Relationship between leaf traits and fire-response strategies in shrub species of a mountainous region of south-eastern Australia

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INTRODUCTION

Fires are an important component of many of the world’s ecosystems (Bond and Keeley, 2005; Bowman et al., 2009). Plant species possess a range of strategies which allow them to persist under different types of fire regimes, particularly in relation to fire intensity and frequency (Cary and Morrison, 1995; Iwasa and Kubo, 1997; Gill and Allan, 2008). Two important traits are the capacity of individual plants to resprout, and recruitment from seed. Species are often classified into groups based on the presence or absence of these two traits (Noble and Slatyer, 1980; Gill, 1981; Bond and van Wilgen, 1996; Pausas et al., 2004). Obligate resprouters can survive fire by producing new growth after being damaged, whereas obligate seeders lack resprouting ability or the means to protect critical plant tissues, and are killed by fire, relying solely on seed recruitment after fire for their persistence. A third group, facultative resprouters, can persist after fire by both vegetative resprouting and seed recruitment. Within these groups, variability in fire-responses can arise because of factors such as ecotypic variation, plant age and height, and variation in fire regimes (Benwell, 1998; Gill and Allan, 2008; Vivian et al., 2008).

For a plant to persist after fire by resprouting or by recruitment from seed, resources must be differentially allocated within the plant to ensure that these strategies are successful (Bellingham and Sparrow, 2000; Vesk and Westoby, 2004). An advantage of resprouting after fire is that a plant can quickly reoccupy space (Bond and Midgley, 2001) but this requires that resprouters have sufficient stored resources for mobilization to support new growth after fire. Comparative studies have often found resprouters to have larger and deeper root systems, with higher starch concentrations, than closely related non-resprouters (Pate et al., 1990; Schwilk and Ackerly, 2004; Knox and Clarke, 2005). In some groups of resprouters, post-fire growth arises from a woody mass of dormant buds in lignotubers (Noble, 2001; Cruz et al., 2003). However, this investment may result in resprouters being slower growing, shorter in height and with a longer maturation period compared with non-resprouters (Pausas et al., 2004; Benwell, 2007; Pratt et al., 2007). For obligate seeders, seed recruitment in the post-fire environment confers advantages such as access to increased availability of some nutrients for seedling growth, soil sterilization and a short-term reduction in competition, herbivory and seed predation (Florence and Crocker, 1962; O’Dowd and Gill, 1984;
The allocation of resources to ensure successful post-fire recruitment of obligate seeders includes high seed production rates, fast seedling growth and high seedling densities (Bellairs and Bell, 1990; Benwell, 1998; Pausas et al., 2004; Knox and Clarke, 2005).

Relationships between fire-response strategies and other traits have been widely examined in fire-prone Mediterranean-type climates (e.g. Keeley, 1986; Pate et al., 1990; Pausas, 2001; Bukh et al., 2007). The world’s four fire-prone Mediterranean-climate ecosystems (California, the Mediterranean basin, south-western Australia and the South African Cape) are regions of high plant diversity and endemism with relatively high fire frequencies, particularly in the two southern hemisphere regions where fire intervals range from 10 to 20 years (Cowling et al., 1996). Fires, along with the strong seasonal variation between winter rainfall and summer drought, are considered to play an important role in determining vegetation dynamics and the distribution of fire-response types in landscapes (Carrington and Keeley, 1999; Bond et al., 2005). In particular, many obligate seeders have been shown to exhibit functional traits that confer drought tolerance, such as longer leaf life span, low specific leaf area and tolerance of leaf turfgor loss (Ackerly, 2004; Bond et al., 2005). This may be related to the lower root-to-shoot investment often found in obligate seeders, which limits access to soil water (Pausas et al., 2004). However, these trait associations are not always evident or consistent across climatic regions, habitats or taxonomic groups (Palacio et al., 2007; Tolsma et al., 2007). In particular, in non-Mediterranean-type climates, where drought may be less pronounced, there may be less differentiation between fire-response types in traits related to drought stress (Tolsma et al., 2007). Hence, testing trait correlations across a comprehensive range of ecosystems and climate regions is important in understanding whether patterns have relevance at a global scale (Pausas et al., 2004).

