Soil P availability limits legume persistence and distribution in the fynbos of the Cape Floristic Region

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Psoralea fleta, Bainskloof Pass (Photo: C.H Stirton)

Scientific discovery has always relished its serendipitous side but had we been satisfied simply with the outcomes of trial and error we would not be where we are today.

D.T Clarkson

Declaration

I know the meaning of plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own. The thesis is submitted for the degree of Master of Science in the Department of Botany, University of Cape Town. It has not been for any degree or examination at any other university.

Simon Power

Abstract

Legumes are unable to persist through post-fire succession in fynbos vegetation of the CFR, unlike species in families such as Proteaceae and Restionaceae. The majority of fynbos legumes are seeders (> 75%), which tend to be shorter-lived than co-occurring resprouters. Seeders are likely to have a higher nutrient requirement than resprouters, given that they tend to invest more biomass-above ground, grow faster and produce larger amounts of seed. In the oligotrophic soils occupied by fynbos, symbiotic N_2-fixation enables legumes to overcome low N availability but not low P availability. I hypothesized that: (1) legumes are less effective at acquiring P from sparingly soluble sources compared with members of Proteaceae and Restionaceae (non-legumes); (2) legume seeders occupy soils with a higher nutrient status than resprouters. P-acquisition strategies of legumes and non-legumes were assessed when supplied either 1 or 10 mg P kg^{-1} of sparingly soluble P (75% $FePO_4$ and 25% $Ca_5(PO_4)_3(OH)$). The soil nutrient status of the habitat of seeders and resprouters from legume genera *Otholobium* and *Psoralea* were characterized. Legumes were predominantly reliant on mycorrhizae for P-acquisition, although *Aspalathus* did produce root clusters. In contrast, root clusters were common in Restionaceae and Proteaceae. *Aspalathus* root clusters released carboxylates at lower concentrations and lower efficacy than did Proteaceae. Species with root clusters have a significant advantage over species that lack them for P-acquisition from sparingly soluble sources. Legume seeders were found to occupy soils with a higher pH, [NO_3^-], exchangeable [Ca], CEC but lower [Fe] than resprouters. Some legume seeders (e.g. *Otholobium* spp.) occupied soils with a higher P availability than resprouters, but the majority of fynbos species, including *Psoralea* spp. occur on soils where P availability is low. Resprouters also appeared to be adapted to low P soils. Their association with oligotrophic soils is probably the ancestral condition. Supporting this contention they had a weak capacity to down-regulate P-uptake when P was readily available. In addition to the lack of effective root adaptations, legume leaves had a higher specific leaf area, associated with a lower degree of sclerophylly than did non-legume species, implying a weaker capacity to conserve nutrients. Thus the majority of fynbos legumes are probably unable to persist through post-fire succession because of a combination of low soil P availability, the lack of effective P-acquisition strategies for recovering sparingly soluble P and a weak capacity to conserve nutrients relative to co-occurring non-legumes.

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Chapter 1

General Introduction

Fynbos, a vegetation-type native to the Cape Floristic Region (CFR), inhabits oligotrophic soils (Stock and Lewis, 1986; Witkowski and Mitchell, 1987). Given their ability to fix atmospheric N_2, legumes should have a competitive advantage over non-fixing species in these soils. Legumes are, however, largely absent from mature fynbos vegetation (+10 years post-fire), being abundant only in the early stages of post-fire succession (Kruger, 1979a; Hoffman *et al*., 1987; Cocks, 1994). Thus in fynbos, legumes appear to be post-fire colonizers that are unable to persist through post-fire succession. Given their potential competitive advantage in the face of nitrogen limitation, the aim of this study was to determine why legumes are unable persist through post-fire succession in fynbos?

The Cape Floristic Region

The CFR, located at the south-western tip of South Africa (Fig 1.1), encompasses two Mediterranean-type ecosystems (MTE's), specifically fynbos and succulent karoo. Other MTE's include chapparal of California, maquis and garrigue of the Mediterranean Basin and, kwongan and mallee of Australia. The climatic regime of the western half of the CFR is typical of an MTE, being characterized by cool wet winters and hot and dry summers (Köppen, 1931; Specht, 1979; Specht and Moll, 1983). In contrast, the eastern half receives rainfall throughout the year (Fig 1.1; Deacon *et al*., 1992; Cowling *et al*., 1997; Goldblatt and Manning, 2000; Rebelo *et al*., 2006). Average rainfall tends to range from 100 mm in the arid interior to 1300 mm at high altitudes (Goldbaltt and Manning, 2000; Rebelo *et al*., 2006). Given the climatic regime, plant growth tends to occur in spring and early summer, while considerable drought stress is experienced towards the end of summer (Kruger, 1979b; Stock and Allsopp, 1992). Besides climatic regime, other factors such as high species richness, sclerophyllous vegetation, low soil nutrient status and the occurrence of fire, characterize the CFR.

Although the CFR is the smallest region to encompass MTE's, occupying 90 000 km^2 (0.04% of earth's surface; Goldblatt, 1978; Cowling *et al.*, 1992, 1996), it boasts a high level of plant species richness. There are approximately 9000 (69% endemic) vascular plant species, with 20% of species located in Asteraceae and Fabaceae (Goldblatt and Manning, 2000). A large number of species are also located in Iridaceae, Azioaceae, Ericaceae, Proteaceae and Restionaceae (Goldblatt and Manning, 2000). The regional diversity of the CFR is two to three times higher than other MTE's (Cowling *et al.*, 1996) and is recognized as one of six floral kingdoms (Good, 1974; Takhtajan, 1986), and 25 global biodiversity 'hotspots' (Myers *et al.*, 2000).

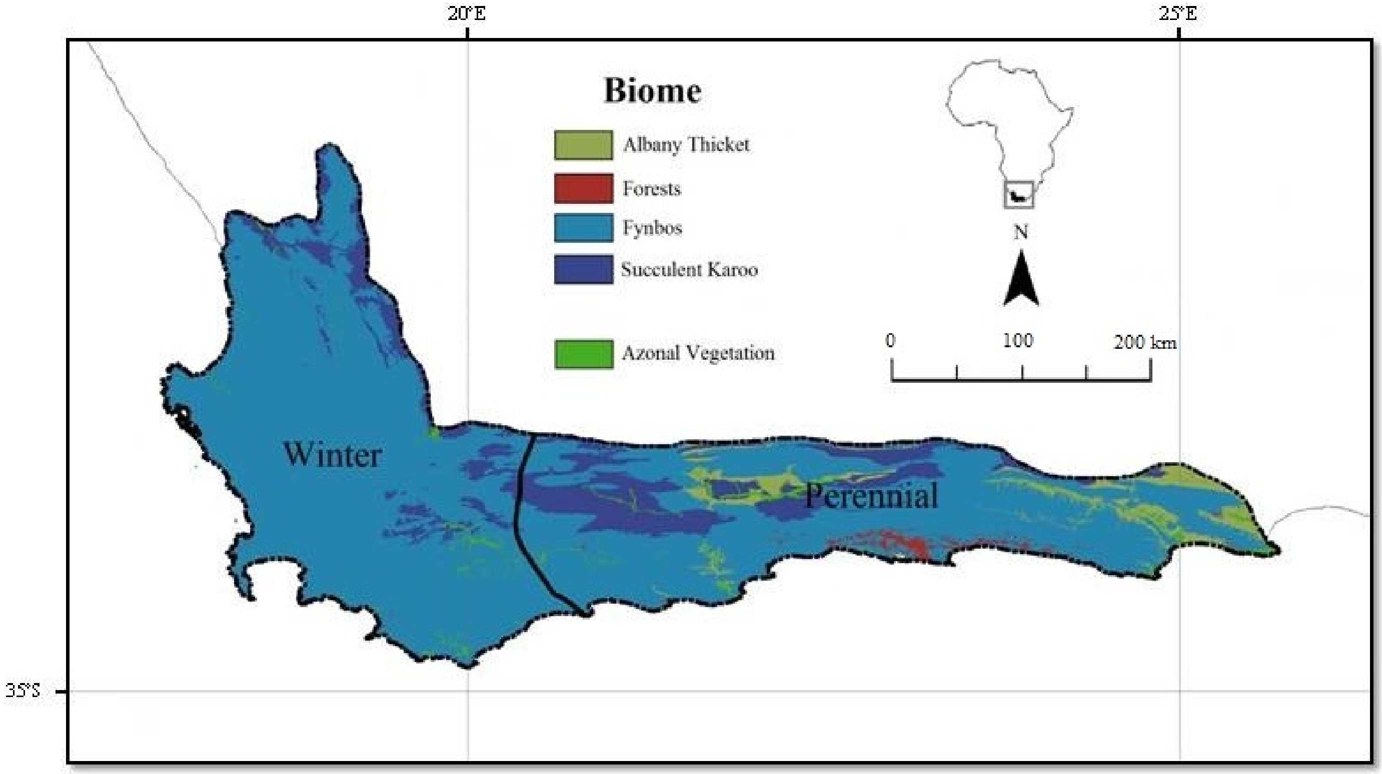

Fig. 1.1 Biomes within the CFR. Azonal vegetation encompasses irregular habitats with special soil forms (e.g. riparian zones, wetlands, saltpans). Solid line separates winter and perennial rainfall zones (modified from Mucina and Rutherford, 2006).

