	1	**<0.0001**
Hydrocotyle pedicellosa	R	S	106	643	7	**<0.0001**
Hydrocotyle peduncularis	R	S	0	38	1	**<0.0001**
Lobelia trigonocaulis	R	unknown	1	122	83	**<0.0001**
Ozothamnus rufescens	OS	H	4	0	10	**0.0043**
Quintinia sieberi	R	unknown	452	5	255	**<0.0001**
Rorippa dictyosperma	OS	unknown	1	3	18	**<0.0001**
Rubus moluccana	R	N	7	5	2	0.2573
Rubus rosifolius	R	N	34	7	31	**0.0001**
Schizomeria ovata	R	unknown	11	2	1	**0.0015**
Solanum aviculare	OS	S	46	29	1	**<0.0001**
Solanum opacum	OS	S	13	1	0	**<0.0001**

Table 4. Species germinated (and their abundance) from the soil seed banks of five communities across burn severity treatments cont.

Species	Fire response[†]	Germination cue[‡]	Unburnt	Low	High	χ^2 probability
Solanum vicinum	OS	S	1	3	6	0.1495
Tasmannia insipida	OS	unknown	6	2	4	0.3678
Zieria sp. aff. *smithii*	OS	H	0	9	4	**0.0091**

Significant values in bold

*exotic species

[†] Fire response refers to R = Resprouter, OS = Obligate seeder (NSWFFRD 2002; Sheringham and Hunter 2002; Clarke *et al.* 2009)

[‡] Germination cue refers to S = Smoke response, H = Heat response, C = Combined Smoke/Heat response and N = No response to heat or smoke. This has been inferred from genera/species reported in the literature (Floyd 1976; Warcup 1980; Hopkins and Graham 1984; Auld and O'Connell 1991; Dixon *et al.* 1995; Keith 1996; Enright *et al.* 1997; Keith 1997; Bell 1999; Read *et al.* 2000; Enright and Kintrup 2001; Wills and Read 2002; Hill and French 2003; Tang *et al.* 2003; Thomas *et al.* 2003; Clarke and French 2005; Ooi *et al.* 2006; Thomas *et al.* 2007; Penman *et al.* 2008).

Response to severity: Fire response categories

Resprouting species were proportionally greater in the soil seed bank of long unburnt vegetation across all communities compared to obligate seeding species (Table 5a). In the more fire-prone communities this pattern did not change across the two sampled burn severities (Table 5a: RO, WH). The soil seed bank of the Dry Sclerophyll Forest community showed a different pattern with a significantly greater proportion of resprouting species emerging from the soil seed bank at low severity but not at high severity (Fig 5a). In contrast, proportionally fewer resprouting species were detected in the mesic communities as severity increased and this was significant for the obligate seeders emerging from the soil seed bank of the Wet Sclerophyll Forest community (Fig. 5b; Table 5a).

In the soil seed bank of unburnt vegetation, the abundance of resprouters was proportionally greater across all communities than obligate seeding species (Fig 6; Table 5a). Based on proportional representation of individuals, resprouting individuals from three communities significantly decreased with increasing severity (Fig. 6a, c, d; Table 5b). This pattern was only detected in the soil seed bank of the Rainforest community in the low severity treatment (Fig 6e; Table 5), whereas the pattern was apparent in the Rocky Outcrop community regardless of severity treatment with significantly more resprouting individuals emerging across all treatments (Fig. 6b; Table 5).

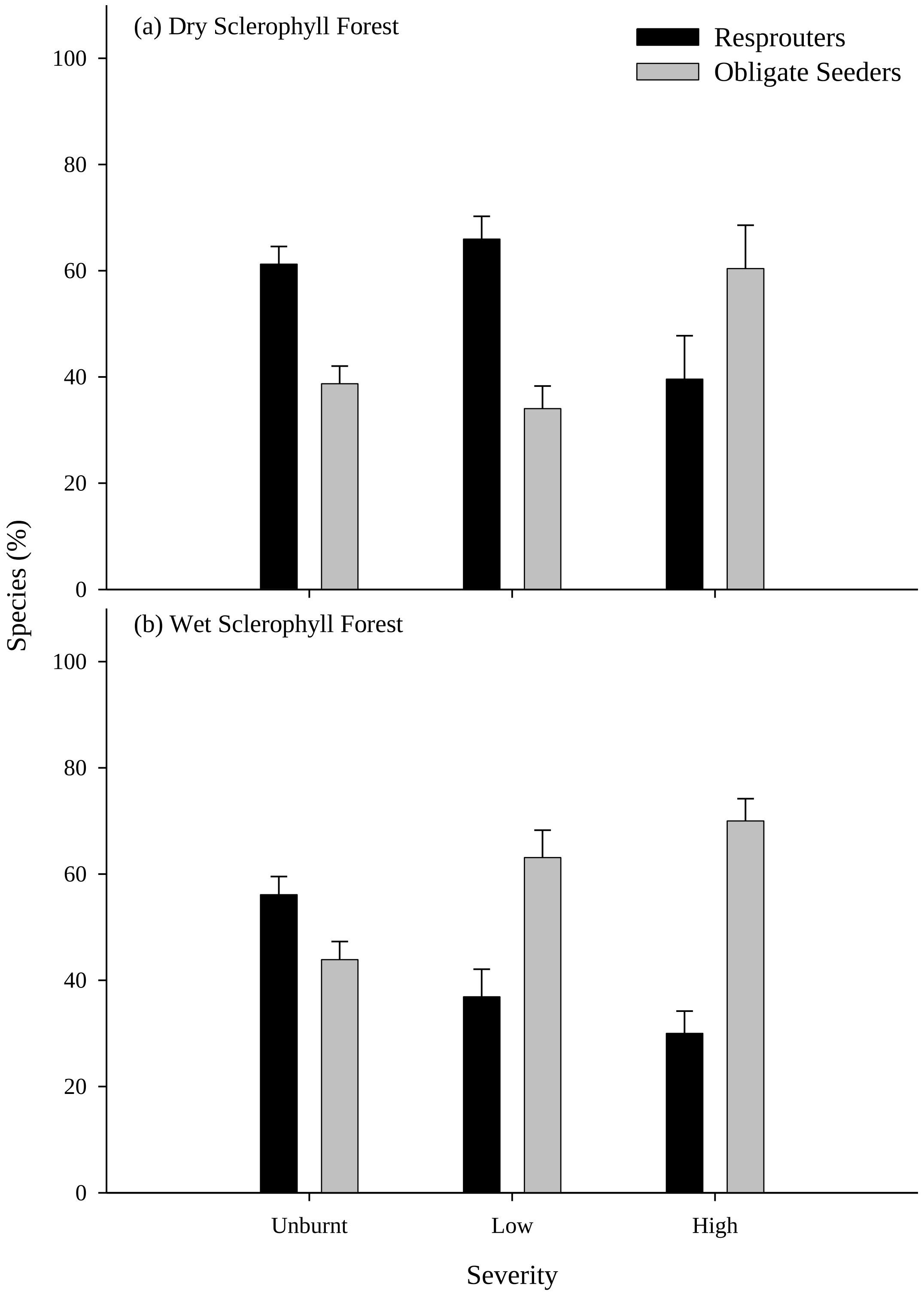

Fig. 5. Effect of burn severity: Percentage of species (+ s.e.) emerging from the soil seed bank of the (a) Dry Sclerophyll Forest and the (b) Wet Sclerophyll Forest communities based on their sprouting ability.

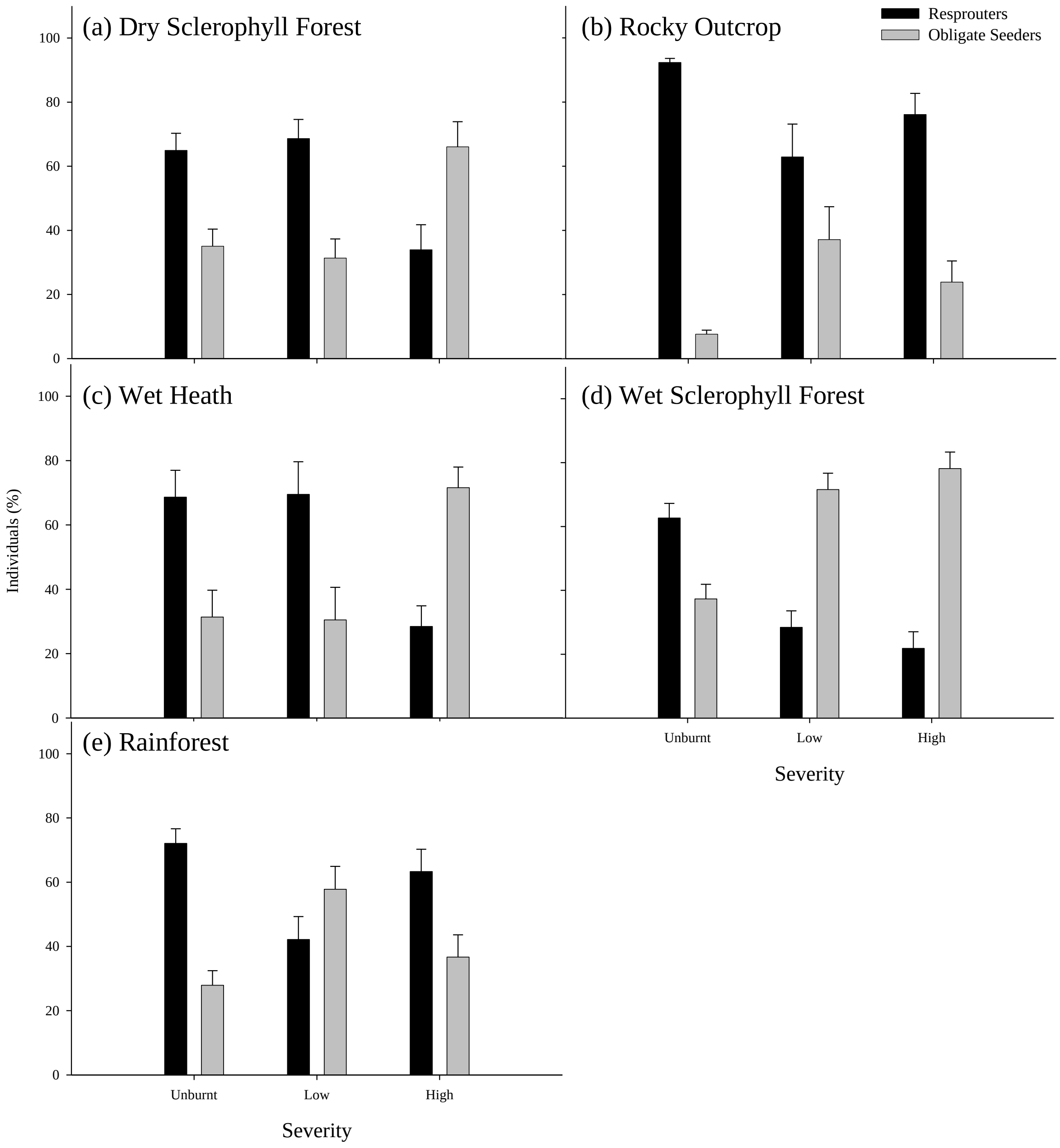

Fig. 6. Effect of burn severity: Percentage of individuals (+ s.e.) emerging from the soil seed bank of the (a) Dry Sclerophyll Forest (b) Rocky Outcrop, (c) Wet Heath, (d) Wet Sclerophyll Forest and (e) Rainforest communities based on their sprouting ability.

Table 5. Proportion of (a) species richness and (b) abundance based on fire response sampled from different severities within each community.*

	Fire response[†]	Unburnt	Low severity	High severity	G test probability
(a) Species richness					
Dry Sclerophyll Forest					**0.0003**
	OS	39	34	60	
	R	61	66	40	
Rocky Outcrop					0.0674
	OS	30	46	36	
	R	70	54	64	
Wet Heath					0.0765
	OS	30	35	45	
	R	70	65	55	
Wet Sclerophyll Forest					**0.0005**
	OS	44	63	70	
	R	56	37	30	
Rainforest					0.4748
	OS	48	52	57	
	R	52	48	43	
(b) Abundance					
Dry Sclerophyll Forest					**≤ 0.0001**
	OS	35	31	66	
	R	65	69	34	
Rocky Outcrop					**≤ 0.0001**
	OS	8	37	24	
	R	92	63	76	
Wet Heath					**≤ 0.0001**
	OS	31	30	72	
	R	69	70	28	
Wet Sclerophyll Forest					**0.0005**
	OS	37	72	78	
	R	63	28	22	
Rainforest					**≤ 0.0001**
	OS	72	58	63	
	R	28	42	37	

*Proportions were calculated as means for the fire response in each severity treatment.

[†] R = Resprouter, OS = Obligate seeder. Significant values in bold.

Discussion

Seedling emergence from a soil seed bank is important for the recovery of communities following a fire event, especially for those species relying on regeneration from a soil seed bank. The magnitude of soil heating increases with fires of increasing intensity and the proportion of the soil seed bank germinating after the passage of fire and ultimately the size of the seed bank which remains dormant depends on the extent of soil heating during the fire (Auld 1987; Whelan 1995). Species with a soil seed bank are at risk of population extinction, where soil heating is high due to the consumption of large quantities of ground fuel, when fire residence times are long. Conversely, species that have germination promoted by heat are also vulnerable to population extinction, where inadequate soil heating occurs due to the low consumption of ground fuel, when fire residence times are short (Keith 1996). The soil seed banks of five communities were sampled more than six years post-fire in areas which had experienced different burn severities. How well the soil seed bank recovered during this time is dependent on whether seeds survived soil heating during the fire and remained dormant, along with the accumulation of seed of species regenerating in a post-fire environment. In this study, burn severity had an affect on the seed bank of species of different communities in a variety of ways. Soil seed banks in unburnt sites were generally more speciose and abundant than sites burnt during the fires of 2002 (Fig. 1; Table 1). This suggests that, six years post-fire, the burnt areas had not yet fully replenished their soil seed banks. This is also illustrated by the separation of the unburnt sites from the burnt sites based on species composition (Fig. 2). Generally, fire with increasing severity had depleted the number and abundance of species emerging from the soil seed bank across all communities although this trend was non-significant ($P > 0.05$). A similar trend was also detected when species were categorised on life forms; abundances of all life forms were greater in the soil seed bank of unburnt vegetation compared to burnt vegetation (Fig. 3a). Strong site effects and large amounts of variation within treatments are likely to contribute to the non-significant result (Fig. 1; Table 1).

In an immediate post-fire environment, species regenerating from the soil seed bank are affected by burn severity. Species-specific responses have shown either negative or positive effects of soil heating. In Chaparral communities, for example, there is generally more regeneration where soil heating was low (Tyler 1995; Odion and Davis 2000), whereas those species where germination is promoted by heat had a positive response to high severity fire (Keeley *et al.* 2005). Severity can, therefore, have an impact on post-fire community recovery and composition, particularly for different functional groups (Moreno and Oechel 1991). In this study six years post-fire, burn severity had an impact on the proportion of species germinating with different fire response strategies. In the Dry and Wet Sclerophyll Forest communities, more obligate seeding species germinating at higher burn severities than resprouting species, indicates that changes in the composition of both fire-prone and mesic communities are likely with fires of different severities (Fig. 5; Table 5). However, the extent of this may vary among communities, depending on species' responses to heat and the input of seeds into the soil seed bank following a fire. The Wet Sclerophyll Forest appears to be more sensitive to changes in burn severity, with more obligate seeding species emerging in the low severity treatment (Fig. 5b). Species sampled in mesic communities are killed at lower temperatures than species from fire-prone communities (Hopkins and Graham 1984). The species forming the soil seed bank in the Wet Sclerophyll Forest may be more sensitive to changes in soil temperature during fires, these species having lower lethal temperature thresholds compared to the more fire-prone communities. This is in contrast to other studies that found less resprouting species emerging from the soil seed bank after high intensity fires (Moreno and Oechel 1991), while high severity fires had a positive effect on non-sprouting species with seed dormancy triggered by heat (Keeley *et al.* 2005). This also demonstrates severity can affect communities differently.