In this paper, our aim is to determine whether species growing in a non-Mediterranean climate region exhibit relationships between their fire-response strategy and other functional traits. We focus on shrub species growing in a mountainous region of south-eastern Australia, covering temperate, subalpine and alpine climates (Fig. 1). The fire regime consists of generally high intensity, canopy-consuming fires that occur in the dry summer months, although intensity varies within individual fires, with unburned patches remaining (Williams et al., 2008). Extensive areas of flammable eucalyptus forest in lower-elevation and subalpine foothills of the region carry fires upslope into the alpine treeless zones, resulting in large, landscape-level fires with a frequency of approximately one to several fires per century (Banks, 1989; Zylstra, 2006; Williams et al., 2008). The vegetation of the region is largely resilient to this fire-interval regime, with a mixture of fire-response strategies present (Walsh and McDougall, 2004; Williams et al., 2008). The majority of species have some resprouting capacity, with obligate seeders occurring in smaller numbers, many with long-lived soil or canopy seed banks (Carey et al., 2003; Walsh and McDougall, 2004; Williams et al., 2008). Several obligate seeders are important structural dominants, such as Eucalyptus delegatensis on sheltered slopes in subalpine areas (Vivian et al., 2008), Leptospermum micromyrtus on rock outcrops (Doherty and Wright, 2004a) and Richea continitensis in alpine wetlands (Walsh and McDougall, 2004).

We compared a range of leaf traits between species with different fire-response strategies. Leaf traits are considered to be fundamental components of the ecological strategies of plants, relating to how plants acquire and use resources (Givnish, 1987; Reich et al., 1997; Westoby, 1998; Diaz et al., 2004), as well as playing an important role in influencing the properties of ecosystems (Lavorel and Garnier, 2002). We quantified five leaf traits: specific leaf area, leaf dry-matter content, leaf width, leaf nitrogen content, and leaf carbon to nitrogen ratios (Cunningham et al., 1999; Wright et al., 2001; Cornelissen et al., 2003; Diaz et al., 2004). These traits reflect a spectrum of leaf economics, ranging from a strategy of rapid resource uptake in leaves with a greater investment into leaf photosynthetic area than structure, and high nitrogen content, to the conservation of resources in longer-lived leaves that have a greater investment into structural attributes than area (Reich et al., 2003; Diaz et al., 2004; Wright et al., 2004). In fire-prone ecosystems, correlations of these leaf traits with fire-response strategies may reflect different trade-offs and resource acquisition strategies associated with post-fire seed recruitment and resprouting. Furthermore, changes in the assemblage of fire-response types, and hence changes in the leaf traits within a community, could influence ecosystem properties, such as flammability (Lavorel and Garnier, 2002; Scarrf and Westoby, 2006).

The occurrence of unplanned, widespread fires across the region in 2003 created an opportunity to obtain fire-response trait data in an area where this information is lacking for many species (Vivian et al., 2010). This allowed us to allocate species to fire-response types using field-collected data, and to incorporate any within-species variation in fire-responses. We took two approaches to classifying species into fire-response groups. Firstly, we divided species into two groups: resprouters and obligate seeders (or non-resprouters). However, associations between fire-responses and other functional traits may be more evident when considering resprouting and seed recruitment in combination (Pausas et al., 2004). Therefore, as a second approach we subdivided resprouters into those that also recruited from seed (facultative resprouters) and those that did not (obligate resprouters). Previous studies have found facultative resprouters to be more similar to obligate seeders in some traits, yet more similar to obligate resprouters in other traits (Saura-Mas and Lloret, 2007; Pratt et al., 2008). The central questions addressed are: (1) Do shrub species with different fire-response strategies also differ in their leaf traits? (2) If so, are the differences identified similar to those found previously in Mediterranean-type climates?

MATERIALS AND METHODS

Study area

The study was carried out in south-eastern Australia (Fig. 1). The study area covers a large part of the Australian Alps in New South Wales (NSW) and the Australian Capital Territory (ACT), the South Eastern Highlands bioregion, and extends...
into the South Western Slopes bioregion at its most northern extent (Fig 1; NSW National Parks and Wildlife Service, 2003). Elevation ranges from 500 m to over 2000 m a.s.l. The north of the study area is located in a temperate climatic zone, characterized by warm summers with mean maximum temperatures of over 30 °C (NSW National Parks and Wildlife Service, 2003). Further south is a transition to increasingly mild summers, with small areas of subalpine and alpine climatic zones at the highest elevations. Mean annual precipitation ranges from 750 mm in the north to over 2000 mm in the south (Houlder et al., 2000). Vegetation community types include woodlands and dry forest at lower elevations, and tall moist forest at mid- to higher elevations (NSW National Parks and Wildlife Service, 2003). Interspersed amongst these are smaller pockets of swamps, grasslands, rock outcrops and heathlands. The southern extent of the study area supports subalpine and alpine vegetation, predominantly Eucalyptus pauciflora (snow gum) woodland, and treeless communities such as

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**Fig. 1.** Location of the study area. (A) The Australian continent, indicating the regional location of the study area as a shaded rectangle in (B). (C) Enlargement of the study area, including the extent of the 2003 fires. ACT, Australian Capital Territory; NSW, New South Wales.
Species selection and sampling strategy