The high species richness in the CFR is associated with a variety of vegetation-types. Four biomes are represented in the region (Fig. 1.1), including Afrotemperate Forest, Albany Thicket, Fynbos and Succulent Karoo (Goldblatt and Manning, 2000; Rebelo *et al.*, 2006). The Fynbos biome occupies the largest area and consists of three vegetation-types: fynbos, renosterveld and western strandveld, of which fynbos is dominant (Cowling *et al.*, 1997;

Rebelo *et al.*, 2006). Fynbos is a fire-prone heathland, containing small-leaved, evergreen sclerophyllous shrubs, typical of MTE's (Kruger, 1983; Cowling and Holmes, 1992; Cowling *et al.*, 1997; Rebelo *et al.*, 2006). In comparison, western strandveld has a lower fire frequency and is typified by broad-leaved shrubs, while renosterveld is distinguished from fynbos primarily by differences in soil fertility and the dominance of small-leaved asteraceous shrubs (Specht and Moll, 1983; Kruger and Bigalke, 1984; Cowling *et al.*, 1997; Rebelo *et al.*, 2006).

The CFR accommodates a mosaic of soil types, derived from various parent materials, including limestone, granite, sandstone and shale (Cowling *et al.*, 1997; Goldblatt and Manning, 2000; Rebelo *et al.*, 2006). Fynbos vegetation predominantly occurs on sandstone-derived soils (Rebelo *et al.*, 2006). These soils are ancient, coarse-grained, highly leached and extremely poor in exchangeable bases, total [N] (0.1–0.2%) and available [P] (0.4–3.7 µg P g^{-1}) (Kruger, 1979b; Stock and Lewis, 1986; Witkowski and Mitchell, 1987). P availability is particularly low in these soils owing to their low pH, and the tendency for P to be locked up in sparingly soluble forms, such as Ca-P and Fe-P (Witkowski and Mitchell, 1987). MTE's generally occupy oligotrophic soils, although chaparral and matorral occupy soils with a higher nutrient status than fynbos or kwongan (Kruger, 1979b; Specht, 1979; Specht and Moll, 1983; Lamont, 1995). In contrast to sandstone-derived soils, limestone-, granite- and shale-derived soils are fine-grained and have a higher nutrient status, containing higher concentrations of N and P (Kruger, 1979b; Witkowski and Mitchell, 1987; Cowling *et al.*, 1997; Rebelo *et al.*, 2006). Apart from the variation between soil types, the nutrient status of CFR soils also fluctuate temporally as a result of fire. Fire, an excellent mineralizing agent, temporarily increases N and P availability post-fire (van Wilgen and Le Maitre, 1981; Brown and Mitchell, 1986; Stock and Lewis, 1986). This flush could dramatically affect species composition immediately post-fire, favouring species with high nutrient demands.

A feature of fynbos species is the presence of specialized root adaptations such as mycorrhizae (arbuscular, ericoid, orchid) and root clusters (cluster/proteoid, capillaroid, dauciform) (Lamont, 1982; Allsopp and Stock, 1993). Mycorrhizal associations are common in Asteraceae, Ericaceae and Fabaceae, whereas root clusters are prevalent in Cyperaceae, Proteaceae and Restionaceae, which are generally non-mycorrhizal (Lamont, 1982; Allsopp and Stock 1993). The advantage of root adaptations such as mycorrihzae and root clusters is their ability to enhance P-acquisition (Lamont, 1982; Marschner and Dell 1994; Smith and Read 1997; Lambers *et al*., 2003, 2006; Lamont, 2003). For instance, root clusters increase root surface area, P-uptake and release of P-solubilizing exudates such as carboxylates and acid-phosphatases (Gardener *et al*., 1983; Dinkelaker *et al*., 1995; Gilbert *et al*., 1999; Grierson and Comerford 2000; Neumann *et al*., 2000; Watt and Evans, 2003; Lambers *et al*., 2006). Thus the presence of specialized root adaptations may be critical to long term persistence in the low P soils of fynbos.

The CFR is characterized by high species turnover along habitat or environmental gradients (beta diversity; Cowling *et al*., 1990). Edaphic diversity has been identified as the primary driver of high beta diversity in the CFR (Cowling, 1990; Cowling *et al*., 1992; McDonald *et al*., 1996). Thus edaphic variation can be considered a major determinant of species distribution in the region. On the Agulhas Plain for example, Richards *et al*. (1995, 1997a) found that the main determinants of fynbos species community boundaries were soil depth, pH and the availability of N and P. The variation in soil nutrient availability between communities suggests variation in plant nutritional requirements. This is supported by transplant experiments done on the Agulhas Plain, where the growth and survival of *Protea* and *Leucadendron* species was greater on native soil types than on neighbouring soil types (Mustart and Cowling, 1993; Richards *et al*., 1997b). The association of different fynbos species with soils of different nutrient status may be driven by differences in nutritional requirements (Richards *et al*., 1997a).

Fire is the predominant form of disturbance in the CFR and is particularly prevalent in fynbos as it is in most MTE's (Specht, 1979; Kruger, 1983; Arroyo *et al*., 1995; Bond and van Wilgen, 1996; Cowling *et al*., 1997; Rebelo *et al*., 2006). Fires in fynbos tend to occur in late summer or early autumn, every 5 to 50 years but more commonly every 15 to 25 years (Kruger and Bigalke, 1984; Le Maitre and Midgley, 1992; Bond and van Wilgen, 1996; Rebelo *et al*., 2006). The intensity of a fire depends on the fuel load and climatic conditions particularly moisture availability and air temperature (Bond and van Wilgen, 1996). In fynbos, fuel load is maximized 21 years after fire (Kruger and Bigalke, 1984). Thus the most intense and devastating fires, which have the greatest impact on the flora, occur in fairly old vegetation during late summer when burning conditions are ideal.

Fire has a major impact on fynbos vegetation through its dramatic ability to affect species composition. The change in fynbos species composition after fire is well documented and is referred to as post-fire succession (Adamson, 1935; Levyns, 1935; Martin, 1966; Kruger, 1979a; Hoffman *et al*., 1987; Cocks, 1994). Post-fire succession consists of a continuum of five step-wise overlapping phases, which are distinguished by species composition patterns (Kruger, 1979a, 1983; Kruger and Bigalke, 1984). The five identified phases are:

1. Immediate post-fire phase (first 12 months): The germination of soil stored seeds and regeneration from vegetative structures occurs. Majority of post-fire annuals/fire-ephemerals and geophytes complete their reproductive cycles.

2. Youth phase (1–5 years): Herbaceous and resprouting shrubs begin to dominate the vegetation. Resprouting species reach reproductive maturity, while short-lived species begin to die out. Taller species begin to emerge from canopy, which reaches pre-fire levels and becomes flammable.

3. Transitional phase (5–10 years): All species reach reproductive maturity. Seeder species emerge from canopy and assume an ascending branching habitat.

4. Mature phase (10–30 years): Seeder species begin to die out with limited germination occurring. Tall shrubs reach their maximum height and flowering activity. Above-ground biomass of the stand is at its greatest.

5. Senescent phase (30–60 years): Mortality of seeder species accelerates with the canopy beginning to open up. Litter accumulation accelerates and there is very little seed germination.

The ability of fire to change species composition in a landscape is a direct consequence of its effect on plant growth, survival and reproduction. In fire-prone ecosystems, plants are either killed by fire and regenerate from a seed bank (seeders) or survive and regenerate from below-ground structures such as lignotubers (resprouters) (Le Maitre and Midgley, 1992; Bond and van Wilgen, 1996; Bellingham and Sparrow, 2000; Bell, 2001; Bond and Midgley, 2001, 2003). In fynbos vegetation, resprouters tend to be longer-lived than co-occurring seeders (Kruger and Bigalke, 1984; Le Maitre and Midgley, 1992; Schutte *et al.*, 1995; Bond and van Wilgen, 1996; Bond and Midgley, 2003). Although the majority of species in the CFR are seeders, the relative proportion of seeders to resprouters varies between families. For example in Fabaceae and Proteaceae, > 75% of species are seeders, whereas in Cyperaceae > 80% of species are resprouters (Le Maitre and Midgley, 1992).