The species composition in this study was affected by burn severity, with samples clustering within each community based on severity type (Fig. 2; Table 2). This result could be attributed to either species being unique to a particular treatment or species present in all treatments but in different abundances. Very few species, however, were unique to one particular burn severity (Table 4); major differences are more likely due to changes in the number of individuals sampled from different severity treatments (Table 4, e.g. *Boronia anethifolia*, *Epacris obtusifolia*).

Weedy Asteraceae species with wind-dispersed seed were the major component of the soil seed bank from both fire-prone and mesic communities that were unaffected by burn severity. Those species in the mesic communities unaffected by burn severity include fleshy-fruited species usually dispersed by animals (Table 4, e.g. *Rubus*, *Tasmannia* species), although patterns were not consistent within all taxa (Table 4, e.g. *Solanum* species). All these species are likely to have been dispersed into the post-fire environment rather than have survived the fire itself and act as opportunistic fire-sensitive invaders (Ferrandis *et al.* 1999). In the Rocky Outcrop community the two species unaffected by burn severity (Table 4, *Mirbelia rubiifolia*, *Laxmannia gracilis*) are likely to have survived the fire and replenished their seed banks because of a short primary (*M. rubiifolia*, three years) and secondary (*L. gracilis*, one year) juvenile periods. Legume species were expected to be greatly affected by soil heating, however, this group was under represented in this study with only three species emerging from the seed bank in significant numbers (Table 4). One species (*M. rubiifolia*) as previously discussed was unaffected by burn severity, whereas the other two species (Table 4, *Acacia melanoxylon*, *Dillwynia phylicoides*) had significantly more emergence in the low severity and unburnt sites compared to the high severity sites (Table 4). This trend for both species is likely to be due to two factors. First, the soil seed banks have been exhausted at high severity sites by the mortality of seed during the fire, by germination from the seed bank, or the lack of detectable residual soil seed bank (Auld and Denham 2006). Second, the detection at high severity sites is minimal because of insufficient time to replenish the seed bank of both species due to their primary (*D. phylicoides*, five years) and secondary juvenile periods (*A. melanoxylon*, > three years). When species were grouped on their requirements to break seed dormancy (Fig. 4), however, heat responders had the opposite trend with greater abundances in the unburnt and high severity sites. Species where germination is promoted by the interaction between heat and smoke were least abundant in the high severity sites, as were the smoke responders, indicating severity may influence the pattern of the residual soil seed bank even for those species that respond to smoke (Auld and Denham 2006). This implies the impact of smoke on promoting germination of soil-stored seed is not uniform across all fire severities.

Recruitment of species from the soil seed bank after a fire will be affected by the extent of soil heating, the heat and smoke requirements that promote germination and the distribution of seeds in the soil profile. The soil seed banks of different species will vary depending on the accumulation of seeds in the soil, seed longevity within seed banks and the proportion of the seed bank that germinates following a fire (Auld 1994). It is therefore difficult studying the impact of burn severity on the soil seed bank in a post-fire environment of more than six years as the rate of recovery will vary for different species.

Recovery over a long-term period may actually result in no difference between areas of different fire severities in terms of species composition and functional groups. The effects of fire severity may be short-lived with communities capable of recovery within 4–5 years post-fire (Odion and Davis 2000; Keeley *et al.* 2008), but with a greater reduction in the residual soil seed bank the time needed for recovery after severe fires may be longer. Where difference may occur is when the fire return interval is not long enough to allow for the replenishment of the seed bank of species which relying on seeds to regenerate following fire. This is particularly important if fires of increasing severity have decreased the size of the residual soil seed bank (Auld and Denham 2006). The assumption has been that species killed by fire with a soil-stored seed bank are able to regenerate from residual seeds in the soil. However, if the entire seed bank has been depleted during a single fire event and the fire-return interval is too short to allow for replenishment of the seed bank, then plant populations become vulnerable to local extinction. Fire management needs to incorporate the interaction between fire frequency and severity to ensure plant populations persist in the landscape. This study demonstrates burn severity can affect species in different communities in a variety of ways. High burn severity generally resulted in a smaller soil seed bank than low burn severity, for a number of species and plant functional groups across all communities, although the extent of differences between burnt and unburnt sites was greater in magnitude.

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Chapter 4. The effect of time since fire on the soil seed banks of five different plant communities.

Introduction

Seed banks are an important mechanism allowing species to persist in a landscape which experiences disturbance events such as fire. Species either have canopy-held (Bradstock and Myerscough 1981; Zammit and Westoby 1987a; Lamont *et al.* 1991) or soil-stored (Auld 1986; Parker and Kelly 1989; Enright *et al.* 2007) seed banks, which buffer plant populations against environmental variability and reduce the risk of species loss and population declines as a result of disturbances (Parker *et al.* 1989; Keith 1996; Thompson 2000). This is particularly important for obligate seeders which are killed by fire and rely on this seed source to regenerate, as opposed to species which resprout and can regenerate vegetatively as well as from seeds (Gill 1981).

Time since fire may affect the species of different plant communities in a number of ways. High and low fire frequency can increase extinction risk through mechanisms such as the death of adult plants, as well as the interruption of maturation and developmental growth of individuals. Low fire frequency can also increase extinction risk through the failure of seed release or seed germination, and the failure of seedling establishment and seed production (Keith 1996). Time since fire can impact on the standing vegetation in some communities, where health and composition benefit from frequent fire (Lunt 1994; Morgan and Lunt 1999), but not for some species in other communities (Keeley and Zedler 1978; Russell and Parsons 1978; Zedler *et al.* 1983; Nieuwenhuis 1987; Penman *et al.* 2009). In different plant communities the impact of time since fire may vary, with species in more fire-prone communities less affected by frequent fire, as resprouting species may dominate (Keeley and Zedler 1978; Fox 1988; Bell 2001), whereas in less frequently burnt communities obligate seeders may dominate (Menges and Kohfeldt 1995). It is also expected seeds of species in mesic communities would be less likely to respond to fire-cues such as high temperatures (Chapter 2; Hopkins and Graham 1984); they have longer maturation periods than more frequently burnt communities. Although species may decline in the standing vegetation with increasing time since fire (Russell and Parsons 1978; Nieuwenhuis 1987; Fox 1988; Penman *et al.* 2009), species with soil seed banks may persist in the landscape as seeds and, therefore, the effect of time since fire may not be as significant as for those species with canopy-held seed banks (Nieuwenhuis 1987). The question is: can soil seed banks buffer plant populations against extinction risk and remain relatively unaffected by time since fire?

Seed banks are known to increase in size with time since fire (Zammit and Westoby 1987b; Zammit and Zedler 1988; Koch *et al.* 2009), however this can vary for individual species depending on including seed accumulation rates, but is not necessarily consistent for species with the same post-fire response (Ne'eman and Izhaki 1999; Clemente *et al.* 2007) or for species occurring in the same habitat (Auld *et al.* 2000). In the soil seed banks of both fire-prone and mesic communities, infrequent fire may result in the decline of species that have short-lived seed banks and are cued by fire to germinate, whereas frequent fire may result in the decline of obligate seeders which have not reached reproductive maturity before any subsequent fire. Key questions then are:

How long after a fire does it take for soil seed banks to be replenished and hence to act as a buffer against the risk of extinction?

Are all soil seed banks long-lived allowing a buffer against the loss of above ground individuals?

This study assessed the impact of time since fire (long unburnt; 6.25 and 4.5 years following fire) on the species present in the soil seed banks of five different plant communities, ranging from those dominated by mesic elements (Wet Sclerophyll Forest (WSF), Rainforest (RF)) to those with strong sclerophyll components (Dry Sclerophyll Forest (DSF), Rocky Outcrop (RO), Wet Heath (WH)). The aim of this study was to determine if there is an immaturity and senescence risk of species in the soil seed bank with respect to fire frequency threshold levels. I specifically asked:

Q.1. Does time since fire affect the key variables of species richness, abundance and species composition of the soil seed banks?

Q.2. Can plant functional groups inform community contrasts in relation to soil seed banks over time?

Methods

Study area

The study was conducted using soil collected from Gibraltar Range – Washpool National Parks, located on the Great Dividing Range in Eastern Australia (29°31’S, 152°18’E). The study area and the characteristics of the five communities sampled in this study have been described in detail in Chapter 1.

Time since fire dataset

The time since fire dataset is a combination of previously collected datasets (Table 1). The effect of time since fire on the soil seed bank of the five communities was determined for 4.5 years since fire (subset of Chapter 1 dataset), 6.25 years since fire (subset of Chapter 3 dataset) and long unburnt (Chapter 3). Although previous fire history is quite heterogeneous across the sites, with variations in fire frequency, and minimum and maximum fire intervals. The methodology for collecting the soil has been described in Chapters 1 and 3. To ensure consistency across this data-set, data from the same low burn severity sites were sampled with the same sampling strategy (the large sample core: 28 x 16 x 5cm: length x width x depth). When low burn severity sites were resampled, soil was taken from areas not previously sampled to ensure this did not influence the detectable seed bank collected 6.25 years after fire.

The treatment of soil samples and the collection of seedling data have also been described in Chapters 1 and 3. Species recorded in the soil seed bank were grouped on their known response to fire (Hunter 1998; NSWFFRD 2002; Sheringham and Hunter 2002; Clarke *et al.* 2009) and their germination cue (Floyd 1976; Warcup 1980; Hopkins and Graham 1984; Auld and O'Connell 1991; Dixon *et al.* 1995; Keith 1996; Enright *et al.* 1997; Keith 1997; Bell 1999; Read *et al.* 2000; Enright and Kintrup 2001; Wills and Read 2002; Hill and French 2003; Tang *et al.* 2003; Thomas *et al.* 2003; Clarke and French 2005; Ooi *et al.* 2006; Thomas *et al.* 2007; Penman *et al.* 2008). The most abundant species from each community were compared on life form patterns, their germination response and the dominant families sampled in each time since fire category. Unidentified species were not included in these comparisons. These comparisons used total abundances to assess general trends with no formal statistical analysis carried out on these data.

Table 1. Fire history for each dataset used in this study across all communities.

Dataset used in this study	Time since last fire DSF[†] RO WH WSF RF	Referred to in text
Pre-critical threshold May 2007 large samples only	All communities 4 years 6 months after fire	4.5 years
Lower fire threshold level (immaturity risk) January 2009 low burn severity sites only	All communities 6 years 3 months after fire	6.25 years
Upper limit fire threshold level (senescence risk) January 2009 – unburnt sites	DSF RO WH: 18/20 years WSF RF: >50 years	Long unburnt

[†] DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Statistical analysis

A three-way general linear model was used to compare differences between time since fire (TSF) and communities. The factors tested in the model were: community, TSF, site (nested in community). As there was a significant interaction between community and TSF, a series of two-way general linear models were used to compare differences between TSF treatments within each community, with the factors TSF and site tested in these models. The variables assessed for all models were: total species richness, and abundance of seedlings emerging from the soil seed bank. Post-hoc Scheffe's test was used to test for differences between levels of a significant factor. All univariate statistical analyses were performed using DataDesk® 6.1 (Velleman 1997). Data were tested for normality using box plots where the response variable was plotted for each community, and transformed to satisfy assumptions of normality where appropriate (Zar 1984).

A two-way PERMANOVA tested differences in species composition between time since fire and communities using a Bray-Curtis similarity matrix. Pairwise tests were used to determine where differences occurred. A similarity percentage (SIMPER) analysis was used to determine the average similarity for each TSF treatment, based on the Bray-Curtis index of similarity, for species emerging from the soil seed bank of each community which contributed most strongly to similarity among samples within each TSF treatment. Analyses were performed on abundance data using PRIMER v6 (Clarke and Gorley 2006) and PERMANOVA+ (Anderson *et al.* 2008) and were transformed where appropriate to improve the underlying distribution.

To determine if germination of species within each community was associated with a particular time since fire treatment, χ^2 analysis was used. The null expectation was no difference in abundance among treatments. Only species that recorded ten or more individuals germinating from the soil seed bank of each community were used in this analysis, as χ^2 analyses are not valid when expected values are low (Zar 1984).

To examine the community response patterns across different functional groupings, the degree to which the proportion of obligate seeders and resprouting species in each community was affected by time since fire was assessed using a log-likelihood ratio analysis.

The log-likelihood ratio analysis statistic (G test) has an approximate distribution of χ^2 (Zar 1984).

Results

Response to time since fire: Species richness and abundance

There was a significant interaction between time since fire and plant community. Time since fire had a variable response on the number and abundance of species emerging from the soil seed bank of each community (Fig. 1; Table 2). Generally, more species emerged in the long unburnt treatments across all communities, with significant increases measured between the long unburnt and 4.5 years after fire in both the fire-prone (Fig. 1a; Table 2: RO, WH) and the mesic communities (Fig. 1a; Table 2: RF). Significant differences were recorded for the number of species emerging 4.5 and 6.25 years after fire for only one community, with species increasing with time since fire (Fig. 1a: RO). Overall, more individuals emerged from the soil seed bank of the long unburnt treatments, except for the Rainforest community (Fig. 1b). Abundance was significantly greater in the long unburnt sites compared to 4.5 years after fire in the fire-prone communities (Fig. 1b; Table 2: DSF, RO). For one community (RO) more individuals emerged from the soil seed bank 6.25 years after fire compared to 4.5 years after fire; this was marginally significant (Fig. 1b: RO, $P > 0.051$). Site was found to be significant for different variables within communities, indicating the patchiness of soil seed banks across sites (Table 2).

Table 2. *F*-statistic and *p*-value of the General Linear Model for (a) species richness and (b) abundance of individuals emerging from the soil seed bank of five communities.

	Factor	d.f.	(a) Species richness		(b) Abundance	
			F	*P*	F	*P*
3-way GLM	Community	4	2.760	0.088	**4.423**	**0.026**
	TSF[†]	2	**93.301**	**< 0.0001**	**30.096**	**0.002**
	Community x TSF	8	**10.206**	**0.010**	**7.993**	**0.017**
	Site (Community)	10	**5.441**	**< 0.0001**	**3.758**	**0.000**
	TSF x Site (community)	5	0.358	0.876	0.281	0.923
2-way GLM						
Dry Sclerophyll Forest	TSF	2	1.558	0.391	**192.11**	**0.005**
	Site	1	0.007	0.934	1.578	0.221
Rocky Outcrop	TSF	2	**98.714**	**0.010**	**117.34**	**0.009**
	Site	1	0.340	0.566	1.596	0.219
Wet Heath	TSF	2	**27.507**	**0.035**	0.472	0.679
	Site	1	**27.113**	**≤ 0.0001**	0.196	0.662
Wet Sclerophyll Forest	TSF	2	3.958	0.202	4.343	0.187
	Site	1	3.282	0.083	3.862	0.061
Rainforest	TSF	2	**36**	**0.027**	0.481	0.675
	Site	1	**24.501**	**≤ 0.0001**	**18.103**	**0.001**