In January 2003, following several months of severe drought, lightning strikes triggered fires that burned through much of the study area over several weeks (Fig. 1; McLeod, 2003). These fires varied in their intensity and severity across the region. Surveys were conducted approx. 1 year later to assess the resprouting and seed recruitment traits of plant species across a wide range of vegetation types and fire severity levels. This monitoring was carried out independently by scientific teams from five organizations: the Commonwealth Scientific and Industrial Research Organisation; NSW Department of Environment, Conservation and Climate Change; ACT Parks, Conservation and Lands; the Australian Alps Liaison Committee and EcoGIS (Crawford, 2004; Doherty and Wright, 2004b; Walsh and McDougall, 2004; Kitchin, 2005; McDonald, 2005). The data from these surveys, which included the observed fire-response traits of all vascular plant species in 284 sites, were collated for analysis in the present study.

We used a number of criteria to select a subset of these species for leaf-trait measurements, including: (a) woody shrubs; (b) fire-response (see text below) and taxonomic relatedness; (c) accessibility; and (d) protection status (we did not sample protected, vulnerable or threatened species). Where possible, species were selected to ensure that there was more than one species, and more than one fire-response type, per family across a range of families (Fig. 2), with the aim of sampling as many species as possible. However, this was not readily achievable because few woody shrubs were observed in the original surveys as being consistent obligate seeders compared with obligate resprouters and facultative resprouters. As such, we included almost all readily accessible obligate seeders recorded in the original surveys. From these criteria we measured leaf traits on 81 shrub species, which represented 47 % of the total number of shrub species in the original surveys.

Leaf sampling was carried out in December 2008 and January 2009. For each of the 81 species, we sampled leaves from populations at locations near to one of the original vegetation survey sites. Widespread species were sampled from more than one location, where possible. Of the 81 species, one species was sampled from three locations, 11 from two locations and the remainder from a single location, giving 94 populations in total (Fig. 2 and Table 1). Within each location, we selected five individual plants of average height and sampled at least two leaves from each individual. Only young but fully expanded leaves, without evidence of herbivore or pathogen damage, were collected. Because the area was burned in 2003, the plant stems (arising from either seedlings or resprouts) were up to 6 years old at the time of sampling. Following standard protocols, leaves were sampled at least 2 h after sunrise and 3 h before sunset (Garnier et al., 2001), kept moist in sealed plastic bags and stored in a refrigerated condition until measurement in a laboratory the following day (Garnier et al., 2001). Phyllodes from Acacia species were treated as leaves.

Classification of fire-responses

A key step in the selection of the species subset was to classify each species according to their fire-response. Many of the study species had been recorded in multiple sites during the original fire-response surveys. Some between-site variation occurred: 23 % of all woody species varied between sites in their resprouting response, and 40 % varied in their seeding response, partly because of variation in fire severity across sites. Classifying species when intraspecific variation occurs can be challenging (Vivian et al., 2010). For this study, we determined the fire-responses of species from sites which had been burned by high-severity fire (i.e. where the canopy had been at least fully scorched), a condition that has previously been used to standardize fire-responses between and within species (Gill, 1981). Four species (5 % of the total) were only recorded in low-severity sites and for these species we allocated the fire-response observed from the low-severity sites. Then the following rules were used to classify each species:

1. Species were classified as obligate seeders if they had been recorded as fire-killed and were regenerating from seed, indicated by the presence of seedlings, in all (high-severity) sites in which they occurred.
2. The remaining species, which had all been recorded as surviving by resprouting, were classified as resprouters.

We then subdivided the resprouters into two further groups:

1a. Facultative resprouters were those species which had been observed resprouting and regenerating from seed, as indicated by the presence of seedlings. Some of the species that were classified as facultative resprouters were observed in some sites as obligate seeding or obligate resprouting, but overall in the study area were recorded as expressing both responses.
1b. Obligate resprouters were those species that exhibited resprouting but had not been recorded as regenerating from seed in any (high-severity) sites. An additional four species were also classified as obligate resprouters because they were recorded as obligate resprouters in all but one site in which they were recorded.

Of the 81 species, which occurred in 15 families, 23 were classified as obligate seeders, 29 as facultative resprouters and 29 as obligate seeders (Table 1). Nineteen species occurred in just one site and were classified with no information on the potential for between-site variability. Consequently, there was less certainty in the fire-response classification of infrequently occurring species.