Seeders and resprouters are distinguished by differences in their allocation of resources to growth, reproduction and investment in vegetative structures such as lignotubers and rhizomes (Stearns, 1976; Keeley and Zedler, 1978; Pate *et al.*, 1985, 1990; Hansen *et al.*, 1991; Bond and van Wilgen, 1996; Bell, 2001). For instance, in comparing congeneric *Banksia* species, Low and Lamont (1990) found that reproductive structures made up 32% of shoot biomass in seeders, whereas in resprouters it was < 20%. However, the shoot: root ratios and root starch concentrations of resprouters are up to four and 20 times greater than in seeders, respectively (Pate *et al.*, 1990; Bell and Ojeda, 1999). Therefore seeders reflect high reproductive effort, whereas resprouters correspond to high below-ground investment.

Legumes in Mediterranean-Type Ecosystems

Fabaceae (legumes) has approximately 19 325 species, making it the third largest plant family on earth, after Orchidaceae and Asteraceae (Lewis *et al.*, 2005). The relatively high species richness of legumes is also reflected in the floras of MTE’s (Rundel, 1989). In California for example, legumes comprise between 6 and 7% of the total flora (Rundel, 1989). Furthermore, Fabaceae is the second largest family in the CFR (760 spp., Goldblatt and Manning, 2000) and South-western Australia (1043 spp., Hopper and Gioia, 2004), respectively.

An important attribute of many legumes is their ability to fix atmospheric N_2. N_2-fixation occurs via a symbiotic relationship between legumes and soil bacteria collectively known as rhizobia (Marschner, 1995; Lambers *et al.*, 2008a). The bacteria fix atmospheric N_2, providing an alternative N supply while in return, the legumes provide the bacteria with carbohydrates. N_2-fixing legumes are located predominantly in the sub-families Mimosoideae and Papilionoideae (Lewis *et al.*, 2005). A large proportion of legume genera native to the CFR possess nodules and thus the ability to fix N_2 (Grobbelaar and Clarke, 1972; Lamont *et al.*, 1982; Grobbelaar *et al.*, 1983; Stirton, 1989). In fynbos and other MTE's with oligotrophic soils, symbiotic N supply should provide legumes with a competitive advantage over non-fixing species (Vitousek and Howarth, 1991; Cocks and Stock, 2001). However, legumes are largely absent from mature vegetation in these ecosystems, although they are common in the immediate and youth phases of post-fire succession (Kruger, 1979a; Shea *et al.*, 1979; Bell and Koch, 1980; Westman, 1981; Hoffman *et al.*, 1987; Cocks, 1994; Kazanis and Arianoustou, 1996, 2004). In fynbos for example, their presence in the early years of post-fire succession is often so distinct that genera such as *Aspalathus*, have been found to dominate a landscape in the first year post-fire but are scarce and often absent five to six years later (Adamson, 1935; Levyns, 1935; Esterhuysen, 1936; Dahlgren, 1963). Hence they are frequently referred to as post-fire colonizers or 'forgotten fynbos' (Kruger, 1983; Cocks and Stock, 1993; Cocks, 1994).

The rise and fall of legume abundance during post-fire succession in fynbos has been well documented. For instance, in lowland fynbos, Hoffman *et al.* (1987) recorded a mean percentage cover of Fabaceae of 5–8 % in the first five years post-fire. However, in stands between the ages of 12 and 19 years, this cover had reduced to 1–2 %, which contrasted with a significant increase in cover of Proteaceae and Restionaceae over the same period. Similarly, in a comparison of adjacent young (1–3 years) and old (10+ years) fynbos sites on various soil types, Cocks (1994) recorded a significant decline in legume cover, but an increase in the abundance of Cyperaceae, Ericaceae, Proteaceae and Restionaceae during post-fire succession. Thus over time legumes are gradually replaced by climax species from genera such as Proteaceae and Restionaceae, which are better able to persist through post-fire succession.

The inability of legumes to persist post-fire is not unique to fynbos, as indicated by studies on other MTE's. For example, in Californian chaparral, Westman (1981) noted a 10% drop in *Lupinus* and *Lotus* species seven years post-fire. These species were virtually absent in stands 20 years and older. Similarly, in the Jarrah forest of South-western Australia, *Acacia*, *Bossiaea* and *Kennedia* species are almost non-existent six years post-fire (Shea *et al.*, 1979; Bell and Koch, 1980). More recently in *Pinus halepensis* forests of the Mediterranean Basin, where fire frequencies are significantly lower than in fynbos or chaparral, Kazanis and Arianoustou, (1996, 2004) observed a dramatic decline in the number of legume species over a 30 year period post-fire. Thus a large proportion of legumes in MTE's appear to be fire-ephemerals that are unable to persist through post-fire succession.

Potential factors constraining legumes in ecosystems

The inability of legumes to persist after fire for long periods in MTE's is puzzling, given their N_2-fixing capabilities in N-impoverished environments. Several review papers have suggested a number of environmental constraints, which could limit the prevalence of legumes in such ecosystems. These include physical constraints such as herbivory, the energetic costs of N_2-fixation, and the low availability of macro-nutrients such as soil P (Vitousek and Howarth, 1991; Vitousek and Field 1999; Sprent, 1999; Vitousek *et al.*, 2002). Other constraints may include limited water availability and competition mediated by the low availability of resources such as light, water and nutrients (Cocks, 1994; Vilá and Sardans, 1999). Therefore several factors could be contributing to the lack of legume persistence in MTE's, including fynbos.

Natural herbivory in MTE's is considered to be low compared with other ecosystems (Morrow, 1983; Johnson, 1992). Herbivory in fynbos vegetation is low because of the low densities of large herbivores and insects (Bigalke, 1979; Johnson, 1992). This low prevalence of herbivores is probably a consequence of several factors including the low nutritional content of foliage, the high degree of sclerophylly and the occurrence of secondary metabolites such as phenolics, which combine to reduce nutritive value and palatability (Morrow, 1983; Campbell, 1986; Johnson, 1992). Little is known about the degree of sclerophylly and the presence of secondary metabolites in legumes. However, legumes are likely to have higher foliar N concentrations compared with non-legumes as a consequence of their enhanced N supply. Therefore, their protein-rich foliage may be susceptible to preferential grazing by herbivores (Vitousek and Howarth, 1999; Vitousek *et al.*, 2002). In the CFR, however, the largest legume genus, *Aspalathus* (Goldblatt and Manning, 2000), is one of the few fynbos taxa that possess spines, which may act as an anti-herbivory mechanism (Johnson, 1992). Accordingly, the low occurrence of herbivores combined with the presence of spines may prevent herbivores from having an adverse effect on legume persistence in fynbos.

Symbiotic N_2-fixation is an energetically expensive process. The fixation process can consume up to 25% of fixed carbon per day, which is significantly more than required for NO_3^- and NH_4^+ acquisition (Gutschick, 1981; Marschner, 1995; Lambers *et al.*, 2008a). This high carbon demand implies a high light requirement, leading to the expectation that legumes are shade-intolerant and weak competitors in low light conditions (Vitousek and Howarth, 1991; Sprent, 1999; Vitousek *et al.*, 2002). Post-fire environments provide high light conditions but as succession proceeds, light penetration diminishes as large over-storey species begin to mature and change light availability (Kruger, 1983; Specht and Moll, 1983). In the later stages of post-fire succession, competition for light will therefore prevent legumes from meeting the costs of N_2-fixation, thus losing their competitive advantage in low N soils. In fynbos, however, under-storey species decline before the cover of over-storey species reaches 30%, implying that factors other than light are contributing to their decline (Kruger, 1983). Moreover, Lambers *et al.* (2008a) suggested that in N-limited ecosystems such as fynbos, the costs of N_2-fixation are likely to be similar to the acquisition of mineral N. Thus the carbon costs of N_2-fixation are unlikely to affect the persistence of legumes in fynbos negatively.