Significant values in bold, [†]TSF = Time Since Fire

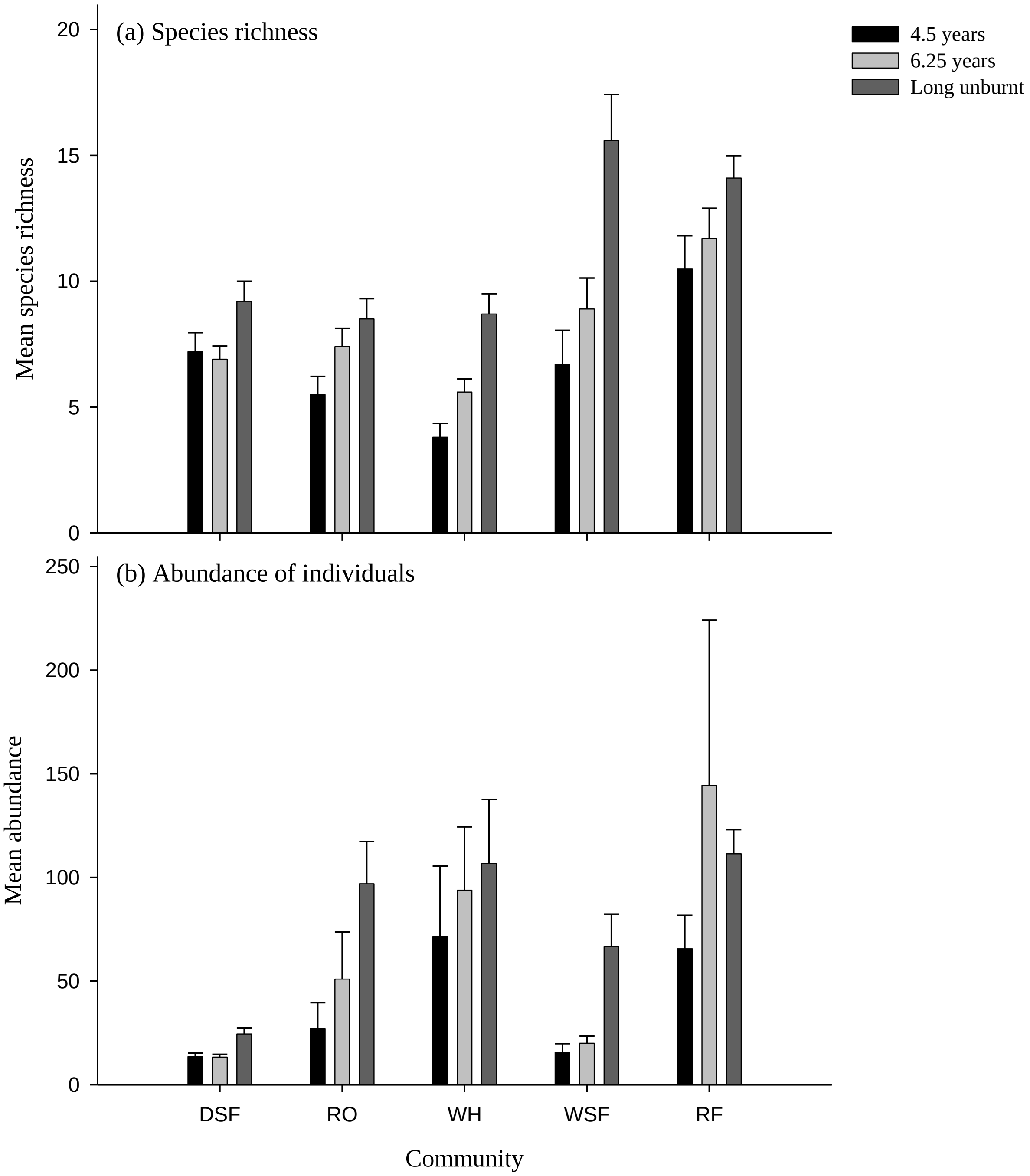

Fig. 1. Effect of time since fire on the emergence of (a) species (+ s.e.) and the (b) abundance of individuals (+ s.e.) from the soil seed bank of five communities. Communities referred to as DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest.

Response to time since fire: Species composition

Soil seed bank samples clustered separately based on the similarity within community type, as well as an effect of time since fire (Fig. 2.). A two-way PERMANOVA used to test the effect of time since fire on the species composition found a significant interaction between community and time since fire, with pairwise comparisons indicating significant differences between long unburnt and recently burnt treatments (Table 3). Differences were non-significant for species composition both 6.25 and 4.5 years following fire (Table 3). Average similarity values (using SIMPER analysis) within time since fire treatments for each community ranged from 17 (in the 4.5 year TSF treatment of WSF) to 44% (in the long unburnt treatment of WH), indicating similarity within TSF treatments was quite variable (Table 4).

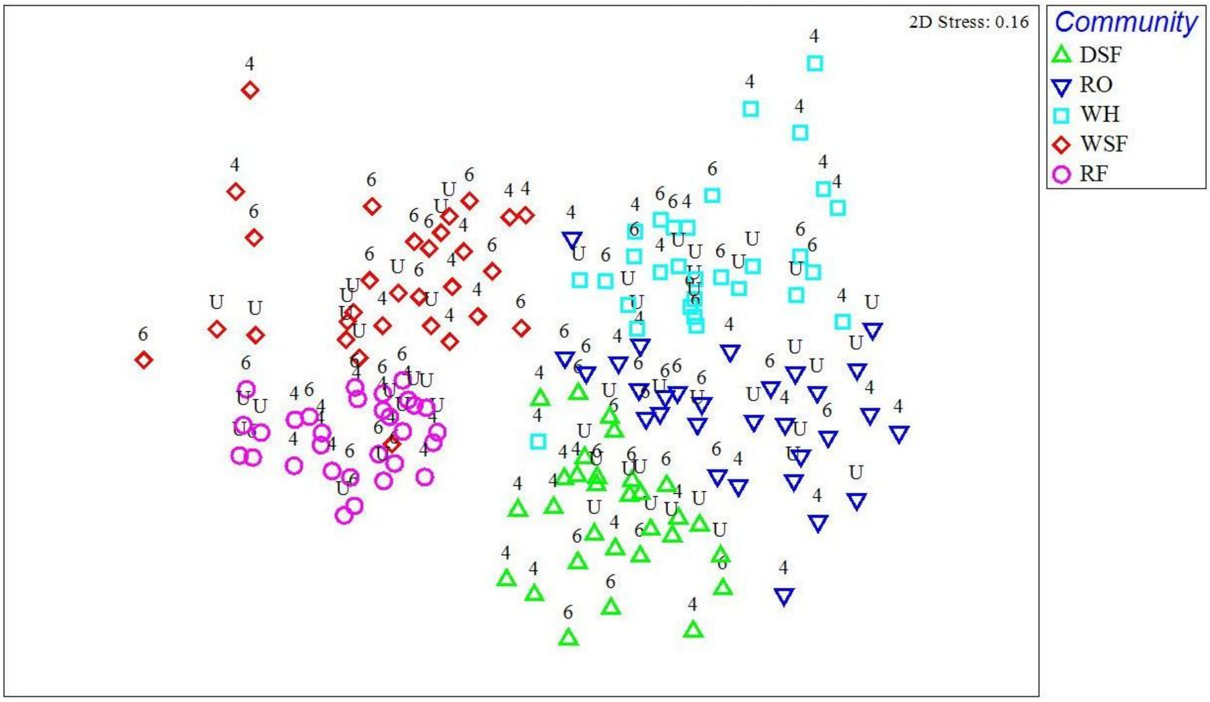

Fig. 2. Ordination of samples (nMDS) in two dimensions for the abundance of species recorded from the soil seed bank of each community within each severity treatment. Resemblance is based on Bray-Curtis similarity index. Stress = 0.16. Communities referred to: DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest , RF = Rainforest. Time since fire referred to as: 4 = 4.5 years, 6 = 6.25 years and U = Long unburnt.

Table 3. PERMANOVA identifying the effect of time since fire on the species composition of the soil seed banks of five communities.

Factor		d.f.	Pseudo F	P (Perm)
Community		4	21.33	**≤ 0.0001**
TSF		2	3.13	**≤ 0.0001**
Community x TSF		8	2.83	**≤ 0.0001**
Pairwise			T statisitc	P (Perm)
DSF[†]	4.5 *vs.* 6.25 years		1.208	0.162
	4.5 *vs.* LU years		2.423	**≤ 0.0001**
	6.25 *vs.* LU years		1.586	**0.007**
RO	4.5 *vs.* 6.25 years		1.252	0.114
	4.5 *vs.* LU years		1.716	**0.002**
	6.25 *vs.* LU years		1.916	**0.001**
WH	4.5 *vs.* 6.25 years		0.942	0.499
	4.5 *vs.* LU years		2.234	**0.000**
	6.25 *vs.* LU years		2.173	**0.000**
WSF	4.5 *vs.* 6.25 years		0.706	0.946
	4.5 *vs.* LU years		1.948	**0.000**
	6.25 *vs.* LU years		1.871	**0.001**
RF	4.5 *vs.* 6.25 years		0.963	0.425
	4.5 *vs.* LU years		2.202	**0.000**
	6.25 *vs.* LU years		1.940	**0.002**

[†] DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, WH = Wet Heath, WSF = Wet Sclerophyll Forest, RF = Rainforest. LU = Long unburnt, TSF = Time Since Fire. Significant values in bold.

Table 4. Average similarity indices (calculated from average Bray-Curtis similarity index) for the soil seed banks of five communities within each time since fire category.

Community	4.5 years	6.25 years	Long unburnt
Dry Sclerophyll Forest	25.56	24.67	40.17
Rocky Outcrop	23.02	33.65	37.98
Wet Heath	26.42	35.04	44.81
Wet Sclerophyll Forest	17.48	19.73	29.48
Rainforest	41.53	38.42	43.42

Response to time since fire: Individual species

The most abundant species sampled from the soil seed banks of the different communities varied with time since fire (Table 5). Response varied from increasing to decreasing abundance or no effect of time since fire. A number of species representing a variety of families and life forms showed no trend in abundance with time since the last fire (Table 5, $P > 0.05$). These species fell into two categories: the first were mostly weed-like obligate seeding species from the Asteraceae family (e.g. *Gamochaeta calviceps*, *G. spicata*), which have an ephemeral seed bank and wind-dispersed seeds. The second were native species from several families with no clear trend in terms of fire response and life form.

Time since fire either increased or decreased the abundance of key family groups and life forms for the most abundant species emerging from the soil seed bank (Fig. 3; Table 5). Tree, grass, graminoid and twiners species had a positive response to time since fire, with proportionally more individuals emerging in the long unburnt treatment compared to the recently burnt treatments. In contrast, the herbaceous species had greatest abundance 6.25 years after fire or in unburnt vegetation, whereas there was no distinct pattern for shrub species which showed similar abundance regardless of time since fire (Fig. 3a). Time since fire had a positive impact on two families (Droseraceae, Ericaceae), with proportionally more individuals emerging in the long unburnt treatment compared to recently burnt treatments (Fig. 3b; Table 5). Two other families (Cunoniaceae, Myrtaceae) had a weak decline in abundance with time since fire, with proportionally more individuals emerging in the recently burnt treatments compared to the long unburnt treatment. Species from the Asteraceae family germinated across all communities indicating their emergence from the soil seed bank was unaffected by time since the last fire. However, in all cases, trends of individual species within each taxa were inconsistent with either a positive, negative or neutral response to time since fire (Table 5). In most cases patterns detected in the family groupings could be attributed to the abundance of a single species (Apicaceae: *Hydrocotyle pedicellosa*, Cunoniaceae: *Ackama paniculata*, Droseraceae: *Drosera spatulata*, Ericaceae: *Epacris obtusifolia*, and Myrtaceae: *Baeckea omissa*; Table 5).

A number of species were unique to a particular burn treatment, with fifteen species from all five plant communities unique to the long unburnt treatment, whereas twelve species were absent from this treatment but present in the recently burnt treatments (4.5 and 6.25 years). The smallest group of species unique to a particular treatment were those absent only from the most recently burnt treatment (4.5 years); there were eight species in this group with the largest number of species representing the Rainforest community (Table 5). It should be noted the trends seen in three species (*Epacris gunnii*, *Goodenia bellidifolia*, *Ziera* sp. aff. *smithii*) were inconsistent between the communities where they occurred; caution should be taken in making generalisations about these three species.

Species grouped based on their requirements to break seed dormancy had a varied response to time since the last fire (Fig. 4). In the shortest period following fire, species that required smoke to germinate their seeds, or had no response to either smoke or heat, where least abundant. The abundance of these two groups increased with increasing time since fire, with both groups being the most abundant in the soil seed bank of the long unburnt vegetation. Species which had a combined response or were heat responders did not vary in abundance with time since fire (Fig. 4).

Nineteen species sampled in more than one community responded differently with time since fire across all communities. Likely difference may have resulted from spatial variability within soil seed banks, indicating caution should be taken in making generalisations about the effect of time since fire on species occurring in different communities.

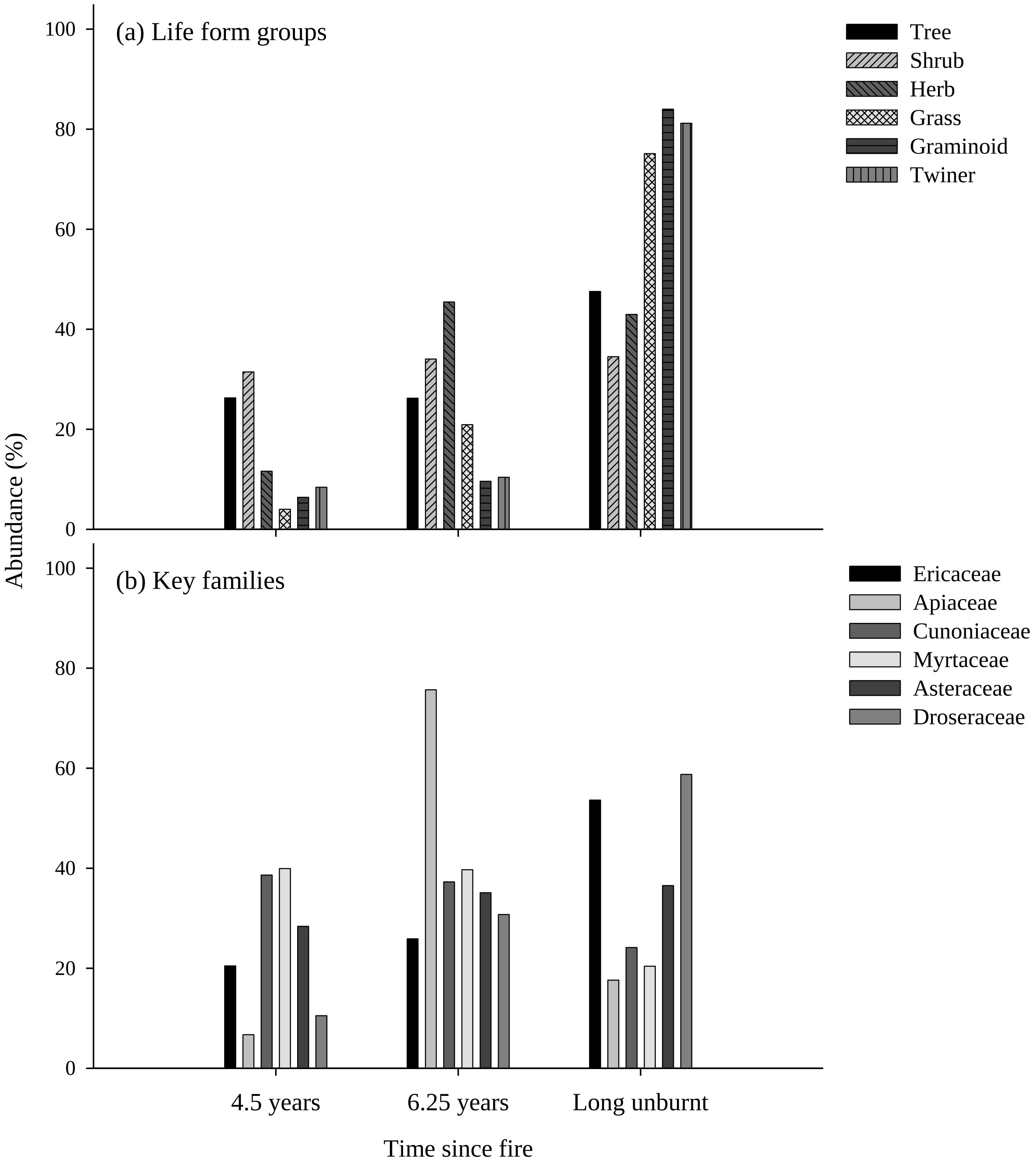

Fig. 3. Effect of time since fire: The percentage of the most abundant species sampled in the soil seed banks of five communities based on (a) life form and (b) key family groups. Percentage abundance is based on groupings among treatments.