Measurement of leaf traits

The one-sided area of each sampled leaf was measured using the imaging processing program ImageJ (Rasband, 1997–2009). Whilst fresh, leaves were placed under a piece of clear perspex alongside a scale bar and photographed using a digital camera. In ImageJ, the photographs of each leaf were converted to a binary image so that the leaf blade was represented as black on a white background. Shadows...
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Fig. 2. Study species (81 in total) represented in a family-level phylogenetic tree. Fire-responses for each species are shown. Twelve species were sampled from more than one location, giving 94 populations in total: *, species sampled from two locations; **, species sampled from three locations.
were removed prior to image conversion. Leaf area and leaf width were measured from the image, with leaf width measured as the diameter of the largest circle that could fit in the boundary of the leaf blade (Reich et al., 1997; Cunningham et al., 1999). Each leaf was then weighed, oven dried at 60 °C for 72 h, and re-weighed. Specific leaf area was calculated as the one-sided area of a fresh leaf divided by the oven-dried mass. Leaf dry-matter content was calculated as the oven dry mass of a leaf divided by its fresh mass (Garnier et al., 2001; Cornelissen et al., 2003).

Leaf nitrogen and carbon content were calculated as the percentage of each element as a proportion of leaf dry mass. Firstly, additional leaves from the five individual plants sampled per location were combined and oven dried at 60 °C for 72 h (Cornelissen et al., 2003). The dried leaf material was then ground to a fine powder using a ball mill and placed in a glass scintillation vial for analysis. Leaf nitrogen and carbon were analysed using Dumas combustion (Carlo Erba EA-1110 CHN-O Elemental Analyser).

### Statistical analyses

The dependent variables for the analyses were leaf dry-matter content, specific leaf area, leaf width, leaf nitrogen content, and leaf carbon to nitrogen ratio. The independent variable, fire-response type, was considered in two ways: (1) as a categorical variable with two levels (obligate seeders vs. all other resprouters); and (2) as a categorical variable with three levels, by subdividing resprouters according to the absence or presence of seed regeneration (obligate seeders vs. obligate resprouters vs. facultative resprouters). For all analyses, the level of replication was the mean of each population (n = 94), with the leaf-trait value for each population calculated from the five individuals sampled (Saura-Mas and Lloret, 2007).

Firstly, we conducted a principal components analysis (PCA) to assess relationships amongst the dependent variables and to determine whether populations could be segregated by their fire-response type, based on the measured traits. t-Tests were used to compare loading scores between obligate seeders and all resprouters, and one-way analysis of variance (ANOVA) was used to compare loading scores between obligate seeders, facultative resprouters and obligate resprouters. Secondly, each leaf trait was compared between fire-response types, using t-tests to compare means between obligate seeders and all resprouters combined, and one-way ANOVA for comparisons between obligate seeders, facultative resprouters and obligate resprouters. Where there were differences between the three categories of fire-response types, we conducted post-hoc Tukey’s HSD tests to determine whether populations could be segregated by fire-response type. Within each divergence, means of leaf-trait values were calculated for each level of fire-response type by averaging across the daughter nodes within each fire-response type. The difference between the means was considered to be a single phyllogenetic-independent contrast. We then tested whether any trends in leaf traits that were found across all species were also evident in the majority of these phyllogenetic-independent contrasts (e.g. Cunningham et al., 1999). Where we had a sufficient number of phyllogenetic-independent contrasts, we used sign tests to determine the significance of the number of contrasts showing the expected trend (i.e. the trend identified across all species) to the total number of contrasts (Webb et al., 2008).

AOT can only be used to calculate phyllogenetic-independent contrasts between pairs of continuous traits, or between continuous and binary traits. Therefore, we used AOT to calculate phyllogenetic-independent contrasts for the comparison of leaf traits between obligate seeders and all resprouters, and calculated phyllogenetic-independent contrasts by hand for the comparison of leaf traits between obligate seeders, facultative resprouters and obligate resprouters (e.g. Cunningham et al., 1999), following the same method used by AOT (Webb et al., 2008). For the three-way comparison, we performed phyllogenetic-independent contrasts only for those leaf traits that were found to be significantly different with the full dataset, and searched for the same trend between fire-response types.

Closely related species may share similar traits because of common ancestry (Silvertown and Dodd, 1996). Hence, trait correlations that are limited to within a clade could be attributed to phylogenetic constraints imposed by the evolutionary history of the particular group, although these traits may still be functionally important (Westoby, 2007). We investigated the effect of phylogenetic relatedness with the assistance of the software package Phylocom (Webb et al., 2008). Firstly, we used Phylomatic, a tool contained in Phylocom, to generate a phylogenetic tree of the study species using information from the angiosperm phylogeny of Davies et al. (2004) (archived at http://svn.phylodiversity.net/tot/megatrees/). Any unresolved relationships between genera within families, and all species within genera, were treated as polytomies. We then used the Analysis of Traits (AOT) module of Phylocom to perform phylogenetic-independent contrasts. Each contrast is a separate evolutionary divergence containing all categories of fire-response type. Within each divergence, means of leaf-trait values were calculated for each level of fire-response type by averaging across the daughter nodes within each fire-response type. The difference between the means was considered to be a single phyllogenetic-independent contrast. We then tested whether any trends in leaf traits that were found across all species were also evident in the majority of these phyllogenetic-independent contrasts (e.g. Cunningham et al., 1999). Where we had a sufficient number of phyllogenetic-independent contrasts, we used sign tests to determine the significance of the number of contrasts showing the expected trend (i.e. the trend identified across all species) to the total number of contrasts (Webb et al., 2008).