Associated with the strong bi-seasonal climate in MTE’s is the potential for drought stress towards the end of summer (Specht, 1979; Specht and Moll, 1983). Summer drought stress may exert a negative effect on nodule fixation and longevity because optimal nodule production and N_2-fixation occurs when soil moisture is high (Lamont, 1983; Grove and Malajczuk, 1992; Serraj and Sinclair, 1996; Cocks and Stock, 2001; Sprent, 2009). However, the production of other root adaptations such as root clusters in Proteaceae are also limited to the wet winter months when nutrient uptake is at its greatest (Lamont, 1982, 2003; Lambers *et al*., 2006). In fynbos, therefore, nutrient uptake during winter is not limited to legumes. Nonetheless, drought might have a detrimental effect on overall persistence. For instance, when tracking the persistence of three *Aspalathus* species post-fire, Cocks (1994) noted that individuals of each species died at the end of summer, implying that they were suffering from water stress. However, Cocks pointed out, that this observation was circumstantial as the effect of water availability on growth was not explicitly tested. Furthermore, numerous legume species only begin to die out five to seven years post-fire, having survived several summer droughts (pers. obs). Thus summer drought is unlikely to explain the failure of legumes to persist in fynbos.

Apart from the potential effects of summer drought stress, Cocks (1994) suggested that levels of soil P may ultimately determine legume persistence in fynbos. Low soil P availability has frequently been attributed to the lack of N_2-fixers in an ecosystem primarily because legumes and N_2-fixation are thought to have high P demands (Vitousek and Howarth, 1991; Vitousek and Field, 1999; Vitousek *et al*., 2002). Several studies indicate that the processes of nodulation and N_2-fixation have high P requirements (Israel, 1987; Sa and Israel, 1991; Ribet and Drevon, 1995). However, other similar studies have shown that increased N_2-fixation, following P-augmentation is a result of stimulated plant growth and not a direct effect of P supply on nodulation and N_2-fixation (Robson *et al*., 1981; Jakobsen, 1985; Høgen-Jensen *et al*., 2002). Furthermore, the P content of nodules in relation to the whole plant is quite small (Sprent, 1999). Thus nodules and N_2-fixation may not have a high P requirement.

In contrast to the N_2-fixation process, the actual growth of N_2-fixing legumes could incur a higher P requirement than that of non-fixers as suggested by their increased growth with an augmented P supply (Robson *et al*., 1981; Jackobsen, 1985; Høgen-Jensen *et al*., 2002). However, in a review Sprent (1999) showed that N_2-fixing legumes and non-fixers have similar P contents when compared as either agricultural or field species, indicating that legumes do not have an inherently high P demand. In addition, Ribet and Drevon (1995) found that *Acacia manguim* had similar P requirements whether the N was supplied as urea or by N_2-fixation. This indicates that legumes reliant on N_2-fixation do not have an elevated P demand. Although legumes might not have an exceptional P requirement, N supply, including

N_2-fixation generally adjusts to P supply in oligotrophic soils (Vitousek *et al.*, 2002). Thus while an ability to fix N_2 may enable legumes to overcome low N availability, low P availability is likely to limit legume growth and their competitive advantage over other species in fynbos.

Thesis Outline

This study investigated the failure of legumes to persist through post-fire succession in fynbos vegetation of the CFR, in relation to soil P availability. I hypothesized that low soil P availability limits legume persistence in fynbos vegetation of the CFR. To test this hypothesis, I divided my thesis into a series of hypotheses. These form the basis of two data chapters.

Chapter two aims to determine whether P-acquisition strategies differ between legumes and non-legumes. I test the hypothesis that legumes lack effective root adaptations required to access sparingly soluble P compared with non-legumes. In addition, I evaluate whether root adaptations differ with regeneration strategies. Specifically I test whether legume and non-legume seeders have more effective root level P-acquisition traits than resprouters. These hypotheses were tested by means of a pot experiment, where congeneric seeder-resprouter pairs from Fabaceae, Proteaceae and Restionaceae were supplied either 1 or 10 mg P kg^{-1} of sparingly soluble P.

Building on the comparison between seeders and resprouters, chapter three aims to establish whether the soils inhabited by legume seeders and resprouters differ with respect to soil nutrient availability and whether this is reflected in their foliar attributes. I hypothesized that seeders occupy soils with a greater nutrient availability and that they have larger leaves with a lower degree of sclerophylly than that of resprouters. To investigate these hypotheses, the soils nutrient status and leaf traits of seeders and resprouters in the legume genera *Otholobium* and *Psoralea* were characterized.

Each research chapter was written as an independent paper thus there is a degree of repetition in the thesis.

Declaration of student contribution to published manuscript

Chapter 2, “Does phosphate acquisition constrain legume persistence in the fynbos vegetation of the Cape Floristic Region”, was published in Plant and Soil (accepted January 2010). I carried out all of the data analysis and also wrote the paper. My co-authors, MD Cramer, GA Verboom and SBM Chimphango provided advice and comments on the manuscript.

Chapter 2

Does phosphate acquisition constrain legume persistence in the fynbos of the Cape Floristic Region?

Abstract

Abundance of Fabaceae declines in representation through post-fire-succession in fynbos vegetation of the Cape Floristic Region (CFR). This reduction in legume occurrence coincides with a known decline in post-fire soil P availability. It was hypothesized that the disappearance of legume species during post-fire succession is due to an inability to acquire P effectively from sparingly soluble sources. P-acquisition strategies and response to P supply were compared between legume (*Aspalathus*, *Cyclopia*, *Indigofera*, *Podalyria*) and non-legume (*Elegia*, *Leucadendron*, *Protea*) genera when supplied with 1 or 10 mg P kg^{-1} dry sand. Each genus consisted of a seeder (non-persistent) and resprouter (persistent) species. Non-legumes showed a greater investment in below-ground biomass, more root clusters, with higher concentrations of carboxylates exuded by cluster roots and carboxylates that were better suited to the mobilization of sparingly soluble P compared with legumes. The growth response to increased P supply was 53% higher in legumes than in non-legumes. The lack of a growth response to an elevated P supply in the non-legumes was attributed to N-limitation. Legume resprouters had a higher investment in cluster root biomass and a lower capacity to down-regulate P-uptake than the seeders. Therefore, the inability to acquire sufficient P from low concentration and sparingly soluble soil P-sources may contribute to the lack of indigenous legume persistence in fynbos vegetation of the CFR.

Introduction

Fabaceae is one of the most species-rich families in the CFR (Goldblatt and Manning, 2000). However, legume species are generally absent or rare in mature (i.e. > 10 years post-fire) fynbos vegetation of the CFR (Kruger, 1979a; Hoffman *et al*., 1987; Cocks, 1994). Surveys on the changes in species composition post-fire conducted in fynbos indicate a decline in legume abundance and a concomitant increase in the abundance of Cyperaceae, Proteaceae and Restionaceae (Hoffman *et al*., 1987; Cocks 1994). This lack of legume persistence through succession has also been reported for other Mediterranean-type ecosystems, such as the chaparral of California (Westman, 1981) and the Jarrah forests of South West Australia (Shea *et al*., 1979; Bell and Koch, 1980). Consequently, legumes are considered to be short-lived, post-fire colonizers in these Mediterranean-type ecosystems (Kruger, 1983; Cocks and Stock, 2001).

The general absence of legumes from mature fynbos vegetation in the CFR is puzzling, since the capacity of most legumes for N_2-fixation may be expected to confer a competitive advantage in the relatively oligotrophic soils that dominate this region (Stock and Lewis, 1986; Witkowski and Mitchell, 1987). The absence of legumes could be limited by (i) the intense energetic costs of N_2-fixation, which potentially reduces their ability to compete in low light circumstances, (ii) physical and ecological constraints such as herbivory of N-replete leaf tissues, and (iii) the low availability of macronutrients such as P or other micronutrients, which may be in greater demand in legumes compared with other species (Vitousek and Howarth, 1991; Vitousek and Field, 1999; Vitousek *et al*., 2002). Cocks and Stock (2001) suggested that post-fire changes in soil nutrient dynamics could be one of the most important factors limiting legumes in fynbos. The post-fire environment provides a temporary flush of nutrient availability, especially P (Brown and Mitchell, 1986) which may sustain legumes until this is depleted.