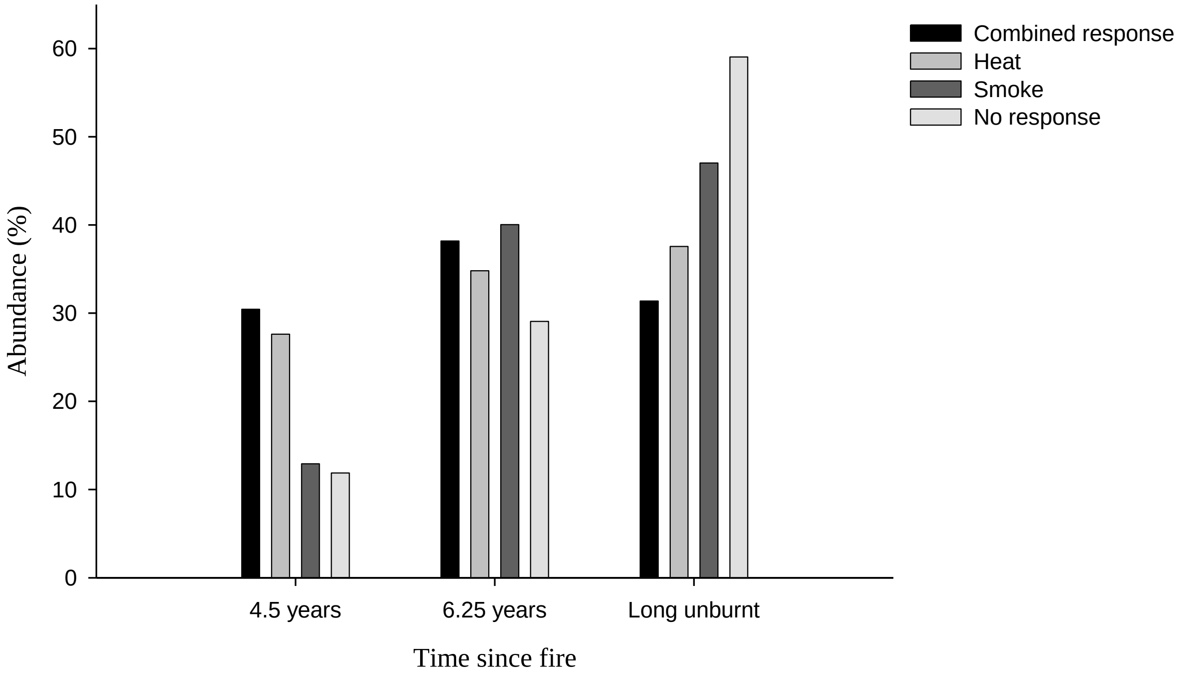

Fig. 4. Responses to time since fire of species grouped on their seed germination cue. Combined heat/smoke category had not distinguished between whether the combined response is unitive or obligatory, independent and additive or synergistic.

Table 5. Species germinated (and their abundance) from the soil seed banks of five communities based on the time since fire treatments. Species had more than 10 seedlings germinating across all treatments.

Species	Fire response†	Germination cue‡	PJP/SJP†	4.5 years	6.25 years	Long unburnt	χ^2 probability
Dry Sclerophyll Forest							
Austrostipa rudis	R	S	-/1?	2	3	8	0.092
Boronia microphylla	R	C	-/2	10	4	1	**0.015**
Bossiaea scortechinii	R	H	5/-	4	3	5	0.779
**Conyza sumatrensis*	OS	N?	-	0	5	12	**0.002**
Dillwynia phylicoides	OS	H	5/-	4	9	14	0.062
Entolasia stricta	R	N	-/0.5	4	14	60	**<0.0001**
Epacris gunnii	R	C	-/2	29	8	0	**<0.0001**
Gamochaeta calviceps	OS	N?	-	7	9	4	0.387
**Gamochaeta spicata*	OS	N?	-	9	5	6	0.522
Goodenia bellidifolia	R	C	2-4/2	7	12	0	**0.003**
Goodenia hederacea	R	C	-/1-2	0	0	13	**<0.0001**
Leptospermum trinervium	R	C	-/2	13	20	34	**0.006**
Platysace ericoides	R	C	-/2	0	9	16	**<0.0001**
Prostanthera saxicola var. *major*	OS	H	3/-	0	0	33	**<0.0001**
Trachymene incisia	R	unknown	-/<2	8	3	0	**0.012**
Rocky Outcrop							
Boronia anethifoia	OS	C	3/	1	6	3	0.150
Calytrix tetragonia	OS	N	6/	0	0	12	**<0.0001**
**Conyza sumatrensis*	OS	N?	-	6	9	1	**0.047**
Drosera spatulata	R	N	-/1	80	148	265	**<0.0001**
Entolasia stricta	R	N	-/0.5	1	22	20	**<0.0001**
Epacris gunni	R	C	-/2	0	0	177	**<0.0001**
**Gamochaeta spicata*	OS	N?	-	3	10	4	0.080
Gonocarpus micranthus subsp. *micranthus*	OS	H	1-2/-	6	0	20	**<0.0001**
Gonocarpus teucrioides	OS	S	2-4/-	14	1	4	**0.001**
Goodenia bellidifolia	R	C	2-4/2	0	0	15	**<0.0001**
Graminoid sp 20	unknown	unknown	-	0	0	24	**<0.0001**
Graminoid sp 23	unknown	unknown	-	0	1	16	**<0.0001**

Table 5. cont.

Species	Fire response[†]	Germination cue[‡]	PJP/SJP[†]	4.5 years	6.25 years	Long unburnt	χ^2 probability
Kunzea bracteolata	R	C	6/-	28	39	104	**<0.0001**
Laxmannia compacta	OS	C	-	10	0	0	**<0.0001**
Laxmannia gracilis	OS	C	-/1	54	179	215	**<0.0001**
Lepidosperma gunnii	R	H	-/1	1	2	14	**<0.0001**
Leucopogon microphyllus	OS	C	4/-	4	29	1	**<0.0001**
Micromyrtus sessilis	R	C	-	0	0	30	**<0.0001**
Mirbelia rubiifolia	OS	H	3/-	31	18	16	**0.047**
Monotoca scoparia	R	H	6/2	0	2	11	**<0.0001**
Platysace ericoides	R	C	-/2	7	5	0	**0.039**
Wet Heath							
Baeckea omissa	R	C	4/1	467	440	21	**<0.0001**
Caesia parvifolia var. *minor*	R?	S	-/1?	0	0	12	**<0.0001**
Drosera spatulata	R	N	-/1	35	189	379	**<0.0001**
Empodisma minus	R	unknown	1/2	13	12	80	**<0.0001**
Entolasia stricta	R	N	-/0.5	1	3	63	**<0.0001**
Epacris gunni	R	C	-/2	25	34	0	**<0.0001**
Epacris obtusifolia	OS	S	5/-	153	194	364	**<0.0001**
Gamochaeta spicata	OS	N?	-	2	4	11	**0.019**
Gonocarpus micranthus subsp. *micranthus*	OS	H	1-2/1	5	25	70	**<0.0001**
Graminoid sp 11	unknown	unknown	-	0	0	14	**<0.0001**
Graminoid sp 14	unknown	unknown	-	1	0	9	**0.001**
Hibbertia sp. aff. *rufa*	R	unknown	-	2	3	5	0.497
Laxmannia gracilis	OS	C	-/1	0	20	0	**<0.0001**
Wet Sclerophyll Forest							
Ackama paniculata	OS		-	3	0	83	**<0.0001**
Archirhodomytrus beckleri	R	unknown	-/6	0	0	60	**<0.0001**
Berbridopsis bechlei	OS	unknown	-	3	6	24	**<0.0001**
Callitriche muelleri	unknown	unknown	-	0	0	18	**<0.0001**
Coronidium elatum	OS	N	-	4	3	4	0.913
Cyperus disjunctus	R	H	-	0	0	38	**<0.0001**
Galium binifolium	OS	S	2/-	3	1	6	0.150
Gamochaeta spicata	OS	N?	-	14	11	10	0.690
Geranium solanderi	R	H	1?/-	2	0	15	**<0.0001**

Table 5. cont.

Species	Fire response[†]	Germination cue[‡]	PJP/SJP[†]	4.5 years	6.25 years	Long unburnt	χ^2 probability
Gonocarpus oreophilus	OS	C	-	19	35	0	**<0.0001**
Goodia lotifolia	OS	H	-/4	5	5	0	0.082
Hedycarya angustifolia	R	unknown	-/6	1	3	10	**0.008**
Hydrocotyle peduncularis	R	S	-	8	10	49	**<0.0001**
Juncus usitatus	R	C	-	1	9	15	**0.003**
Lobelia trigonocaulis	R	?	-	1	0	76	**<0.0001**
Olearia nernstii	OS	N	4/-	1	11	23	**<0.0001**
Oplismenus imbecillis	R	S	-	0	0	71	**<0.0001**
Oxalis exilis	R	S	1/-	7	6	3	0.444
Ozothamnus rufescens	OS	H	5/-	26	22	2	**<0.0001**
Poranthera microphylla	OS	C	1-2/-	5	4	1	0.273
Rubus rosifolius	R	N	-	7	7	6	0.951
Senecio minimus	OS	N	<1/-	0	0	14	**<0.0001**
Solanum aviculare	OS	S	1/-	14	3	4	**0.005**
Viola hederacea	OS	N	1/<1	1	6	30	**<0.0001**
Zieria sp. aff. *smithii*	OS	H	5/-	1	15	13	**0.003**
Rainforest							
Acacia melanoxylon	R	H	-/>3	5	5	38	**<0.0001**
Ackama paniculata	OS	unknown	-	416	394	137	**<0.0001**
Ameilema acuminatum	R	unknown	-	27	20	0	**<0.0001**
Berberidopsis beckleri	OS	unknown	-	13	10	31	**0.001**
Callicoma serratifolia	R	H	-/5	34	41	52	0.143
Cissus hypoglauca	R	unknown	-	1	5	54	**<0.0001**
Clematis aristata	R	C	-	0	0	55	**<0.0001**
Galium binifolium	OS	S	2/-	0	21	1	**<0.0001**
Gamochaeta calviceps	OS	N	-	3	5	4	0.779
**Gamochaeta spicata*	OS	N	-	5	5	8	0.607
Hedycarya angustifolia	R	unknown	-/6	5	5	2	0.472

Table 5. cont.

Species	Fire response[†]	Germination cue[‡]	PJP/SJP[†]	4.5 years	6.25 years	Long unburnt	χ^2 probability
Hydrocotyle laxifolia	R	S	2/-	40	26	0	**<0.0001**
Hydrocotyle pedicellosa	R	S	-	0	643	106	**<0.0001**
Hydrocotyle peduncularis	R	S	-	2	38	0	**<0.0001**
Lobelia trigonocaulis	R	unknown	-	22	122	1	**<0.0001**
Lophostemon confertus	R	unknown	-	3	9	0	**0.005**
Polyscias murrayi	OS	N	-	2	4	4	0.670
Quintinia sieberi	R	unknown	-	0	5	452	**<0.0001**
Rorippa dictyosperma	OS	unknown	1/-	18	3	1	**<0.0001**
Rubus moluccana	R	N	1-5/-	2	5	7	0.257
Rubus rosifolius	R	N	-	8	7	34	**<0.0001**
Schizomeria ovata	R	unknown	-	0	2	11	**<0.0001**
Solanum aviculare	OS	S	1/-	20	29	46	**0.004**
Solanum opacum	OS	S	-	2	1	13	**<0.0001**
Zieria sp. aff. *smithii*	OS	H	5/-	1	9	0	**0.001**

Significant values in bold.

*exotic species

[†] Fire response refers to R= Resprouter, OS = Obligate seeder (NSWFFRD 2002; Sheringham and Hunter 2002; Clarke *et al.* 2009), PJP = Primary juvenile period, SJP = Secondary juvenile period

[‡] Germination cue refers to S = Smoke response, H = Heat response, C= Combined Smoke/Heat response and N = No response to heat or smoke. This has been inferred from genera/species reported in the literature (Floyd 1976; Warcup 1980; Hopkins and Graham 1984; Auld and O'Connell 1991; Dixon *et al.* 1995; Keith 1996; Enright *et al.* 1997; Keith 1997; Bell 1999; Read *et al.* 2000; Enright and Kintrup 2001; Wills and Read 2002; Hill and French 2003; Tang *et al.* 2003; Thomas *et al.* 2003; Clarke and French 2005; Ooi *et al.* 2006; Thomas *et al.* 2007; Penman *et al.* 2008).

Response to time since fire: Fire response categories

Resprouting species were proportionally greater in the soil seed bank of unburnt vegetation across all communities (Table 6a). This pattern changed at sites with a short time since fire interval. For two communities, resprouting species continued to dominate the soil seed bank 4.5 years after fire (Table 6a: DSF, WH) and this pattern became more distinct 6.25 years after fire, with the soil seed bank of all fire-prone communities dominated by resprouting species (Table 6a: DSF, RO, WH). A significant trend was detected in the soil seed bank of the Rocky Outcrop community, with the proportion of resprouting species increasing with time since fire (Fig. 5a; Table 6a). In contrast, proportionally fewer resprouting species were detected in the mesic communities 4.5 and 6.25 years after fire (Table 6a: WSF, RF). The differences across the sampled time since fire was significant in the Wet Sclerophyll Forest community (Fig. 5b: Table 6b).

Based on proportional representation of individuals, resprouters also dominated the soil seed bank of unburnt vegetation sampled across all communities (Table 6b). In the fire-prone communities, resprouting dominated the soil seed bank in recently burnt vegetation e.g. Rocky Outcrop (Fig. 6a; Table 6b). In contrast, there were significantly more individuals of obligate seeders dominating the soil seed banks of the mesic communities (WSF, RF) in the recently burnt vegetation, indicating time since fire had an impact on the relative number of sprouting versus non-sprouting species present in the soil seed bank of these communities (Fig. 6b, c; Table 6b).

Table 6. Proportion of (a) species richness and (b) abundance based on fire response sampled in sites with different fire histories within each community. The fire response of the species in the standing vegetation is shown here as a baseline comparison.*

	Fire response[†]	4.5 years	6.25 years	Long unburnt	G test probability
(a) Species richness					
Dry Sclerophyll Forest					0.210
	OS	27	34	39	
	R	73	66	61	
Rocky Outcrop					**0.002**
	OS	56	46	31	
	R	44	54	69	
Wet Heath					0.193
	OS	23	35	30	
	R	77	65	70	
Wet Sclerophyll Forest					**0.000**
	OS	71	63	44	
	R	29	37	56	
Rainforest					0.378
	OS	58	52	48	
	R	42	48	52	
(b) Abundance					
Dry Sclerophyll Forest					0.102
	OS	22	31	35	
	R	78	69	65	
Rocky Outcrop					**<0.0001**
	OS	44	37	8	
	R	56	63	92	
Wet Heath					0.214
	OS	21	30	31	
	R	79	70	69	
Wet Sclerophyll Forest					**<0.0001**
	OS	83	72	37	
	R	17	28	63	
Rainforest					**<0.0001**
	OS	68	58	28	
	R	32	42	72	

*Proportions were calculated as means for severity treatments.

[†] R = Resprouter, OS = Obligate seeder. Significant values in bold.