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For the analysis of leaf traits between the simpler, two-level fire-response classification (obligate seeders vs. all resprouters), 15 phyllogenetic-independent contrasts were identified, providing a sufficient number of contrasts to determine significance.
using a sign test (e.g. Cunningham *et al.*, 1999). However, for the analysis of leaf traits between fire-response types considered at three levels, only three phylogenetic-independent contrasts were identified.

We also explored a potentially confounded relationship between facultative resprouters and the Fabaceae. Of the 20 study species that were Fabaceae, 15 were facultative resprouters (Fig. 2). Preliminary analyses suggested that leaf nitrogen content was higher in the facultative resprouters, which could be related to the nitrogen-fixing strategy of the Fabaceae rather than to the trade-offs associated with the facultative resprouting strategy more broadly. To determine whether relationships between leaf traits and fire-response types were consistent in other phylogenetic groups, we ran both our trait and phylogenetic analyses using the full dataset as well as with a reduced dataset that excluded the Fabaceae (Table 1; 61 species; 68 populations). Using the reduced dataset, the number of phylogenetic-independent contrasts numbered 14 and 2 for the analysis of fire-response type as a two-level and three-level categorical variable, respectively.

**RESULTS**

The first three axes of the PCA explained over 90% of the variance in leaf-trait values amongst the study populations (Fig. 3 and Table 2). PCA 1 was strongly correlated with leaf nitrogen traits, and PCA 2 was strongly correlated with leaf dry-matter content. The third principal component was most strongly associated with leaf width (Table 2). Facultative resprouters were grouped together along PCA 1, with a significantly higher loading score than either obligate seeders or facultative resprouters, suggesting a higher leaf nitrogen content and a lower relative investment in leaf carbon (Fig. 4). No other clear segregation of species into fire-response types was evident (Figs 3 and 4).

**Table 2. Results from principal components analysis**

<table>
<thead>
<tr>
<th>Principal component</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>2.41</td>
<td>1.21</td>
<td>0.96</td>
</tr>
<tr>
<td>% of variance</td>
<td>48.09</td>
<td>24.16</td>
<td>19.16</td>
</tr>
<tr>
<td>Cumulative % variance</td>
<td>48.09</td>
<td>72.25</td>
<td>91.42</td>
</tr>
<tr>
<td>Loadings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDMC</td>
<td>–0.532</td>
<td>–0.724</td>
<td>0.139</td>
</tr>
<tr>
<td>SLA</td>
<td>0.656</td>
<td>0.352</td>
<td>–0.518</td>
</tr>
<tr>
<td>LW</td>
<td>0.327</td>
<td>0.437</td>
<td>0.811</td>
</tr>
<tr>
<td>LNC</td>
<td>0.871</td>
<td>–0.464</td>
<td>0.087</td>
</tr>
<tr>
<td>C:N</td>
<td>–0.908</td>
<td>0.391</td>
<td>–0.080</td>
</tr>
</tbody>
</table>

Each loading is scaled to its standard deviation. LDMC, leaf dry-matter content; SLA, specific leaf area; LW, leaf width; LNC, leaf nitrogen content; C:N = carbon to nitrogen ratio.
When considering fire-response type as a categorical variable with two levels (obligate seeders vs. all resprouters), leaf nitrogen content was the only leaf trait to vary significantly, being lower in the obligate seeders (Table 3A). This trend was also evident in 12 of the 15 phylogenetic-independent contrasts performed. However, this difference disappeared when the Fabaceae were removed from the analysis (Table 3A).

In contrast, a greater number of leaf traits differed when comparing obligate seeders, facultative resprouters and obligate resprouters. Across all species, facultative resprouters had a higher leaf width than obligate resprouters, a higher leaf nitrogen content than both obligate seeders and obligate resprouters, and a lower leaf carbon to nitrogen ratio than both obligate seeders and obligate resprouters (Table 3B). This difference in leaf width was also evident in all three phylogenetic-independent contrasts. However, the trends in leaf nitrogen content and carbon to nitrogen ratio were not consistent in any of the phylogenetic-independent contrasts (Table 3B). When the Fabaceae were removed from the analysis, the differences in leaf width, leaf nitrogen content and carbon to nitrogen ratio between facultative resprouters and the other two fire-response types remained. In addition, facultative resprouters had a lower leaf dry-matter content than both obligate seeders and obligate resprouters (Table 3B).