A number of species in fynbos possess specialized root adaptations such as root clusters and mycorrhizae, for enhanced P-acquisition (Lamont, 1982, 2003; Allsopp and Stock, 1993; Marschner and Dell, 1994; Smith and Read, 1997; Lambers *et al*., 2003, 2006). Different types of root clusters are commonly found in species from Cyperaceae (dauciform), Proteaceae (cluster/proteoid) and Restionaceae (capillaroid), which persist through post-fire succession, but they are thought to function similarly (Lamont, 1982; Lambers *et al*., 2006). Root clusters facilitate P-acquisition from sparingly soluble sources through increased root surface area, increased P-uptake rate and the release of P-solubilizing compounds such as carboxylates and acid-phosphatases (Gardener *et al*., 1983; Dinkelaker *et al*., 1995; Gilbert *et al*., 1999; Grierson and Comerford, 2000; Neumann *et al*., 2000; Watt and Evans, 2003; Lambers *et al*., 2006). In families where there is a high prevalence of root clusters, such as Proteaceae, mycorrhizal symbioses are relatively uncommon, although there are exceptions (e.g. *Hakea verrucosa*, Boulet and Lambers, 2005). In contrast, many species of Fabaceae have mycorrhizal symbionts (Lamont, 1982; Allsopp and Stock, 1993). In severely P-impoverished soils, root clusters are thought to confer a significant advantage over species that lack them (Lambers *et al*., 2006, 2008). Although cluster roots have been reported in legume genera such as *Cyclopia* (Spriggs, 2004) and *Aspalathus* (Allsopp and Stock, 1993), very little is known about their prevalence in fynbos legumes. Indeed, the inability of legumes to persist into late succession, in contrast to the persistence of Cyperaceae, Proteaceae and Restionaceae, may be associated with a lack of effective P-acquisition mechanisms.

Although Fabaceae decline in abundance through post-fire succession, some species do persist into mature vegetation (Cocks, 1994; Hoffman *et al*., 1987). Unfortunately, data on fynbos species longevity is scarce. Regeneration strategies may, however be a reasonable proxy for longevity because seeders tend to be shorter-lived than co-occurring resprouters in fynbos vegetation (Kruger and Bigalke, 1984; Le Maitre and Midgley, 1992; Schutte *et al*., 1995; Bond and van Wilgen, 1996; Bond and Midgley, 2003). Associated with the differences in longevity between seeders and resprouters are differences in resource allocation, specifically to reproductive and vegetative investment (Kruger, 1983; Bellingham and Sparrow, 2000). Seeders tend to have a high reproductive effort with low vegetative investment, whereas resprouters show the reverse pattern (Keeley and Zedler, 1978; Pate *et al*., 1990; Hansen *et al*., 1991; Le Maitre, 1992; Bell, 2001). For example, in a comparison between a congeneric legume seeder and resprouter, Hansen *et al*. (1991) found that the seeder produced more seeds and that the seeds had higher nutrient concentrations, than the resprouter. Because seedlings need to be supplied with scarce nutrients, a high reproductive effort in oligotrophic soils might render seeders more vulnerable to nutrient limitation than resprouters. The higher shoot : root ratio of seeders relative to resprouters (Pate *et al*., 1990;

Hansen *et al*., 1991; Bell, 2001) also suggests that the former might have reduced capacity for nutrient uptake. Consequently, the shorter life-spans of seeders in relation to resprouters may be a result of inadequate nutrient uptake strategies to sustain their nutritional demands in highly oligotrophic soils.

Two hypotheses were proposed to explain the widespread lack of post-fire persistence of legumes in fynbos of the CFR: (1) Species of Fabaceae are less effective at acquiring P from sparingly sources compared with members of Proteaceae and Restionaceae; (2) seeders (including Fabaceae, Proteaceae and Restionaceae) are less effective at acquiring P from sparingly soluble sources than resprouters. To test these hypotheses, the P-acquisition strategies and responses to P supply were assessed in congeneric seeder-resprouter pairs from Fabaceae, Proteaceae and Restionaceae.

Materials and Methods

Species and growth conditions

Fourteen species indigenous to fynbos vegetation were selected for the study, including eight species from Fabaceae (*Aspalathus nivea* Thunb., *Aspalathus subtingens* Eckl. & Zeyh., *Cyclopia pubescens* Eckl. & Zeyh., *Cycolpia genistoides* (L.) R.Br., *Indigofera cytisoides* (L.)L., *Indigofera filifolia* Thunb., *Podalyria sericea* (Andrews) R.Br., *Podalyria myrtillifolia* (Retz.) Willd.), four from Proteaceae (*Leucadendron coniferum* (L.) Meisn., *Leucadendron spissifolium* (Salisb. Ex Knight) I.Williams ssp. *spissfolium*, *Protea grandiceps* Tratt., *Protea speciosa* (L.)L.) and two from Restionaceae (*Elegia persistens* (Burm.f) Schelpe, *Elegia capensis* Pillans.) (Goldblatt and Manning, 2000; Haaksma and Linder, 2000; Rebelo, 2001). *Podalyria* is abbreviated to *Po*. and *Protea* to *Pr*. Fabaceae species are hereafter referred to as 'legumes' and species from Proteaceae and Restionaceae as 'non-legumes'. Each genus was represented by a 'seeder' and 'resprouter' species, as designated by Goldblatt and Manning (2000), Haaksma and Linder (2000), and Rebelo (2001) (Table 2.1). The seeder-resprouter pairs were selected to be as closely related as possible and all occur on Table Mountain Sandstone substrates in the fynbos.

All species were grown from seed (Silverhill Seeds and Books, Cape Town, South Africa) except *E. capensis* and *E. persistens* which were obtained as seedlings from National Botanical Gardens Nursery (Kirstenbosch, South Africa). Legumes seeds were scarified in 98% H_2SO_4 for 30 min to break dormancy and then rinsed six times before soaking in smoke water (CAPE Seed Primer, National Botanical Gardens, Kirstenbosch) for 24 h. The *Protea* and *Leucadendron* seeds were placed directly into the smoke water and left to soak for 24 h before being planted into trays of acid washed sand where they were kept moist until emergence.

Two weeks after emergence, eight seedlings of each species were removed and transplanted into 3 kg pots containing an acid washed mix of coarse and fine sand (1:1). To ensure mycorrhizal and rhizobial inoculation, 150 g (i.e 5% of sand mix) of soil (pH 6.5, N 0.1%, total P 32.7 mg kg^{-1}, Bray II P 6.3 mg kg^{-1}), collected from various sites across the CFR was mixed with the sand. The substrate mix (acid washed sand and inoculum soil) was mechanically mixed with either 1 or 10 mg P kg^{-1} dry sand of sparingly soluble P for 30 min. The sparingly soluble P consisted of 75% $FePO_4$ (AR, Sigma, F1523) and 25 % $Ca_5(PO_4)_3(OH)$ (AR, Sigma C3161), which resembles the approximate ratio found in Table Mountain Sandstone-derived soils (Witkowski and Mitchell 1987). Thus, while all plants were supplied 0.32 mg kg^{-1} Bray II P (from inoculums), actual total P supplied was 2.6 and 11.6 mg kg^{-1} for the low-P (1 mg kg^{-1} treatment) and high-P (10 mg kg^{-1} treatment) plants, respectively.

The pots were supplied a nutrient solution containing (µM): $Ca(NO_3)_2$, 400; K_2SO_4, 200; $MgSO_4$, 54; $MnSO_4$, 0.24; $ZnSO_4$, 0.10; CuSO4, 0.02; H_3BO_3, 2.4; $NaMoO_4$, 0.03; Fe-EDTA, 10 to 70% field capacity twice a week. The pots were also wetted to 70% field capacity once a week with de-ionized water. All the pots were randomly arranged on trolleys in a well ventilated glasshouse situated at the University of Cape Town (S 33° 57.353'; E 018° 27.742’). Pots and trolleys were re-arranged every 2 weeks. Plants were grown for 6 months from November to May with an average temperature of 27°C and a range of 20 – 35 °C. The average relative humidity in the glasshouse was 40%.

Nutritional analysis of substrate

The acid washed sand and inoculum soil were air-dried and sieved (1 mm mesh) prior to analyses. pH was determined by shaking 2 g of material in 20 mL 1*M* KCl at 180 rpm for 60 min, centrifuging at 10 000 *g* for 10 min and measuring the pH of the supernatant. Total N was determined by digestion with a Leco FP528 N Analyzer (Leco Corporation). Available P was determined by extracting 2 g of soil in Bray II solution (Bray and Kurtz, 1945) and then filtering through Whatman No.2 filter paper. The filtrate was analyzed colorimetrically using the Malachite Green method (Motomizu *et al.*, 1983).

Plant biomass

At harvest the plants were separated into leaves, stems and roots. Where cluster roots were present in the 1 mg P kg^{-1} treatment, 2–3 individual clusters with rhizosphere sand still intact were separated from the root system for subsequent exudate collection. The remaining root system was gently excavated under running water. A sample of the roots, consisting of 50 randomly selected 0.5–1.0 cm pieces were stored in 10% ethanol for mycorrhizal assessment. Each organ was then weighed and dried at 80°C for 48 h. After drying the material was then re-weighed and milled using a ball mill (MM200, Retsch®, Haan, Germany).