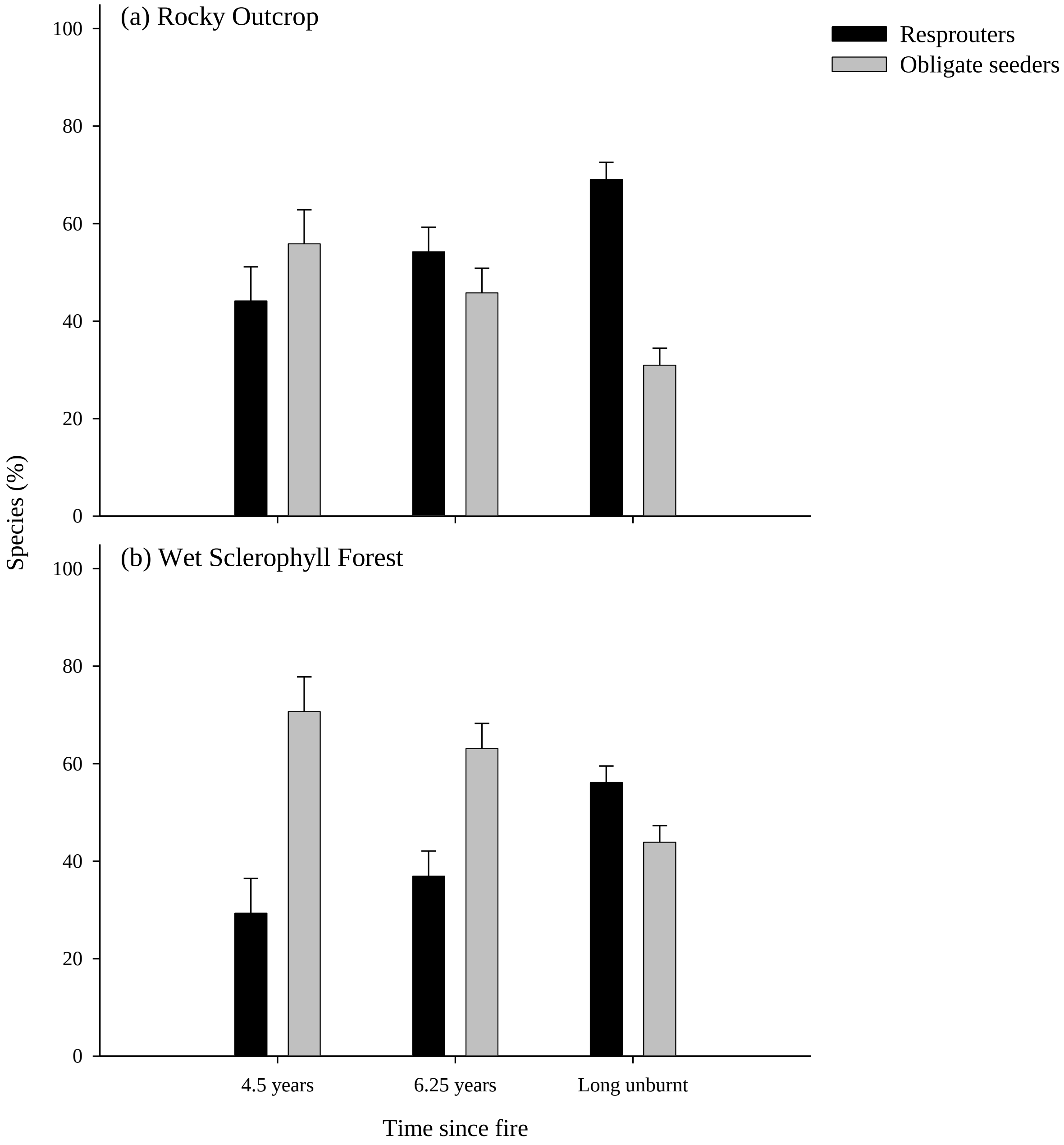

Fig. 5. Effect of time since fire: Percentage of species (+ s.e.) emerging from the soil seed bank of the (a) Rocky Outcrop and the (b) Wet Sclerophyll Forest communities based on their sprouting ability.

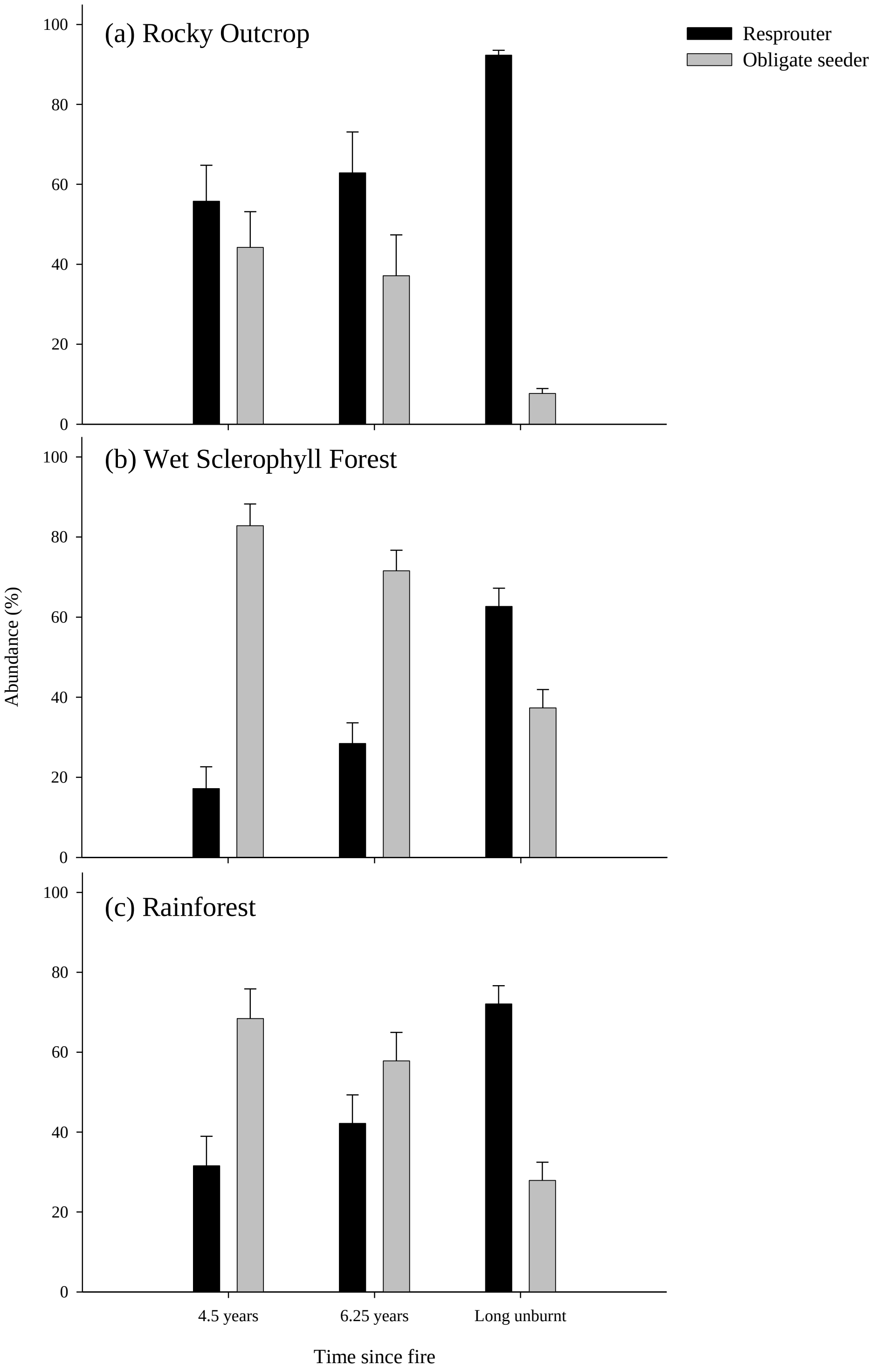

Fig. 6. Effect of time since fire: Percentage of individuals (+ s.e.) emerging from the soil seed bank of the (a) Rocky Outcrop (b) Wet Sclerophyll Forest and (c) Rainforest communities based on their sprouting ability.

Discussion

Soil seed banks are an important source of seed for the regeneration of species following a disturbance event, such as fire, and are particularly important for species that are killed during such a disturbance event. Seed bank accumulation in species can vary due to differences in seed input and seed longevity (Pierce and Cowling 1991; Auld *et al.* 2000; Holmes and Newton 2004; Clemente *et al.* 2007). This may affect the persistence of species and survival of populations with increasing and decreasing time since fire. In this study the soil seed banks of five communities were sampled in sites burnt 4.5, 6.25 years following fire and in long unburnt sites. Time since fire had an affect on the seed bank of species of different communities in a variety of ways. For many species, their seed banks had not been replenished at sites with a short time since fire interval, with the exception of the Rocky Outcrop community, suggesting the rate of recovery was greater in this community for some species. However, most species across all communities would require more time to replenish their seed banks and are at risk of local extinction if the fire return interval is too short. In the long-term, it is generally thought soil seed banks can buffer against the loss of above ground individuals (Keith *et al.* 2001); this would be the case for only some species in this study. A number of species could not be detected in long unburnt sites, suggesting they do not have long-lived seed; their seed banks would not buffer against their loss from the landscape.

Differences occurred mainly between long unburnt and the shortest fire interval (4.5 years), with soil seed banks in unburnt sites generally more speciose and abundant compared to recently burnt sites (Fig. 1; Table 1). There was no significant difference for the number and abundance of species emerging from the soil seed bank 4.5 and 6.25 years after fire, with the exception of the Rocky Outcrop community, where significantly more species and individuals were detected in the soil seed bank 6.25 years after fire. Differences were also detected for species composition between long unburnt and recently burnt sites (Fig. 2; Table 3), suggesting seed banks had not been replenished in recently burnt sites. Generally there was a greater abundance in unburnt sites when species were categorised on life forms (Fig. 3a). Shrub species were similar in abundance regardless of time since fire and herbaceous species were more abundant 6.25 years following fire (Fig. 3). The trend for key family groups was highly variable, with some families (Fig 3b: Ericaceae, Asteraceae and Droseraceae) more abundant in long unburnt sites compared to recently burnt sites, whereas the opposite was true for other families (Fig. 3b: Cunoniaceae, Myrtaceae). Apiaceae was the only family that was most abundant 6.25 years following fire (Fig. 3b) and this could be attributed to the abundance of *Hydrocotyle pedicellosa* in the soil seed bank of the Rainforest community (Table 5). Differences were also detected for species groups based on their seed germination requirements (Fig. 4; Table 5). Generally all groups increased with time since fire, with smoke responders and species with seed that do not respond to either smoke or heat the least abundant 4.5 years following fire and the most abundant in the long unburnt vegetation. This suggests frequent fire may threaten species in these two groups if the fire return interval is too short for these taxa to replenish their seed bank.Those species with a combined response or a heat response may remain unaffected due to the presence of a residual seed bank (Auld and Denham 2006). A decline in smoke responders has been detected in similar fire-prone communities (Penman and Towerton 2008; Penman *et al.* 2011), where soil heating failed to reach temperatures to trigger germination in some species, but smoke present possibly broke seed dormancy and promoted germination in other species.

Time since fire also had an impact on the number of species and abundance of individuals with different sprouting abilities. In the Rocky Outcrop community, significantly more resprouting species germinated in long unburnt and 6.25 years after fire, whereas in the Wet Sclerophyll Forest community, significantly more resprouting species germinated in the long unburnt vegetation (Fig. 5; Table 6a). A similar trend was detected in the proportion of individuals with different sprouting abilities. In the fire-prone communities, resprouters were proportionally greater in abundance in the soil seed bank than obligate seeders, regardless of time since fire, although in the Rocky Outcrop community this was only strong in the unburnt sites. In the mesic communities, obligate seeders were significantly greater in abundance in the soil seed banks from recently burnt vegetation but not in the seed bank of long unburnt vegetation (Fig 6; Table 6). This indicates obligate seeders in the mesic and Rocky Outcrop communities may be at greater extinction risk if they are unable to reach maturity and replenish their seed bank before the next fire (Keith 1996).

Episodes of frequent fire may change the composition of some communities with resprouting species dominating regardless of time since fire (Fox 1988). However, obligate seeders that have been found to decline with frequent fire are all known to have canopy-held seed banks (Fox and Fox 1986; Nieuwenhuis 1987; Cary and Morrison 1995). It is this group of species likely to be the first lost under a regime of frequent fire. In this study, some obligate seeding species were present in the soil seed bank across all communities in the recently burnt sites (Table 6); herbaceous species were the main drivers of this pattern. Maturity rates are known to be longer for woody species than for herbaceous species (Benson 1985; Keith 1996). For species in this study with known maturation rates (Juvenile period: Table 5), herbaceous species generally flowered within five years of fire. The woody species in this study generally take five or more years to flower after fire. In the context of the soil seed bank of these communities, it is specifically woody obligate seeding species with maturation rates greater than five years, and germination promoted by smoke, or a neutral response to heat or smoke (Fig. 4; Table 5, e.g. *Calytrix tetragonia*), most at risk of extinction where fire return intervals are too short.

Both seed death during a fire and germination of seeds after a fire will deplete the seed available in the soil. This may explain the low number of species and individuals detected in the shortest interval after fire in this study and in studies of other communities (Ferrandis *et al.* 1999; Auld and Denham 2006; Koch *et al.* 2009). In this study, herbaceous species were the main drivers for differences in species composition between recently burnt and long unburnt sites, in a similar manner to that found by Wills and Read (2007). The peak in herbaceous species seen 6.25 years after fire (Fig. 3) were driven by two species, *Drosera spatulata* and *Hydrocotyle pedicellosa*, both of which are likely to have a short maturity rate and the ability to replenish their seed banks rapidly.

The species composition in this study was affected by time since fire, with samples clustering within each community based on time since last burn (Fig. 2; Table 3). This result could be attributed to either species being unique to a particular treatment, or species being present in all treatments but at different abundances. Many species were unique to one treatment (Table 5). Strong site effects (Table 2) and a lack of significant difference in species composition between 4.5 and 6.25 years after fire (Table 3) may indicate differences are due to site effects rather than being a true effect of time since fire. The sites used to measure 4.5 and 6.25 years time since fire are relatively close to each other in all communities, although sampled in different locations within the sites. Species unique to the long unburnt sites may not indicate the effect of time since fire, but rather reflect site differences in terms of species composition.

Where species were detected in all sampled times since the last fire (Table 5), patterns of species abundance varied from increasing, decreasing or being unaffected by time since fire. There were no trends detected for these species in terms of life forms or sprouting ability. Overall more species increased with time since fire across all communities, supporting the findings of other studies that have also found seed banks increase in size with time since fire (Zammit and Westoby 1987b; Zammit and Zedler 1988; Koch *et al.* 2009).

Although vegetation structure and composition may change due to short intervals between fires in some communities (Zedler *et al.* 1983; Nieuwenhuis 1987; Maliakal *et al.* 2000), soil seed banks may still buffer species and populations against the risk of extinction (Keith 1996). Seed persistence is known to vary between species due to seed dormancy and decay rates, and the greatest extinction risk is for those species with short-term or transient soil seed banks and when the fire free interval exceeds their life-span (Auld *et al.* 2000). In the study by Nieuwenhuis (1987), both the resprouters and obligate seeders found to be absent above ground from infrequently burnt sites are all known to have soil-stored seed banks. This indicates these species may still be persistent in the habitat as seeds in the soil. In this study, twelve species were absent from the long unburnt sites but present in the recently burnt sites, suggesting these species may have short-term seed banks resulting in their absence from areas experiencing infrequent fire. In contrast, fifteen species were only present in the long unburnt sites suggesting these species may take a long time to replenish their soil seed banks due to long maturation rates. The eight species absent from the sites burnt 4.5 years ago but present in the other two fire treatments, would be at risk if the fire return interval was less than five years as they would have insufficient time to replenish their seed bank. These species were predominately from the seed bank of the Rainforest community.

This study has demonstrated time since fire can affect the soil seed banks of five communities. The soil seed banks in long unburnt vegetation were generally larger for a number of species and plant functional groups across all communities, indicating many species were not at risk of extinction through senescence. For some taxa, however, there may be a senescence risk. The presence of soil seed banks in recently burnt vegetation demonstrated some species were able to rapidly replenish their seed banks. Increases of these taxa, and additions of new taxa with increasing time since fire, indicates the threshold of community persistence is likely to be beyond the 6.25 years examined in this study.

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Chapter 5. Germination response of legume seeds subjected to simulated post-fire soil temperatures.

Introduction

The germination of species with a persistent soil seed bank is promoted by fire cues such as heat (Auld and O'Connell 1991; Gashaw and Michelsen 2002), and smoke (Dixon *et al.* 1995; Keeley and Fotheringham 1998), as well as their interactions, during and after the passage of fire (Thomas *et al.* 2003; 2007; Moreira *et al.* 2010). Soil heating during the passage of fire breaks seed dormancy and triggers germination, or promotes germination, for seeds from the soil seed bank (Whelan 1995; Ooi 2007). Soil temperatures measured during fires vary in magnitude and duration, resulting in spatial patchiness due to the variability in the maximum temperatures reached at any particular point on the fire ground (Raison *et al.* 1986; Bradstock and Auld 1995; Keeley and McGinnis 2007; Penman and Towerton 2008). Seeds in the soil seed bank are exposed to a range of temperatures which may either fail to break dormancy or promote germination because they are too low, break dormancy and initiate or promote germination, or kill seeds outright because the temperatures are too high. For seeds which remain dormant after the passage of fire, post-fire soil temperatures may play a vital role in triggering their germination (Santana *et al.* 2010).