**DISCUSSION**

Our aim was to determine whether shrub species growing in a non-Mediterranean climate region exhibited a relationship between their fire-response strategy and other functional traits. We compared leaf traits in a large number of shrub species from 15 families. However, differences were only evident when we considered facultative resprouters as a separate fire-response type to obligate resprouters. Facultative resprouters had wider leaves, with higher nitrogen content and a lower carbon to nitrogen ratio, than obligate seeders and obligate resprouters. The latter two fire-response types were similar in the leaf traits measured. Furthermore, when considering all resprouters as one group, the only leaf trait that differed in comparison to obligate seeders was leaf nitrogen content, an effect that disappeared when we removed the Fabaceae from the analysis. These results are in contrast to those previously found in many studies exploring patterns in Mediterranean-type climates. As such, this study provides important information on the limits to trait co-occurrences across different ecosystems (Pausas et al., 2004).

The investment a plant requires for ensuring successful post-fire resprouting and/or seeding has been found to be associated with trade-offs that are manifested in a range of co-occurring morphological, life history and physiological traits. Biogeographical studies, as well as experimental research, suggest that these sets of co-occurring traits can lead to particular fire-response types becoming dominant across gradients of resource availability and under particular fire regimes (Pausas et al., 2004; Knox and Clarke, 2005; Lloret et al., 2005). For a resprouter, the costs of allocation to storage reserves may become inefficient where soil fertility is low.
et al. Saura-Mas under recent, drier Quaternary climates (Verdu, 2000; 2009). However, in the present study, there were no differences evident between obligate seeders and all resprouters, or between obligate seeders and obligate resprouters, in a range of leaf traits. This suggests that potential influential factors such as climate, soil fertility and fire frequency, are exerting similar effects on species’ ecological strategies, irrespective of the constraints and trade-offs that may be associated with obligate seeding and resprouting. Similarly, a study of alpine grassland species in a nearby region found no differences in root morphology and carbohydrate concentrations between obligate seeders, obligate resprouters and facultative resprouters (Tolmsa et al., 2007). This suggested that the characteristics of the alpine environment, such as short growing season, were the major influence on root traits, equally affecting species with different fire-response strategies (Tolmsa et al., 2007).

Our study included samples from lower, warmer, elevations as well as alpine habitats. In all these locations seasonal differences in precipitation are not as marked as in Mediterranean climates. Even at high altitudes, only around 56% of the annual precipitation falls in winter months (April–September; data from the Bureau of Meteorology www.bom.gov.au), compared with over 75% – and up to 95% – in fire-prone Mediterranean regions (Cowling et al., 2005). As such, in our study, summer water deficits are less likely to play a role in influencing species’ traits, resulting in reduced pressure for those species that may have limited access to soil water (e.g. obligate seeders) to compensate with drought-tolerant leaf traits. Examination of root traits between fire-response groups was the same as was found across all species) out of the total number of contrasts. Significance level for PICs, using a sign test, is shown for (A), where ns = non-significant, * = P < 0.05.

### Table 3. Comparison of trait values between fire-response types: (A) obligate seeders compared with all resprouters; and (B) obligate seeders (OS) vs. obligate resprouters (OR) vs. facultative resprouters (F)

<table>
<thead>
<tr>
<th>Trait</th>
<th>SLA (mm² mg⁻¹)</th>
<th>LDMC (%)</th>
<th>LW (mm)</th>
<th>LNC (%)</th>
<th>C : N</th>
</tr>
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<tbody>
<tr>
<td>(A) Obligate seeders compared with all resprouters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obligate seeders</td>
<td>8.85 ± 0.79</td>
<td>44.93 ± 1.89</td>
<td>8.77 ± 1.87</td>
<td>1.42 ± 0.08</td>
<td>37.39 ± 2.05</td>
</tr>
<tr>
<td>Resprouters (all)</td>
<td>9.07 ± 0.65</td>
<td>46.86 ± 1.67</td>
<td>13.34 ± 2.02</td>
<td>1.55 ± 0.10</td>
<td>37.46 ± 2.29</td>
</tr>
<tr>
<td>Facultative seeders</td>
<td>9.07 ± 0.75</td>
<td>42.29 ± 1.51</td>
<td>13.34 ± 2.02</td>
<td>1.55 ± 0.10</td>
<td>37.46 ± 2.29</td>
</tr>
<tr>
<td>F</td>
<td>0.359</td>
<td>2.1172,91</td>
<td>5.0472,91</td>
<td>1.4585,91</td>
<td>2.8629,91</td>
</tr>
<tr>
<td>P</td>
<td>0.699</td>
<td>0.126</td>
<td>0.008</td>
<td>0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Post-hoc comparison</td>
<td>–</td>
<td>–</td>
<td>F &gt; OR</td>
<td>F &gt; OR,OS</td>
<td>F &lt; OS,OR</td>
</tr>
<tr>
<td>PIC: C/Ftot</td>
<td>8/14 ns</td>
<td>7/14 ns</td>
<td>0.008</td>
<td>0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