Foliar nutritional analysis

Foliar concentrations of B, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn were measured by dry-ashing pulverized leaf material at 480°C for 8 h and dissolving with a 1:1 (v/v) of HCl according to Kalra (1998). Assessment of the element concentrations in solution was performed using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia). Foliar [N] was determined using mass spectrometry. Between 1.900 and 2.100 mg of milled leaf sample was weighed into a 8 x 5 mm tin capsule (Elemental Microanalysis Ltd, Okehampton, UK). The tin capsules were then combusted in a Thermo Flash EA 1112 series elemental analyzer coupled to a Delta Plus XP isotope ratio mass spectrometer via a Thermo Finnigan Conflo III control unit (all from Thermo Electron Corporation, Milan, Italy). The values were determined using International Atomic Energy Authority standards.

Assessment of mycorrhizal infection

Stored roots were cleared in 10% KOH for 20 min at 60°C, then rinsed and acidified with 1*M* HCl. The roots were then stained in 0.5% (w/v) Trypan Blue (Koske and Gemma, 1989) at 60°C for 20 min and destained with 50% lactic acid. Percentage colonization was assessed using the gridline intercept method (Giovannetti and Mosse, 1980). The percentage was calculated as the proportion of infected to non-infected root interceptions.

Cluster root exudates

The separated cluster roots were washed for 30 min in vials containing 20–30 mL of 0.2 m*M* $CaCl_2$ on an orbital shaker. The resulting solution was filtered through 0.22 μm Acrodisc syringe filters (Pal-Gelman, Ann Arbor, MI, USA) and stored at -20°C. The stored samples were analyzed for organic acids using High Performance Liquid Chromatography (HPLC). Only samples where cluster roots appeared to be white/grey at harvest were analyzed. The analysis was performed with an Agilent 1100 binary pump (Agilent Technologies Inc., Santa Clara CA, USA) and a 717 autosampler (Waters, Milford MA, USA). A 40 µL subsample of exudate solution was injected into a reverse phase C-18 column (300 x 7.8 mm, Aminex HPX-87H, Biorad, CA, USA). The mobile phase was 5 m*M* H_2SO_4, with a flow rate of 0.5 mL min^{-1} at 45°C. The run time for each sample was 30 min. Detection of the organic acids was done at 210 nm (Refractive index analyzer**,** Waters). Organic acids in the samples were identified by comparing the retention times of the samples against the retention times of standards (acetic citric, lactic, malic and succinic acid; Central Drug House Ltd, Delhi, India). Identity of the organic acids was confirmed by comparing the absorption spectra of the sample between 200 and 300 nm, with that of the corresponding standard. The amounts of organic acids were calculated by contrasting the peak heights of the samples against the standards. Data were processed using Millenium software (Waters).

Statistical analysis

All variables, apart from percentage data were log transformed. Percentage data were arcsine square root transformed (Sokal and Rohlf, 1995). All statistical analyses were conducted in Genstat 11.1 (Lawes Agricultural Trust; Rothamsted Experimental Station). A three-way linear mixed effects model using restricted maximum likelihood was used to test differences between groups, regeneration strategies and P treatments, using the Wald χ^2 statistic. The fixed factor was a three way interaction between groups, regeneration strategies and P treatments. The random factor was species nested within genera. A two-way ANOVA was used to determine the interaction between species and P treatment, which was not possible in the mixed model. Where significant differences between groups (legumes and non-legumes), regeneration strategies, species and P treatments were found, differences between the predicted means that exceed twice the standard error of difference were used to separate significantly different means at $P \leq 0.05$ For variables involving cluster roots or mycorrhizae, statistical analysis was performed only on species having the designated root adaptation.

Results

Biomass accumulation

Overall, legumes supplied with 10 mg P kg^{-1} accumulated significantly more biomass (53%, $\chi^2 = 6.43$, $P < 0.05$) than those supplied with 1 mg P kg^{-1} (Figs. 2.1 and 2.2a). A significant statistical interaction between species and P treatments was evident for all of the legumes ($F_{7,110} = 2.39$, $P < 0.05$), except *C. genistoides*, for which no significant differences between treatments were apparent (Fig. 2.2c). *I. cytisoides* and *I. filifolia* were the only species to show a significant ($P < 0.05$) decline in biomass with an increase in P supply, possibly due to slight toxicity developing at the higher levels of P supply (Fig. 2.2c). In contrast, *L. spissifolium* was the only non-legume species whose biomass significantly ($P < 0.05$) increased with an elevated P supply (Fig. 2.2c). Overall, both seeders and resprouters responded positively to increased P supply ($\chi^2 = 31.96$, $P < 0.001$, Fig. 2.2b). However, this is a reflection of the response shown by five of the eight legumes species and the non-legume resprouter *L. spissifolium* (Fig. 2.2c).

Fig. 2.1 The growth response of a legume and non-legume species when supplied the low and high P treatments.

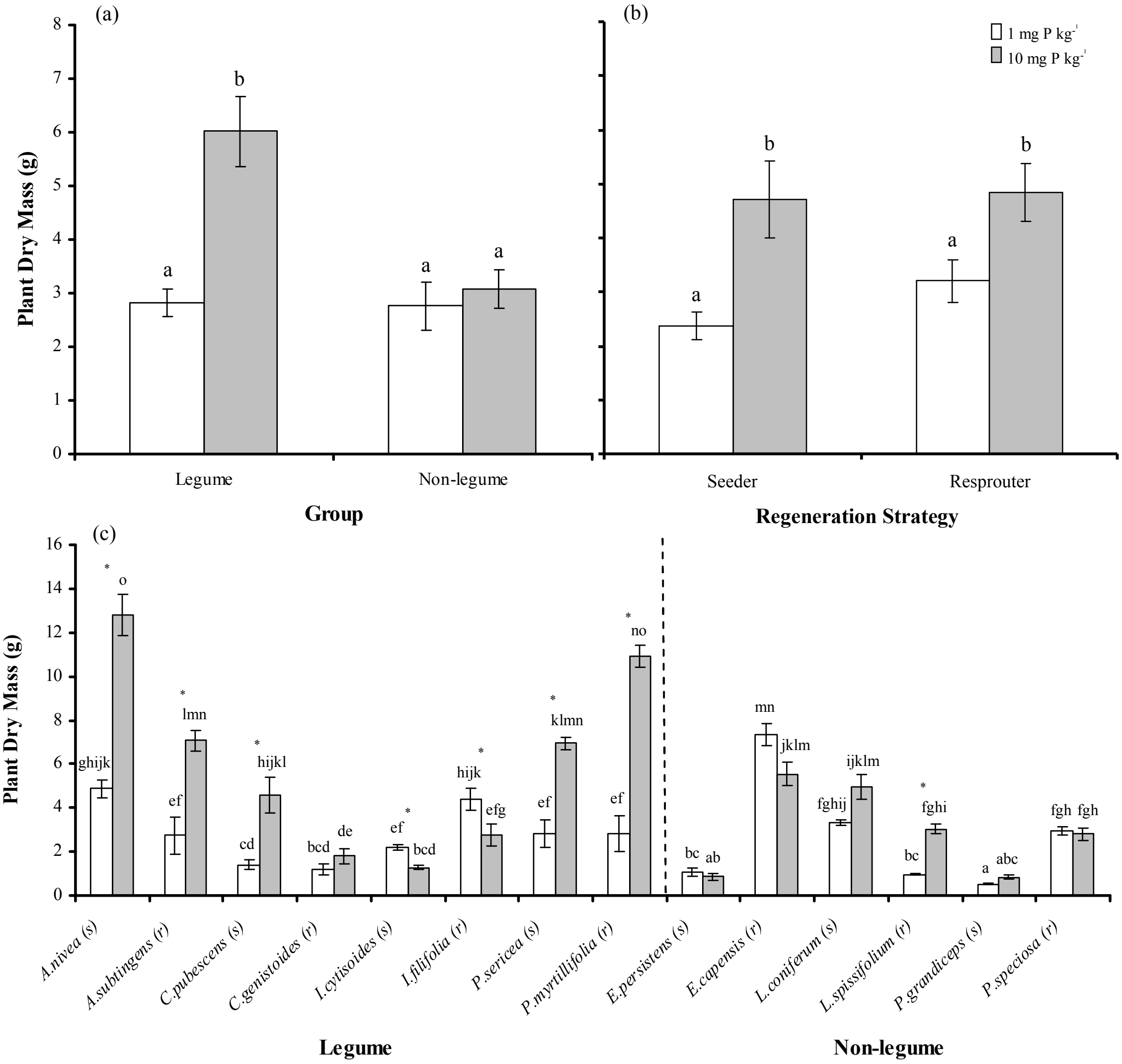

Fig. 2.2 Biomass accumulation of (a) legumes and non-legumes, (b) seeders and resprouters, (c) legume and non-legume species supplied with 1 or 10 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figures (a) and (b) denote significant differences between groups, regeneration strategies and P treatments ($P < 0.05$) from a linear mixed model (fixed factor: group x regeneration strategy x P treatment, random factor: species nested in genus). Significant differences between species and P treatments ($P < 0.05$) in figure (c) are indicated by different letters, from a two-way ANOVA (factors: species and P treatment). Differences between P treatments within a species are highlighted by an asterisk. All tests were conducted on transformed. Regeneration strategies are indicated by (s) = seeder, (r) = resprouter.