During a fire the soil temperatures experienced are related to fine fuel consumption, with the depth of heating significantly affected by the amount of fine fuels consumed on the ground (Bradstock and Auld 1995). Fine fuel consumption during a fire determines whether temperatures are sufficient to break dormancy in hard-seeded species and at what depth seedlings emerge. After the passage of fire, soil temperatures may increase due to removal of canopy vegetation during the fire. In the post-fire environment of eastern Australia, maximum soil temperatures are known to reach between 45–70°C during summer (Tothill 1969; Auld and Bradstock 1996), whereas summer soil temperatures do not normally exceed 40°C in unburnt areas (Auld and Bradstock 1996). Differences are likely to be as a result of vegetation loss during a fire and an increase in solar radiation on the soil surface (Orme and Leege 1976) or soil albedo (Post *et al.* 2000). During a hot summer season, where air temperatures may exceed 40°C, this peak in maximum soil temperatures may be very important for seed survival and the persistence of seed in the soil seed bank. Exposure to heat is known to increase germination of hard-seeded species by breaking physical seed dormancy. Martin *et al.* (1975) developed a hypothetical model for the germination response of hard-seeded species to heat. The model included: a range of temperatures that resulted in low germination, a range where temperatures broke seed dormancy and germination increased rapidly, a range where maximum germination occurred, and finally the lethal range where seeds were killed (Fig. 1). This modelled pattern of germination response to heat for physically dormant species has been found in a range of legumes, however, the shape and position of the curve on the temperature axis may vary for different species (Auld and O'Connell 1991). Temperatures above 40°C are known to break seed dormancy for some species, however, in different studies the optimal temperatures where the highest germination was achieved ranged from 60–100°C, depending on the duration of heating and the species assessed (Floyd 1966; Auld 1986b; Auld and O'Connell 1991; Granstrom and Schimmel 1993; Herranz *et al.* 1999; Gashaw and Michelsen 2002; Luna *et al.* 2007; Hanley 2009). Soil temperatures measured in a post-fire environment rarely exceed 70°C (Raison *et al.* 1986; Auld and Bradstock 1996), and are therefore not likely to promote the germination of those species requiring a particular temperature threshold to break seed dormancy (i.e. greater than 70°C). For those species where the temperature threshold for breaking seed dormancy lies between 40–70°C, some effect of post-fire soil temperature is possible. In addition, seeds of different sizes are known to respond differently over a large range of temperatures, with small-seeded species more resilient to high temperatures (Hanley *et al.* 2003).

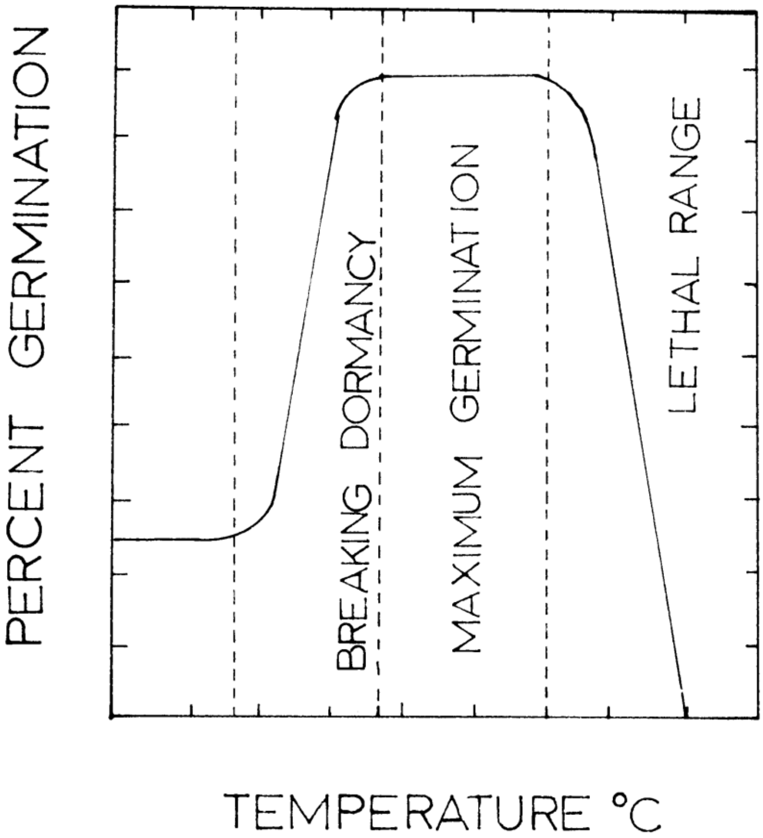

Fig. 1. Hypothetical model for the germination response of hard-seeded species to heat (Adapted from Martin *et al.* 1975).

The germination response of seeds to varying amounts of heat (across temperatures and durations) varies from species to species (Floyd 1966; Auld and O'Connell 1991; Hanley *et al.* 2003; Thomas *et al.* 2007). There has been limited work investigating the temperature experienced in a post-fire environment (Auld and Bradstock 1996) and, subsequently, the effect of solar radiation on the germination of species from the soil seed bank (Warcup 1980; Ooi *et al.* 2009). This study assessed the impact of post-fire temperatures (which may be elevated due to increased solar radiation) on seed germination. The aim was to simulate the effect of temperature on soil seed banks in a post-fire environment. I used the seeds of nine Fabaceae species using four levels of heat (up to 70°C, the maximum observed in the field in the limited work in comparable habitats in southeastern Australia). Although well studied, Fabaceae species present a good model to use as they have a known dormancy-breaking response to heat in general, (Martin *et al.* 1975; Auld and O'Connell 1991; Herranz *et al.* 1998; Hanley 2009). However, I included species with a range of seed sizes as the influence of seed size on germination response to post-fire soil temperatures has not been tested for the Fabaceae beyond the work of Hanley *et al.* (2003). I asked:

Q.1. Do predicted elevated soil temperatures after fire affect the germination or viability of seeds that have their dormancy broken during a fire?

Q.2. For seeds that do not have their dormancy broken during a fire, can dormancy be broken as a result of increases in soil temperatures due to solar radiation post-fire? Can seed viability also be affected?

Q.3. Can seed size be used as a predictor for responses 1 and 2 above?

Q.4. What are the implications of soil heating, via increased solar radiation after a fire, on the persistence of soil seed banks?

Methods

Germination experiment

Seeds of nine Fabaceae species were used for this experiment (Table 1). Seeds from six species (*Acacia falciformis, A. terminalis, A. venulosa, Dillwynia phylicoides, Gompholobium latifolium* and *Goodia lotifolia*) were collected from Gibraltar Range National Park (29°31’S, 152°18’E) during January 2008; *A. ulicifolia* was also collected from the same location during January 2009. Seeds were collected from multiple plants and pooled into representative species blocks. Seeds from *A. melonoxylon* and *A. viscidula* were supplied by the Armidale Tree Group, Mann St. Armidale, from collections made in the Northern Tablelands Bioregion within the preceding five years. Species were selected to represent a range of different seed sizes and fire response. Members of the Fabaceae family are known to have seed dormancy broken by heat (Auld and O'Connell 1991). Mean seed mass was determined by weighing 20 seeds of each species (Table 1).

To simulate seed dormancy being broken by fire, half the seeds of each species were scarified using sandpaper to break the seed coat. The other half of the seeds of each species had their seed coat left intact, the equivalent of seed dormancy not being broken. Five replicates of 20 seeds of each species from each of the two dormancy treatments were then dry heated in paper bags in five separate ovens at 50, 60 and 70°C for three hours each day for five days. The assumption being that a rainfall event would occur within a week and that a five day interval was considered appropriate. The control treatment, simulating unburnt vegetation, was stored at room temperature (19°C) during this time. The temperature range was selected to simulate soil temperatures experienced in a post-fire environment where the canopy has been removed by fire (Auld and Bradstock 1996), with the assumption being that seeds experiencing these temperatures were located between a depth of 2cm from the soil surface. Once the heat treatment had finished, seeds were placed in Petri dishes on germination pads with a moist sponge underneath the pad and watered with distilled water when required. A replicate Petri dish containing 20 seeds from each heat/dormancy treatment was placed in one of five germination cabinets set at a 12-hour light/dark cycle with a temperature range 26/13°C. During the experiment one of the cabinets malfunctioned with the dark cycle temperature dropping to a minimum of -9°C: this affected the viability of seeds which had already imbided, producing spurious results. The data from this cabinet were excluded from the final analysis. Germination was scored when the radicle emerged. The trial was run for a period of 85 days. After this time seeds which had not germinated were tested for viability by using the cut test (Ooi *et al.* 2004). Seeds which were firm, with no obvious signs of damage were considered viable.

Statistical analyses

A two-way general linear model was used to compare differences in total number of seeds germinating between scarification and heat treatments for each species using Datadesk®6.1 (Velleman 1997). Since seed viability was high for all species tested (Table 1) no corrections were made to the viable seed percentages. Post-hoc Scheffe's test was used to determine differences between heat treatments within scarified and non-scarified seeds for each species. Data were tested for normality using box plots and transformed where appropriate (Zar 1984).

To assess the linear relationship between seed germination and seed size (seed weight) among four different heat treatments, an ordinary least squares regression (OLS) within the SMATR program was used (Falster *et al.* 2006; Warton *et al.* 2006). A permutation test (n = 1000 iterations) was done to compare the common slope among heat treatments. When differences were found a post-hoc test determined where slopes were different. If no differences were found between heat treatments, a test for a shift in elevation between heat treatments was carried out using WALD statistic (Falster *et al.* 2006; Warton *et al.* 2006).

Table 1. Nine legume species used to determine if post-fire soil temperatures affect germination rates.

Species	Habitat[†]	Germination cue[‡]	Fire response[‡]	Seed storage[‡]	Seed weight (mg±s.e.)	Viability (%±s.e.)	Non-dormancy (%±s.e.)
Acacia falciformis	WSF	Heat	Rs	P soil	31.5 (0.0)	100	14 (3.3)
A. terminalis	DSF	Heat	Sr	P soil	30.9 (0.0)	97.0 (1.2)	13.0 (3.4)
Goodia lotifolia	WSF	Heat	S/R	P soil	26.6 (0.0)	96.0 (1.9)	17.0 (4.4)
Acacia venulosa	DSF/ RO	Heat	S	P soil	14.0 (0.0)	92.0 (4.1)	1.0 (1.0)
A. ulicifolia	DSF	Heat	Sr/R	P soil	12.6 (0.0)	100	1.0 (1.0)
A. melonoxylon	WSF/ RF	Heat	Rs/S	P soil	10.2 (0.0)	100	4.0 (1.9)
A. viscidula	DSF/ RO	Heat	S	P soil	9.6 (0.0)	99.0 (1.0)	8.0 (6.7)
Gompholobium latifolium	DSF	Heat	Sr	P soil	4.6 (0.0)	99.0 (1.0)	3.0 (2.0)
Dillwynia phylicoides	DSF	Heat	Sr/S	P soil	2.7 (0.0)	98.0 (2.0)	4.0 (1.0)

[†] DSF = Dry Sclerophyll Forest, RO = Rocky Outcrop, RF = Rainforest, WSF = Wet Sclerophyll Forest.

[‡] Sourced from (NSWFFRD 2002). Fire response: S = killed by fire, R = Resprouts following fire, Rs= Resprouts, but sometimes killed, Sr = Killed, but sometimes resprouts, S/R= documented fire response with no clear trend. Seed storage: P soil = persistent soil.

Results

Heat had a significant effect on the germination of seeds of different species compared with unheated controls (Fig. 2). For five out of the nine species studied, there was a significant interaction between scarification and heat treatments (Fig. 2e-h; $P < 0.01$). Heating did not affect the germination of non-dormant seeds for all nine species (Fig. 2; $P > 0.05$), with most seeds germinating across all four heat treatments. Germination was generally uniform across all scarified treatments. For dormant seeds, heating of the smaller-seeded species (*Acacia ulicifolia*, *A. melanoxylon*, *A. viscidula*, *Gompolobium latifolium* and *Dillwynia phylicoides*) resulted in more germination compared with unheated controls, with varied responses between individual species (Fig. 2.). Species with a broad response to heat (*Gompolobium latifolium, A. melanoxylon*) had significantly greater germination at 50, 60 and 70°C than compared to the unheated control. However, *G. latifolium*, did not exhibit a significant difference among heat treatments and *A. melonoxylon* did not exhibit a significant difference between 60 and 70°C. Germination of the four largest-seeded species (*Acacia falciformis, A. terminalis, Goodia lotifolia* and *A. venulosa*) was unaffected by heat ($P > 0.05$).

There was a significant relationship between seed germination and seed size among the four heat treatments (Fig. 3, Test Stat. = 55.79, $P < 0.001$, Fig. 3). The linear relationship between seed germination and seed size was negative for seeds exposed to 70°C (Slope = -0.4459, $R^2 = 0.502$, $P < 0.0001$) and 60°C (Slope = -0.3450, $R^2 = 0.34$, $P < 0.0001$), positive for the unheated control (Slope = 0.106, $R^2 = 0.442$, $P < 0.0001$); no relationship was found for seeds exposed to 50°C (Slope = -0.0897, $R^2 = 0.049$, $P = 0.193$). Comparisons of slopes between heat treatments found no difference between 70 and 60°C ($P = 0.386$), but both were different to each of the 50°C treatment ($P < 0.05$) and the unheated control ($P < 0.001$). For the two temperature treatments where there was no significant difference in slope (60 and 70°C), there was a significant difference in the elevation of the slope (WALD Stat = 5.47, P = 0.019), with more germination occurring at 70°C compared to 60°C across all seed sizes (Fig. 3).

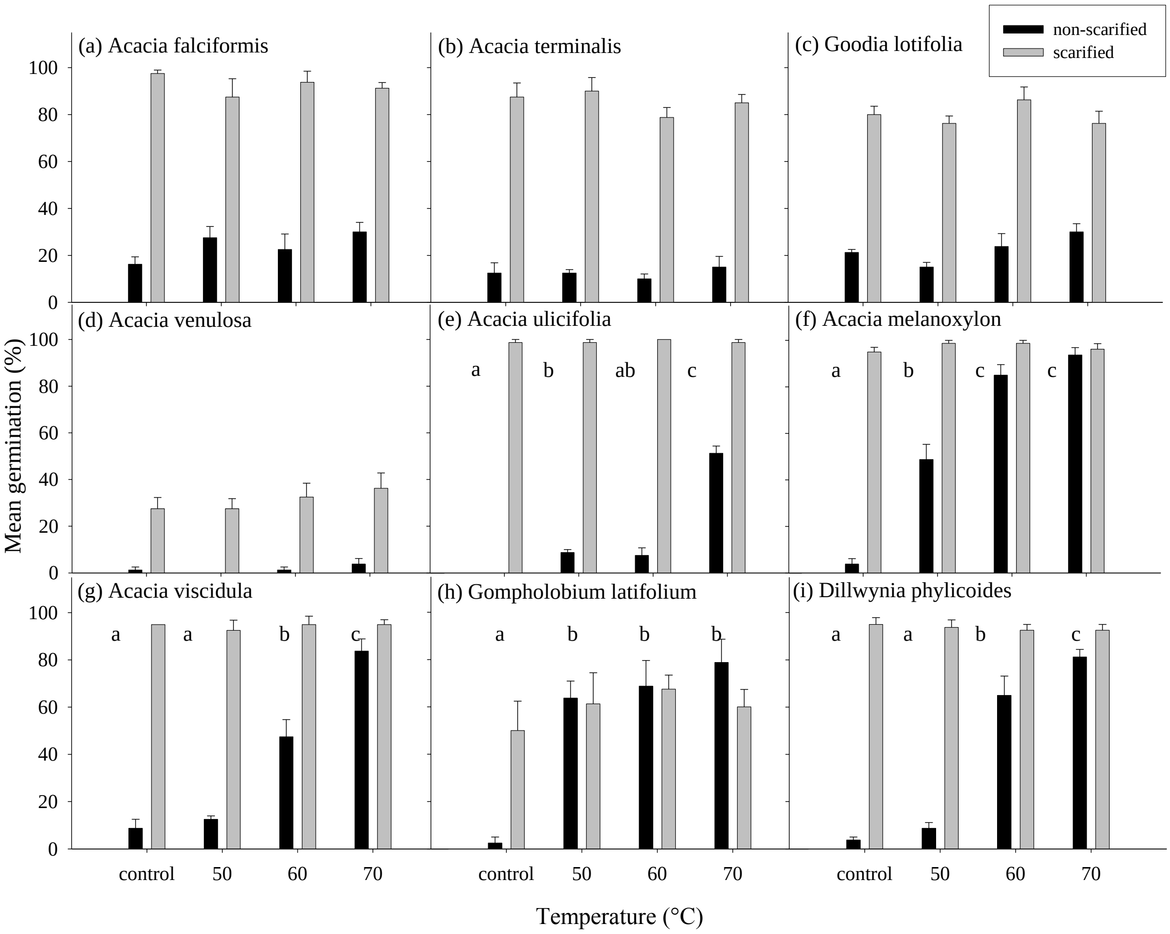

Fig. 2. Mean germination (± s.e.) of nine legume species for a range of temperatures simulating post-fire soil temperatures. Letters represent post-hoc test comparisons for non-scarified seeds. Different letters indicate significant differences, $p < 0.05$.