| Phylogenetic-independent contrasts (PICs): C/Ftot = number of positive contrasts (contrasts in which the direction of difference in trait means between fire-response groups was the same as was found across all species) out of the total number of contrasts. Significance level for PICs, using a sign test, is shown for (A), where ns = non-significant, * = P < 0.05. |

<table>
<thead>
<tr>
<th>Trait</th>
<th>SLA (mm² mg⁻¹)</th>
<th>LDMC (%)</th>
<th>LW (mm)</th>
<th>LNC (%)</th>
<th>C : N</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B) Obligate seeders vs. obligate resprouters vs. facultative resprouters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obligate seeders</td>
<td>8.85 ± 0.79</td>
<td>44.93 ± 1.89</td>
<td>8.77 ± 1.87</td>
<td>1.42 ± 0.08</td>
<td>37.39 ± 2.05</td>
</tr>
<tr>
<td>Obligate resprouters</td>
<td>9.07 ± 0.65</td>
<td>46.86 ± 1.67</td>
<td>13.34 ± 2.02</td>
<td>1.55 ± 0.10</td>
<td>37.46 ± 2.29</td>
</tr>
<tr>
<td>Facultative resprouters</td>
<td>9.07 ± 0.75</td>
<td>42.29 ± 1.51</td>
<td>13.34 ± 2.02</td>
<td>1.55 ± 0.10</td>
<td>37.46 ± 2.29</td>
</tr>
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<td>7/14 ns</td>
<td>0.008</td>
<td>0.001</td>
<td>0.004</td>
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</tbody>
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Phylogenetic-independent contrasts (PICs): C/Ftot = number of positive contrasts (contrasts in which the direction of difference in trait means between fire-response groups was the same as was found across all species) out of the total number of contrasts. Significance level for PICs, using a sign test, is shown for (A), where ns = non-significant, * = P < 0.05.

F: Degrees of freedom; P, significance level (highlighted in bold where P < 0.05); SLA, specific leaf area; LDMC, leaf dry-matter content; LW, leaf width; LNC, leaf nitrogen content; C : N, carbon to nitrogen ratio.

(Wisheu, 2000), or where fire frequencies are very high or very low (Iwasa and Kubo, 1997; Bellingham and Sparrow, 2000). The larger and more extensive root systems of many resprouters may also provide greater access to soil moisture, resulting in more resprouters in wetter parts of landscapes (Keeley, 1986; Pate et al., 1990, 1991; Bell et al., 1996; Verdu, 2000). In the Mediterranean basin, drought-tolerant traits in obligate seeders may be due to the evolution of this strategy under recent, drier Quaternary climates (Verdu, 2000; Saura-Mas et al., 2009). However, in the present study, there were no differences evident between obligate seeders and all resprouters, or between obligate seeders and obligate resprouters, in a range of leaf traits. This suggests that potential influential factors such as climate, soil fertility and fire frequency, are exerting similar effects on species’ ecological strategies, irrespective of the constraints and trade-offs that may be associated with obligate seeding and resprouting. Similarly, a study of alpine grassland species in a nearby region found no differences in root morphology and carbohydrate concentrations between obligate seeders, obligate resprouters and facultative resprouters (Tolmsa et al., 2007). This suggested that the characteristics of the alpine environment, such as short growing season, were the major influence on root traits.
Mediterranean regions, although they may be more similar to those in the Californian chaparral (Cowling et al., 1996; Keeley and Fotheringham, 2001). As such, very short (or very long) time periods between fires are likely to be of less influence in determining plant strategies. However, at a within-landscape scale, fire regimes may vary considerably, such as occurring at a lower frequency on rock outcrops or in wetter habitats (Gill and Bradstock, 1995). Further investigation of co-occurring traits at the habitat scale may therefore aid in understanding relationships between fire-responses and broader plant strategies.