Nutrient acquisition strategies

Cluster roots were observed on *Aspalathus*, *Leucadendron* and *Protea*, and capillaroid roots on *Elegia*. The cluster roots of *A. nivea* (Fig. 2.3) and *A. subtingens* were similar in appearance to those in Proteaceae, as described by Purnell (1960). However, as in *Lupinus albus* (Neumann *et al*., 2000.), the lateral roots within the clusters of the legumes were less tightly packed than in Proteaceae. Although Spriggs (2004) recorded cluster roots in *Cyclopia*, none were observed in this study, possibly because there was sufficient P in the growth medium to inhibit cluster root formation in this species. Arbuscular mycorrhizae, along with fine endophytes, were present on the roots of all of the legume species, including the cluster root bearing *Aspalathus* species. Cross sectioned nodules on all of the legumes appeared red, indicating the presence of leghaemoglobin and thus active N_2 fixation. There was no differentiation in root adaptations between congeneric species or P treatments.

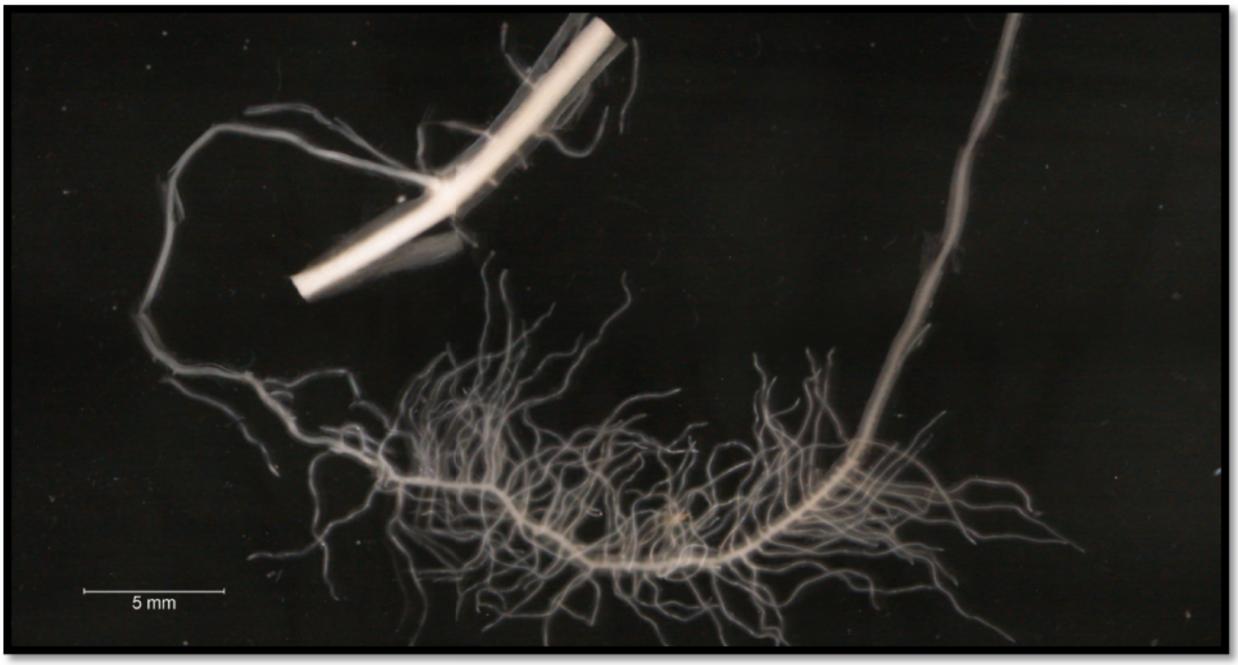

Fig. 2.3 Cluster root of *Aspalathus nivea*, grown in sand.(Photo: P Muller)

Overall, the legumes had a shoot : root ratio of 2.2, which was significantly ($\chi^2 = 7.33$, $P < 0.05$) greater than shown by non-legumes (1.2). Although there were significant differences between seeders and resprouters in five congeneric pairs ($F_{7, 110} = 35.76$, $P < 0.001$), there was no significant overall difference between the shoot : root ratios of seeders and resprouters (Table 2.1). The difference in P treatments did not affect the shoot : root ratio in any of the species (Table 2.1). Increased P supply did, however reduce cluster root : root ratios ($F_{3, 46} = 8.68$, $P < 0.001$) in all of the cluster root species, except *L. coniferum* and *Pr. grandiceps* (Table 2.1). In *Pr. grandiceps*, this ratio actually increased with increasing P supply. Interestingly, increased P supply reduced the cluster root : root ratio in resprouters ($\chi^2 = 17.44$, $P < 0.001$), but not in seeders. Moreover, the resprouter *A. subtingens* had a significantly ($P < 0.001$) higher cluster root : root ratio than the seeder *A. nivea* when supplied with 1 mg P kg^{-1} (Table 2.1).

Percentage mycorrhizal colonization indicated a significant interaction between legume species and P treatment (Table 2.1). All species except *C. genistoides*, *I. filifolia* and *Po. sericea* showed a significant ($F_{4, 64} = 2.96$, $P < 0.05$) reduction in percentage colonization with increased P supply. Overall, the percentage colonization in seeders (32%) and resprouters (29%) was similar but increased P supply resulted in a significant ($\chi^2 = 44.44$, $P < 0.001$) decline of 14% and 24% for the seeders and resprouters respectively.

Table 2.1 Effect of P supply on the shoot : root and cluster root : root biomass (dry weight) ratios and mycorrhizal colonization of the 14 species (regeneration strategies are indicated by (s) = seeder, (r) = resprouter). The values are mean ± SE. Letters within a column denote significant differences between species from a two-way ANOVA (factors: species and P treatment) performed on transformed data. Bold type indicates significant differences between P treatments within species. The absence of cluster roots/mycorrhizae is indicated by a '-'.

Species	**P ($mg\ kg^{-1}$)**	**Shoot: Root**	**Cluster Root: Root**	**Mycorrhizal (%)**
Fabaceae				
Aspalathus nivea (s)	1	1.7 ± 0.3^{defg}	**0.20 ± 0.03^{a}**	**44 ± 2^{fg}**
	10	2.0 ± 0.1^{defg}	**0.09 ± 0.01^{cd}**	**26 ± 2^{bcd}**
Aspalathus subtingens (r)	1	1.9 ± 0.3^{defg}	**0.51 ± 0.03^{g}**	**31 ± 4^{de}**
	10	1.8 ± 0.1^{defg}	**0.10 ± 0.01^{ab}**	**20 ± 3^{ab}**
Cyclopia pubescens (s)	1	1.4 ± 0.1^{def}	-	**45 ± 5^{g}**
	10	2.12 ± 0.2^{def}	-	**29 ± 3^{bcd}**
Cycolpia genistoides (r)	1	1.5 ± 0.2^{de}	-	43 ± 4^{fg}
	10	1.8 ± 0.1^{de}	-	34 ± 5^{defg}
Indigofera cytisoides (s)	1	3.7 ± 0.3^{h}	-	**40 ± 3^{efg}**
	10	4.4 ± 0.2^{h}	-	**20 ± 3^{abc}**
Indigofera filifolia (r)	1	1.9 ± 0.1^{fg}	-	29 ± 3^{cde}
	10	2.2 ± 0.2^{fg}	-	27 ± 45^{bcd}
Podalyria sericea (s)	1	2.3 ± 0.4^{g}	-	30 ± 4^{de}
	10	2.2 ± 0.1^{g}	-	26 ± 2^{bcd}
Podalyria myrtillifolia (r)	1	1.5 ± 0.2^{def}	-	**34 ± 3^{def}**
	10	1.9 ± 0.1^{def}	-	**15 ± 2^{a}**
Restionaceae				
Elegia persistens (s)	1	1.3 ± 0.2^{c}	-	-
	10	0.8 ± 0.1^{c}	-	-
Elegia capensis (r)	1	0.35 ± 0.03^{a}	-	-
	10	0.3 ± 0.1^{a}	-	-
Proteaceae				
Leucadendron coniferum (s)	1	1.0 ± 0.1^{c}	0.17 ± 0.01^{cd}	-
	10	1.03 ± 0.09^{c}	0.14 ± 0.02^{bc}	-
Leucadendron spissifolium (r)	1	1.58 ± 0.30^{d}	**0.23 ± 0.04^{de}**	-
	10	1.67 ± 0.22^{d}	0.17 ± 0.04c	-
Protea grandiceps (s)	1	0.93 ± 0.10^{b}	**0.30 ± 0.02^{ef}**	-
	10	0.68 ± 0.05^{b}	**0.53 ± 0.04^{g}**	-
Protea speciosa (r)	1	1.90 ± 0.17^{efg}	**0.43 ± 0.07^{fg}**	-
	10	2.08 ± 0.16^{efg}	**0.25 ± 0.02^{de}**	-