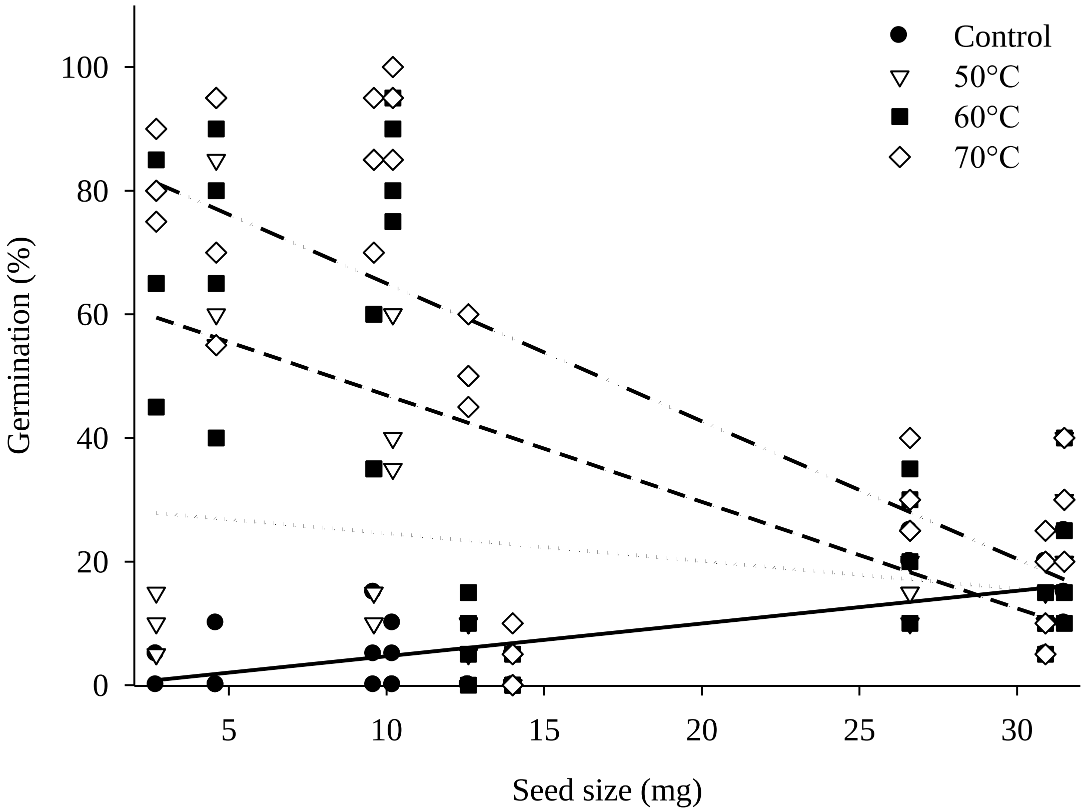

Fig. 3. Relationship between germination and seed size (mg) for all species in response to temperatures simulating post-fire soil temperatures. Regression line for each heat treatment is as follows: Control (———), 50°C (··········), 60°C (– – – – ·) and 70°C (— · · —).

Discussion

Post-fire germination of seeds from the soil seed bank is important for the recovery of individuals relying on a soil seed bank to re-establish following a fire event. Germination of seeds, which remain dormant following a fire due to low temperatures not sufficient to break seed dormancy (Martin *et al.* 1975), may in turn be triggered by increased post-fire soil temperatures as a result of soil heating by solar radiation. This would deplete the available residual seed bank in species where dormancy is broken at temperatures similar to those recorded in a post-fire environment. In this study, simulated post-fire soil temperatures influenced the germination of seed of heat-cued species with a persistent soil seed bank in different ways. For seeds that may not have their dormancy broken by heat exposure during a fire, simulated post-fire soil temperatures up to 70°C will significantly increase the germination of species with relatively small- to medium-sized seeds and as a consequence reduce the available residual soil seed bank. For seeds that may have had their dormancy broken during a fire, post-fire soil temperatures will not affect the germination and viability of these seeds.

Dormant seeds of five species had a significant response to heat, with the greatest germination occurring at higher temperatures compared to the unheated control (Fig. 2). This can be seen quite distinctly in two species (*Acacia visidula* and *Dillwynia phylicoides*), where both had a narrow response to heat with significantly more germination at 60 and 70°C than at 50°C or in the unheated control. *Acacia ulicifolia* germination also only responded to a narrow range of heat, with significantly higher germination at 70°C compared to either 60° or 50°C.

Two species (*Gompholobium latifolium* and *A. melanoxylon*) recorded increased germination over all sampled temperatures (50–70°C), indicating the range of temperatures that will break seed dormancy was greater than for any other species in this study. The temperatures that initiate germination, result in optimal germination, or kill seeds (although not seen in this study) will vary for different species (*sensu* Fig. 1). The broad germination response of the five smallest-seeded species was very similar, with temperatures being sufficient to trigger germination in most species, the amount of germination increasing significantly with increasing temperature (Fig. 2). In a scenario where both soil heating during a fire and post-fire soil temperatures breaks seed-dormancy, a considerable proportion of the soil seed bank would germinate leaving very little residual seed in the soil, except at depths below which there is no temperature effect. We know maximum soil temperatures during a fire decline with depth of soil (Beadle 1940; Bradstock and Auld 1995) and seedling emergence also declines with increasing depth of soil (Auld 1986a; Bond *et al.* 1999). It is predicted those species most at risk are small-seeded obligate seeders, which rely on seeds in the top two centimetre of soil for regeneration following fire, combined with having seed dormancy broken after exposure to temperatures between 50–70°C for a long duration. It is generally assumed soil seed banks provide a buffer between fire events (Keith *et al.* 2001), however, in this scenario these species are behaving more like species with a canopy-held seed bank where all seed are released following a fire (Lamont *et al.* 1991).

The risk of depletions of residual soil seed banks in the post-fire environment does not apply to all taxa with a soil seed bank. The dormant seeds of four species (*A. falciformis*, *A. terminalis*, *Goodia lotifolia* and *A. venulosa*), remained unaffected by different levels of heat in this study ($P > 0.05$), with low germination recorded across all treatments, indicating the dormancy breaking zone (Fig. 1; Martin *et al.* 1975) was not yet reached for these species. Previous work has indicated that, certainly for at least one of these species (*A. terminalis*), heating of seeds at 70°C or above is required to break dormancy, with optimal germination occurring at much higher temperatures for short durations than recorded in this study (Auld and O'Connell 1991). It is therefore predicted that dormant seeds of these species in a post-fire environment would not be affected by fluctuations in soil temperatures and they would not have reductions in the magnitude of their residual soil seed banks. This should enable a relatively greater persistence in the landscape compared to the species with lower temperature thresholds for breaking seed dormancy. The germination of seeds seen here in the large-seeded species (26.6–31.5 mg), although not significant (Fig. 2. – control treatments), could be attributed to the germination of soft-coated seeds and may be responsible for the maintenance of the population of species in fire free periods, where they utilise gaps in vegetation cover to establish individuals at suitable sites (see Table 1 – non-dormant seed).

Seed germination showed a negative relationship with seed size when assessed at high soil temperatures (60–70°C), indicating that, in post-fire environments, where soil temperatures reach 60°C and above, the species germinating are likely to be small-seeded (Fig. 3). Most studies have reported maximum germination for larger-seeded Fabaceae species at temperatures of 80°C or above; greater temperatures than applied in this study. Small-seeded Fabaceae species appear to be able to germinate across a wider range of different temperatures (Floyd 1966; Auld and O'Connell 1991; Hanley *et al.* 2003; Santana *et al.* 2010) than large-seeded species. This would suggest the post-fire germination of large-seeded species would be dependant solely upon the degree of soil heating during the passage of fire. Again this reinforces the idea that large-seeded species will have a relatively more stable residual seed bank in a post-fire environment, assuming that some seed remain dormant due to insufficient heating of the soil during a fire.

Soil temperatures during prescribed burning rarely reach a maximum temperature above 60°C (Penman and Towerton 2008), and it is predicted that soil temperatures would only be sufficient to trigger germination of seed from a subset of species in the soil seed bank. Increased solar radiation on the soil surface, as a result of gap creation due to canopy loss during a fire event, may result in increased post-fire soil temperatures. A greater proportion of small-seeded species are cued to germinate at lower temperatures compared to the proportion of large-seeded species (Auld and O'Connell 1991). This study has demonstrated that, if soil temperatures have reached 50°C and above, dormant seed of small-seeded species (<12.6mg) are capable of germinating. Low severity fires followed by increased post-fire soil temperatures may result in germination of those species that have their dormancy broken at relatively low temperatures. Any subsequent moderate-high severity fire, which heats the soil to greater depths (see Fig 3.11b, Whelan 1995), may result in the depletion of the seed bank unless it has been replenished depending on the primary or secondary juvenile periods of the species. If the soil seed bank was not replenished, these species would have a limited buffer between fire events and would be at risk of local extinction, especially where the fire return interval is too short.

Comparison of summer temperatures in unburnt vegetation (<40°C; Auld and O'Connell 1991; Auld and Bradstock 1996) indicate soil temperatures would be insufficient to break seed dormancy and stimulate the germination of seeds of the large-seeded species in this study. Likely differences between soil temperatures measured in burnt and unburnt vegetation could be attributed to less exposure of the soil surface to solar radiation because of shading from established vegetation in unburnt areas. It is therefore pertinent to ask whether this trend will continue in a changing climate.

Climate change is expected to increase average air temperatures 2–5°C over the coming century (AAS 2010). Increases in air temperature have already been seen, with the last decade being the warmest on record. These increases in air temperature are likely to result in increases in soil temperatures. In the arid zone of Australia, Ooi *et al.* (2009) predicted an increase in soil temperature with a changing climate, would result in a depletion of the residual soil seed bank because taxa in this environment are sensitive to increases in soil temperatures. Predictions for the New England Tablelands region of northern New South Wales are that air temperatures will increase 2–5°C (DECC 2008). It is likely this region would experience a greater occurrence of higher soil temperatures especially in a post-fire environment, when conditions are more likely to be warm and dry. Using the model for arid environments (Ooi *et al.* 2009), it is predicted with a changing climate there would be a shift in the germination response of both large- and small-seeded species. For those species that had the broadest germination response to temperatures used in this study (*Acacia melanoxylon* and *Gompholobium latifolium*), more germination is likely to occur if soil temperatures increase due to solar radiation. It is also possible that, under a climate change scenario where soil temperatures increase for a region, small-seeded species will reach the lethal response zone (see Fig. 1, Martin *et al.* 1975), resulting in the death of seeds. Species from different habitats may respond differently to changes in soil temperatures. For equivalent durations of heating, species from wetter habitats (Floyd 1966; 1976) reach the lethal response zone at lower temperatures (95–100°C) compared to species from drier habitats (Auld and O'Connell 1991), which have a broader germination response curve, and reach the lethal response zone at much higher temperatures (100–120°C). Both these conditions will contribute to a depletion of the soil seed bank, and may threaten the persistence of these species in the landscape, especially for small-seeded species in wetter habitats. Those large-seeded species (14–31.5 mg), that did not have their seed dormancy broken at the high temperatures used in this study, may have their seed dormancy broken due to an increase in soil temperatures under a changing climate. However, it is unlikely soil temperatures will reach the lethal response zone for these species.

Loss of seed dormancy as a result of heat exposure is well known in legume species (Martin *et al.* 1975; Shea *et al.* 1979; Auld and O'Connell 1991), however, understanding the mechanism that controls this process is unclear, and does not necessarily follow a general pattern within the taxa and among plant communities. In some legume species, seed size is related to seed coat thickness, with large-seeded species having a thicker seed coat than smaller-seeded species (Cavanagh 1980; 1983; Morrison *et al.* 1992). In this study, I found small-seeded species had lower temperature thresholds than larger-seeded species, which could be attributed to the thickness of the seed coat. The relationship between seed size and seed coat thickness in determining temperature thresholds is still not fully understood and this is an area of future research that needs to be examined in more detail, especially with regards to its consistency among vegetation communities. Two other explanations for this result are also possible. First, differences in temperature threshold in species may also be due to differences between resprouting and seeder species, with the expectation that resprouting species are less tolerant to thermal shock (Bell and Williams 1998). Second, responses to temperature changes in soil may be different between communities, with the expectation that species from fire-prone communities would be more tolerant to thermal shock than those species in less fire-prone communities, which experience infrequent fire and have species capable of germinating between inter-fire periods. Further investigation using congeneric species with different sized seeds and species from different habitats to test this is warranted.

This study predicts the potential depletion of soil seed banks of some species in fire-prone habitats. Simulated post-fire soil temperatures affected the germination of dormant seed by breaking seed dormancy in hard-seeded species, with small-seeded species affected more by changes in simulated soil temperatures compared to large-seeded species. This will ultimately have an impact on the persistence of the soil seed banks of species which have a broad germination response to increasing soil temperatures in fire-prone habitats.

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Conclusion

The principal aim of this thesis was to compare the characteristics of soil seed banks and their relationship to the standing vegetation among five different plant communities; and assess the response of these soil seed banks to different aspects of fire, such as soil heating, burn severity, time since fire and post-fire environmental conditions. In this concluding chapter I present summaries of the main findings of my thesis and the significance of these finding in the context of fire management in plant communities. I also address the limitations of this work and potential future research directions.

Characteristics of the soil seed bank and their comparison to the standing vegetation

In this thesis I studied the soil seed banks of five different plant communities, ranging from those dominated by mesic elements with low fire frequency (Wet Sclerophyll Forest, Rainforest), to those with a strong sclerophyll component and relatively high fire frequency (Dry Sclerophyll Forest, Rocky Outcrop, Wet Heath). The soil seed banks of these communities were characterised by similar components, with shrub and perennial species being the most abundant life form and life history, respectively (Chapter 1). Generally, graminoids, herbs and shrubs were the most abundant and speciose life forms within the fire-prone communities (DSF, RO, WH), whereas herbs, shrubs and trees were the most abundant and speciose life forms within the mesic communities (WSF, RF). Tree and twiner species were more speciose and abundant in the Rainforest community than in the fire-prone communities (DSF, RO, WH). The estimated seed density of the soil seed bank for these communities ranged from 143–834 seeds/m^2, with the Rainforest and the Wet Health communities having the greatest density and the Dry Sclerophyll Forest community having the least: this is similar to densities measured for the soil seed banks of other communities (e.g. Hopkins and Graham 1984; Enright *et al.* 1997; Wang 1997; Wills and Read 2002).