Facultative resprouters were identified in our study as exhibiting differences in leaf traits compared with other fire-response groups. In general, trait covariation has been less frequently explored in facultative resprouters than in obligate seeders and obligate resprouters, despite facultative resprouters being an important component of plant communities in many fire-prone ecosystems (e.g. Buhk et al., 2007; Pratt et al., 2008). Buhk et al. (2007) suggested that facultative resprouters may be limited by the necessity for investment into resources that support both post-fire recruitment and resprouting, in contrast to obligate resprouters and obligate seeders, which only invest in one or other strategy. Similarly, Higgins et al. (2008) proposed that for resprouters, selection favours increased adult survival rates, and increased seed production and dispersibility in seeders, such that investment into either resprouting or reproduction by seed is promoted, rather than a strategy to maximize both. Various costs associated with this double strategy of post-fire seed recruitment and resprouting have been identified elsewhere. Pratt et al. (2008) found that seedlings of woody facultative resprouters were the least tolerant of water stress in southern California. In the South African Cape Floristic Region, Verdaguer and Ojeda (2002) recorded higher root starch and greater root to shoot ratios in seedlings of the facultative resprouter form of Erica species, compared with seedlings of the obligate seeder form. Lower post-fire seedling densities have also been identified in facultative resprouting species compared with closely related obligate seeders (Benwell, 1998; Vivian et al., 2008).

In this study, the combination of traits identified in facultative resprouters – wider and more nitrogen-rich leaves – suggests a strategy along the leaf economics spectrum of rapid resource uptake through higher photosynthetic rates, short leaf life-spans and a lower investment in leaf structure, in comparison to that of other fire-response types (Reich et al., 1997; Wright et al., 2004). This combination of traits could be indicative of a strategy that requires facultative resprouting plants to quickly acquire resources, particularly immediately after fire, in order to compete with obligate seeders and resprouters, as a result of trade-offs associated with the costs of supporting both post-fire resprouting and seeding. When we considered resprouters as one group, these differences largely disappeared. Resprouters had a higher nitrogen content, including in 12 out of 15 of the phylogenetic-independent comparisons. However, when the Fabaceae were removed, this was not significant. Again this suggests that for the shrub species in the study area, any variability in the expression of leaf traits is only evident when resprouters are subdivided according to whether they also regenerated from seed. This result reflects those of Pausas et al. (2004), who considered fire-responses in a hierarchical manner. In a review covering a range of taxonomic groups and regions, trait correlations and landscape patterns depended on whether fire-responses were considered as two functional types (resprouters vs. non-resprouters) or as four functional types (the combination of post-fire seeding and resprouting) (Pausas et al., 2004). It is possible that further subdivisions in fire-response classifications could reveal meaningful differences in the trade-offs associated with particular strategies. For example, within resprouting plants, differences in the location of sprouts (e.g. from roots or from stem-derived structures such as rhizomes or lignotubers) may influence resource allocation within the plant body (Palacio et al., 2007).

Of the 29 facultative resprouters included in our study, 15 were Fabaceae, including members of the subfamilies Mimosoideae (the acacias) and Faboideae. The functional importance of the leaf traits identified in the facultative resprouters could be more directly related to ancestral characteristics of this family, particularly the nitrogen-fixing strategy of the Fabaceae (Cornelissen et al., 1997; Wright et al., 2005), rather than associated with any trade-offs or costs of the facultative resprouting strategy more broadly. However, differences in leaf width, leaf nitrogen content and carbon to nitrogen ratios were also evident when the Fabaceae were removed from the analysis. Greater leaf width was also evident in the two phylogenetic-independent contrasts conducted. Furthermore, across the remaining 68 populations, leaf dry-matter content was lower in facultative resprouters, again suggesting a greater investment towards rapid biomass production rather than leaf structure and nutrient retention. The consistent nature of these results suggests that these patterns are robust to phylogenetic relatedness.

Understanding the relationships between the fire-response strategies of plants and co-varying traits will improve our knowledge of the functional role of species in fire-prone ecosystems. In particular, variability in fire regimes can promote, reduce or even remove species with particular combinations of fire-response traits (Gill and Bradstock, 1995; Morrison et al., 1996; Bradstock and Kenny, 2003; Burrows and Wardell-Johnson, 2003). Hence, if species with particular fire-response strategies have recurring associations with other traits, such as leaf attributes, this could facilitate prediction of other consequences of changes in fire regimes, such as on ecosystem function. For example, at a landscape level, any changes in the distribution and dominance of fire-response types with divergent leaf traits may also alter ecosystem flammability and fuel structure (Scarf and Westoby, 2006; Saura-Mas et al., 2010). In addition, understanding consistent associations between fire-response strategies and other traits could simplify the selection of traits used to summarize plant strategies and the development of plant functional groups. However, although the evidence for covariation amongst fire-response strategies and a range of other traits have been building across an array of taxonomic groups and geographic locations, much of this research has been conducted in Mediterranean-climate regions. Our study suggests that these relationships may not be consistent in other areas. Understanding the limits to the extent of trait associations is a critical part of understanding the role of plant functional traits and disturbance-response strategies globally.
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