There were clear differences between the number and composition of species recorded in the standing vegetation and the soil seed bank of all communities (Chapter 1). More species were recorded in the standing vegetation than the soil seed bank of all communities, except the Rainforest community. In both fire-prone and mesic communities, similarity in composition between the standing vegetation and the soil seed bank was quite low. I attributed this to the large number of species unique to either the standing vegetation or the soil seed bank of each community. Species were absent from the soil seed bank for a number of reasons. Some species with canopy-held seed storage were not detected in the soil seed banks, however their absence was not unexpected due to the nature of seed release immediately following a fire event. In contrast, other species missing from the seed bank are known to have a persistent soil-stored seed bank and their absence may be indicative of uneven distribution of their seed in the soil. Other reasons for this pattern could be the lack of response to germination cues, i.e. inappropriate cues and/or failure to break seed dormancy even though seeds may be present in the soil. Some species absent from the soil seed bank experiment in Chapter 1 were detected in experiments described in Chapter 2, indicating some species may require higher soil temperatures to break seed dormancy: an assessment that was outside the scope of the work described in Chapter 1. In other instances, the transient nature of seeds of some species accounted for their absence from the soil seed bank. A small number of species, where little was known about their type of seed bank and dormancy requirements, were also absent, limiting any conclusions that could be made for these species. Species absent from the standing vegetation could be grouped into two categories. The first, were weedy Asteraceae species with wind-dispersed seed. The second group were native species with no clear trend in terms of life form or fire response. Both these groups are likely to be ephemeral in their above-ground presence.

Trends in species and community composition

The impact of fire severity on both the presence and abundance of species varied across community types. In fire-prone communities, species that responded positively to fire increased with increasing severity of fire. In mesic communities, those species that responded negatively to fire decreased with increasing severity. Heating soil to simulate the effect of different fire severities (Chapter 2) demonstrated some species respond to heat (in combination with smoke) with enhanced germination at high temperatures, while other species have limited germination at high temperatures. Species within communities that were considered more fire-prone generally had a positive response to heat. Species in the more mesic, less fire-prone communities, responded negatively to heat, with the exception of legume species, which responded the same across all communities. Although some legume species germinated at a range of temperatures (e.g. *Goodia lotifolia*), the majority of germination occurred at the highest temperatures, indicating hard-seeded species are likely to be depleted from the soil seed bank of all communities following a fire event that has heated the soil to moderate temperatures. This also indicates that fires that producing this amount of heat are needed to maintain these species. Germination response for taxa in the family Fabaceae may also be dependent on seed size (Chapter 5). Ericaceae species also had a similar response to heat. Both *Epacris* species (*E. gunnii* and *E. obtusifolia*) recorded greater germination in the high heat treatment; this agrees with other published studies (Enright and Kintrup 2001; Penman *et al.* 2008).

Heating soil at different temperatures did not affect the broad species composition among treatments within community type, with samples across treatments clustering tightly based on plant community type. However, within each community the abundance of individual species changed among heat treatments. Generally, tree and twiner species with a soil seed bank responded negatively to heat, whereas shrub, grass and graminoid species had a positive response to high soil temperatures. Herbaceous species germinated regardless of heat treatments (Chapter 2).

In Chapter 3, I demonstrated burn severity can affect soil seed banks across different communities in a variety of ways. High burn severity generally resulted in a smaller soil seed bank for a number of species, and plant functional groups, across all communities; although the extent of differences between burnt and unburnt sites was greater in magnitude. Soil seed banks in unburnt sites generally were more speciose and abundant than sites burnt during the fires of 2002. This suggests that six years post-fire that species in the burnt areas had not yet fully replenished their soil seed banks. This was also illustrated by the separation of the unburnt sites from the burnt sites based on species composition, although very few species were unique to one particular burn severity class; major differences are more likely due to changes in the number of individuals sampled from different severity treatments. Generally, increasing severity of fire led to a reduction in the number and abundance of species emerging from the soil seed bank across all communities some six years after fire, although this trend was non-significant ($P > 0.05$). A similar trend was also detected when species were categorised on life forms. Abundances of all life forms were greater in the soil seed bank of unburnt vegetation than burnt vegetation (Chapter 3). Weedy Asteraceae species with wind-dispersed seed were the major component of the soil seed bank unaffected by burn severity from both fire-prone and mesic communities. Those species in the mesic communities unaffected by burn severity included fleshy-fruited species usually dispersed by animals, although patterns were not consistent within all taxa. All these species are likely to have been dispersed into the post-fire environment rather than survived the fire itself and act as opportunistic fire-sensitive invaders (Ferrandis *et al.* 1999a).

When species were grouped on their requirements to break seed dormancy, those species known to respond to heat showed greater abundances in the unburnt and high severity sites (Chapter 3). Species that have germination promoted by the interaction between heat and smoke were least abundant in the high severity sites, as were the smoke responders, indicating severity may influence the pattern of the residual soil seed bank even for those species that respond to smoke (Auld and Denham 2006). This implies the impact of smoke on promoting germination of soil-stored seed is not uniform across all fire severities.

Recovery over a long-term period in terms of species composition and functional groups may actually result in no difference between areas of different fire severities. The effects of fire severity may be short-lived with elements of communities capable of recovery within 4–5 years post-fire. However, given the greater reduction in the residual soil seed bank after severe fires, there may be a longer time needed for recovery following such fires. Fire severity impacts may occur when the fire return interval is not long enough to allow for the replenishment of the seed bank of species relying on seeds to regenerate following fire. This is particularly important in fires of increasing severity or where post-fire soil temperatures (Chapter 5) have decreased the size of the residual soil seed bank. The assumption was that species killed by fire, but with a soil-stored seed bank, are able to regenerate from residual seeds in the soil. If the entire seed bank, however, has been depleted during a single fire event and the fire-return interval is too short to allow for replenishment of the seed bank, then plant populations become vulnerable to local extinction. Fire management needs to incorporate the interaction between fire frequency and severity to ensure plant populations persist in the landscape.

Time since fire had a variety of impacts on the seed banks of species across the different communities. Differences mainly occurred between long unburnt and the shortest fire interval (4.5 years), with soil seed banks in unburnt sites generally more speciose and abundant than in those recently burnt sites (Chapter 4). There was no significant difference for the number and abundance of species emerging from the soil seed bank 4.5 and 6.25 years after fire, with the exception of the Rocky Outcrop community where significantly more species and individuals were detected in the soil seed bank 6.25 years after fire. Differences were also detected for species composition between long unburnt and recently burnt sites, suggesting seed banks had not been replenished in recently burnt sites. When species were grouped on life form, there generally was a greater abundance in unburnt sites, although shrub species were similar in abundances regardless of time since fire and herbaceous species were more abundant at sites 6.25 years after fire.

Differences were also detected for species groups based on seed germination requirements and time since fire. Generally all groups increased with time since fire, with the smoke responders and species with seed that does not respond to either smoke or heat being the least abundant 4.5 years following fire and the most abundant in the long unburnt vegetation. This suggests frequent fire may threaten species in these two groups, if the fire return interval is too short for these taxa to replenish their seed bank, especially following a high severity fire (Chapter 3). Those species with a combined response or a heat response may remain unaffected due to the presence of a residual seed bank (Auld and Denham 2006).

In this study a number of taxa were consistently the most abundant species sampled within each community, regardless of treatments (Chapters 2–4). For some species, trends were consistent between communities, whereas for others they were not. Also a large number of the most abundant species sampled from the soil seed bank of the five plant communities did not respond differently across the treatments tested. Variable responses of species to different levels of soil heating, burn severity, and time since fire, enables a degree of buffering against the impact of subsequent fire events on the soil seed banks of different communities, thus enabling species to persist in the landscape.

Trends in species with different sprouting abilities

Resprouters were more abundant than obligate seeders across all communities, except the Wet Sclerophyll Forest (Chapter 1). Resprouting shrubs were most abundant in the fire-prone communities (DSF, RO, WH) and the least abundant in the mesic communities (WSF, RF). This indicates communities that experience fire on a more regular basis are not only dominated by resprouting shrubs but also have a soil seed bank containing these shrub species. When overall proportions of resprouters and obligate seeders were examined among communities (Chapter 1), there were clear trends based on fire frequency between communities, with resprouters more abundant in the fire-prone communities (DSF, RO, WH) and obligate seeders more abundant in the mesic communities (WSF, RF).

When the effect of heat on the soil seed banks of five different communities was examined for species with different sprouting abilities (Chapter 2), only one community (WSF) recorded significant differences in the proportion of species based on their sprouting ability across different heat treatments; high temperatures increased the proportion and abundance of obligate seeding species recorded in that community. Over time, this may result in changes in species composition, with obligate seeding species increasing their relative abundances across the landscape where fires of higher severity have occurred. Given that the most abundant species in Wet Sclerophyll Forest were long-lived obligate seeders, it is likely changes in species composition will be long-term, assuming the fire return interval is long enough to enable seed bank accumulation.

During a fire that has heated soil to high temperatures, more germination from the soil seed bank would occur in the fire-prone communities, whereas seed from some species in the mesic communities would be killed by the high temperatures. For those species relying on regeneration from seed in the soil to persist in the landscape, this may result in a decline of their soil-stored seed bank. A similar trend was detected for the effect of burn severity on species with different sprouting abilities (Chapter 3). In this study, six years post-fire, burn severity had an impact on the proportion of species germinating with different fire response strategies. In the Dry and Wet Sclerophyll Forest communities more obligate seeding species germinated at higher burn severities than resprouting species, indicating changes in the composition of both fire-prone and mesic communities are likely with fires of different severities. The Wet Sclerophyll Forest appears to be more sensitive to changes in burn severity, with more obligate seeding species emerging in the low severity treatment. Species sampled in mesic communities are killed at lower temperatures than species from fire-prone communities (Hopkins and Graham 1984). Therefore, species forming the soil seed bank in the Wet Sclerophyll Forest may be more sensitive to changes in soil temperature during fires, these species having lower lethal temperature thresholds compared to species from the more fire-prone communities.

Population sizes of obligate seeder species would decline and sprouting species would dominate if fire return intervals were insufficient for species to accumulate a seed bank (Keeley and Zedler 1978), especially where a high severity fire, which resulted in heating of soil to significant depths, had exhausted the soil seed bank either by seed death or seed germination (see Fig. 3. 11B, Whelan 1995). Alternatively, soil which remained unheated or did not reach sufficient temperatures to stimulate germination would buffer any species loss from the landscape after a subsequent fire event because the seed would remain dormant (Auld 1987). Time since fire also had an impact on the number of species and abundance of individuals with different sprouting abilities (Chapter 4). In the Rocky Outcrop community significantly more resprouting species germinated in long unburnt and 6.25 years after fire, whereas in the Wet Sclerophyll Forest community significantly more resprouting species germinated in the long unburnt vegetation. A similar trend was detected in the proportion of individuals with different sprouting abilities. In the fire-prone communities, resprouters were proportionally greater in the soil seed bank than obligate seeders, regardless of time since fire. For the Rocky Outcrop community, however, this was only strong in the unburnt sites. In the mesic communities (WSF, RF), obligate seeders were significantly greater in abundance in the soil seed banks from recently burnt vegetation, but not in the seed bank of long unburnt vegetation. This indicates obligate seeders in the mesic and Rocky Outcrop communities may be at greater extinction risk if they are unable to reach maturity and replenish their seed bank before the next fire (Keith 1996).

Immaturity and senescence risk of species with a soil-stored seed bank.

Time since fire may affect the species of different plant communities in a number of ways. High and low fire frequency can increase extinction risk through mechanisms such as the death of adult plants, as well as the interruption of maturation and developmental growth of individuals. Low fire frequency can also increase extinction risk through the failure of seed release or seed germination, and the failure of seedling establishment and seed production (Keith 1996). In this study some obligate seeding species were present in the soil seed bank across all communities in the recently burnt sites, however, herbaceous species were the main drivers of this pattern (Chapter 4). Maturity rates are known to be longer in woody species than in herbaceous species (Benson 1985; Keith 1996). For species in this study with known rates of maturation (Chapter 4), herbaceous species generally flower within five years of fire, whereas those species that take five or more years to flower after fire were generally woody species. In the context of the soil seed bank of these communities, it is specifically woody obligate seeding species, with maturation rates greater than five years, and germination promoted by smoke or a neutral response to heat or smoke, that are the most at risk of extinction where fire return intervals are too short.

Both seed death during a fire and germination of seeds after a fire will deplete the seed available in the soil and may explain the low number of species and individuals detected in the shortest interval after fire in this study, and in studies of other communities (Ferrandis *et al.* 1999b; Auld and Denham 2006; Koch *et al.* 2009). Twelve species were absent from the long unburnt sites but present in the recently burnt sites, suggesting these species may have short-term seed banks resulting in their absence from areas experiencing infrequent fire. In contrast, fifteen species were only present in the long unburnt sites; these species may take a long time to replenish their soil seed banks due to long maturation rates. The eight species absent from the sites burnt 4.5 years ago but present in the other two fire treatments would be at risk if the fire return interval was less than 5 years. With such a short fire return interval they would have insufficient time to replenish their seed bank. These species were predominately from the Rainforest community.

My research has demonstrated time since fire can affect the soil seed banks of the five communities studied. The soil seed banks in long unburnt vegetation were generally larger for a number of species and plant functional groups across all communities, indicating species were not at risk of extinction through senescence. The presence of soil seed banks in recently burnt vegetation also demonstrated a number of species were able to persist in the landscape.

Study limitations

This PhD project was initiated to assess the persistence of species with soil-stored seed banks in the context of fire severity and was part of a larger ARC Linkage Grant (LP0775145). The major aim of the ARC Linkage project was to determine the effect of plant persistence from plot to landscape scale. The ARC Linkage project had three main themes: 1) spatial analysis of fire severity, 2) the persistence ability of soil-stored seed, and 3) the persistence ability of sprouting species in relation to fire severity and intervals. The PhD project was associated with the second theme of the ARC Linkage project. As part of the obligations of the ARC Linkage project, habitat and site were selected to satisfy the overall themes of the project. Differences between sites were recorded for communities across different variables in most of the studies in this thesis (Chapters 1–4). The significant site effect recorded for most communities would lend support to the conclusion that the soil seed bank was quite variable within each community and this variation is likely to influence the effect of heat, burn severity and time since fire on the species composition of the soil seed banks, making it difficult in some cases to draw any significant trends from the data. For future work, increasing the number of sites sampled and decreasing the amount of replication within each site may strengthen the power of the research design.

The second major limitation of this study relates to the assessment of soil seed banks of different plant communities in relation to fire. Recruitment of species from the soil seed bank after a fire will be affected by the extent of soil heating, the heat and smoke requirements that promote germination, and the distribution of seeds in the soil profile. Also the soil seed banks of different species will vary, depending on the accumulation of seeds in the soil, seed longevity within seed banks and the proportion of the seed bank that germinates following a fire (Auld 1994). It is therefore difficult to study the impact of fire on the soil seed bank of different plant communities in a post-fire environment of more than six years as the rate of recovery varies for different species. However, broadscale studies, such as mine, allow observations of patterns across many taxa or functional groups, rather than relying on individual species comparisons.

Future research

One of the major difficulties I had with this PhD project was the set-up of field-based experiments. Two field-based burn experiments were planned for the second year of the project. The first proposed to manipulate fire intensity, by the addition of fine fuel to some plots, was to measure natural recruitment in the field; the second, using the same burn plots were to measure recruitment in relation to landscape heterogeneity by using inorganic artificial structures and buried seed of known species. However after initial set up, the burn was delayed for 12 months because of either unfavourable weather conditions or the lack of availability of fire-fighting personnel; I had to abandon these experiments due to the time constraints of PhD candidature. Future research in this area should re-examine these experiments, using the addition of seed of the most abundant species I sampled from both the fire-prone and mesic communities to determine if fire severity has an impact on these species. The addition of seed would help control for the spatial variability detected in most communities (see above comments in study limitations). This work could test whether fire severity has an impact on buried seed survival across the landscape; and test whether seed of different sizes are affected by fire severity at various soil depths.

Other areas of future work should also examine whether the size of the burn area has an impact on the soil seed bank. This is particularly important for species recruitment from unburnt areas following fire and should be related to whether the extent of soil heating changes with the size of a burn. This would enhance our understanding of the factors that influence community structure and also predict how these communities may respond to fire severity.

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