On average, the concentration of cluster root carboxylates released by the legumes was 0.8 mmol g^{-1} dry weight, which was significantly lower ($\chi^2 = 5.81$, $P < 0.05$) than the carboxylate concentration released by the cluster roots of the non-legumes (18.3 mmol g^{-1} dry weight). Furthermore, the composition of the carboxylates released by legume cluster roots predominantly consisted of lactate (90% of total carboxylates released) whereas the composition varied greatly between the individual non-legume species (Table 2.2). In *L. coniferum* and *L. spissifolium,* citrate (68%) and lactate (48%) were the largest components, respectively. In *Pr. grandiceps* the carboxylates largely consisted of acetate (33%), citrate (31%) and lactate (34%), while in *Pr. speciosa*, acetate (45%) and succinate (49%) were the dominant components. There were no consistent differences in carboxylate composition between seeders and resprouters (Table 2.2). Only the *Protea* resprouter released significantly ($F_{5,17} = 12.36$, $P < 0.001$) higher concentrations of carboxylates in comparison to the *Protea* seeder (Table 2.2).

Table 2.2 The concentration and composition of cluster root rhizosphere carboxylates from two legume and four non-legume species when supplied with 1 mg P kg^{-1} dry sand. The values are mean ± SE. Letters denote significant differences in rhizosphere carboxylate concentrations between species ($P < 0.001$) from a one-way ANOVA conducted on transformed data.

Species	**[Carboxylate] (mmol g^{-1} d.w)**	**Carboxylate composition (%)**				
		Acetate	**Citrate**	**Malate**	**Lactate**	**Succinate**
Fabaceae						
A. nivea (s)	0.8 ± 0.1^a	3.4	4.0	0	92.6	0
A. subtingens (r)	0.9 ± 0.1^a	7.9	0.9	0	87.4	3.8
Proteaceae						
L. coniferum (s)	10.6 ± 1.7^b	1.7	67.6	5.0	10.9	14.7
L. spissifolium (r)	34.6 ± 17.0^b	10.2	12.6	23.2	47.7	6.4
Pr. grandiceps (s)	1.6 ± 0.3^a	33.0	30.8	2.5	33.7	0
Pr. speciosa (r)	22.4 ± 12.3^b	45.4	0.2	0.8	4.8	48.7

Foliar N and P nutrition

Overall, foliar [N] was significantly (66% $\chi^2 = 34.04$, $P < 0.01$) higher in legumes than in non-legumes (Fig. 2.4a). There was no significant difference in foliar [N] between seeders and resprouters (Fig. 2.4b). However, all of the legume resprouters showed a significant ($F_{7, 110} = 4.76$, $P < 0.001$) increase in foliar [N] with a concomitant increase in P supply, while the seeders did not (Fig. 2.4c). The non-legume *L. spissifolium* was the only species that had a significantly ($P < 0.001$) lower [N] at 10 mg P kg^{-1} compared with 1 mg P kg^{-1} (Fig. 2.4c).

On average, the legumes had significantly (53%, $\chi^2 = 6.72$, $P < 0.05$) higher foliar [P] than the non-legumes (Fig. 2.5a). However, expressed per leaf area, foliar [P] did not differ significantly between legumes (58 ± 4 mg m^{-2}) and non-legumes (53 ± 8 mg m^{-2}). Overall, increased P supply did not change the [P] of either the legumes or non-legumes. However, increased P supply did significantly ($F_{7, 110} = 4.43$, $P < 0.001$) increase foliar [P] in both legume and non-legume resprouters, apart from *A. subtingens* (Fig. 2.5c). *L. coniferum* was the only seeder to show a similar increase in foliar [P] with P (Fig. 2.5c).

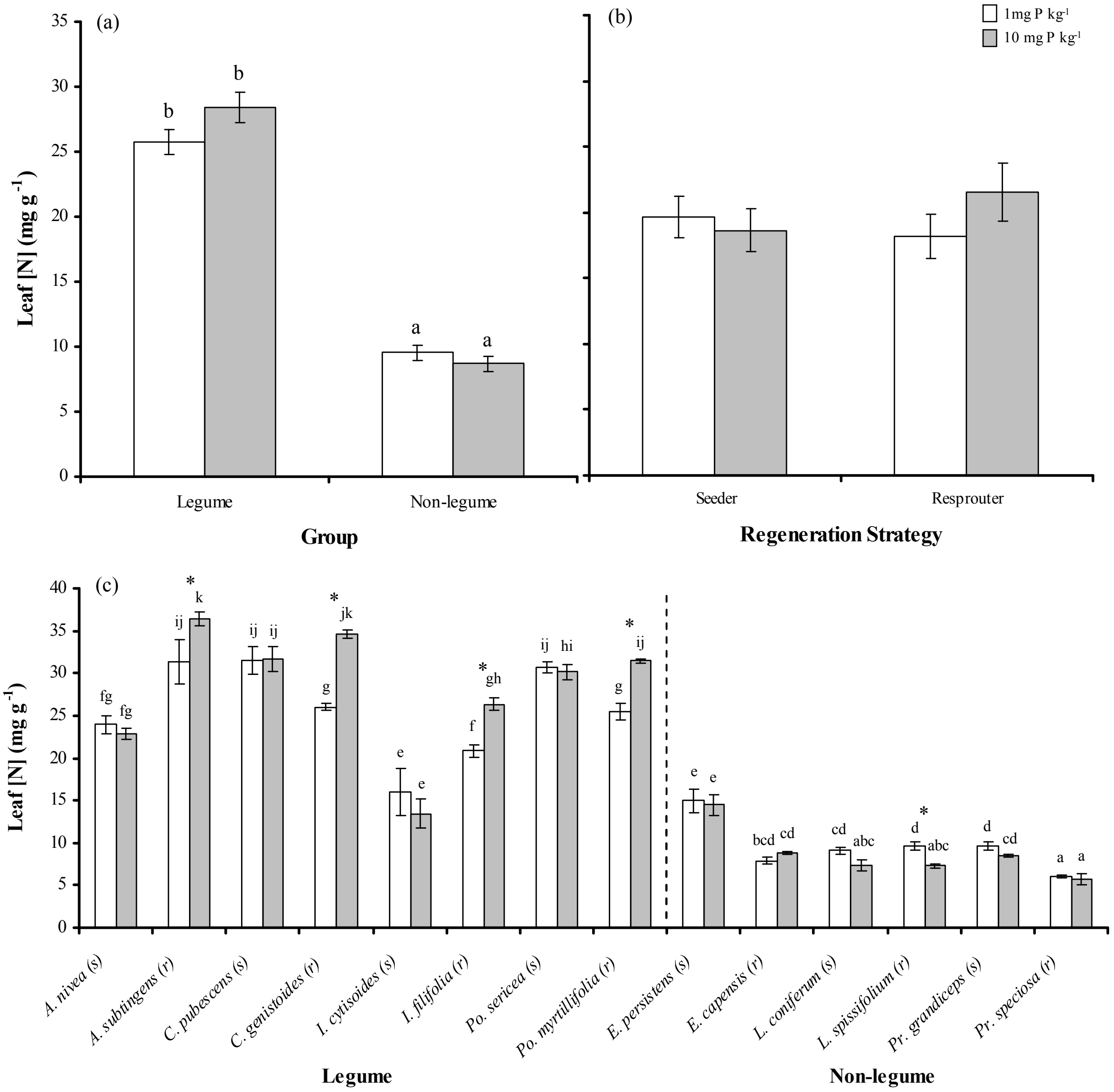

Fig. 2.4 Foliar [N] of (a) legumes and non-legumes, (b) seeders and resprouters, (c) legume and non-legume species supplied with 1 or 10 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figure (a) denote significant differences between groups ($P < 0.001$) from a linear mixed model (fixed factor: group x regeneration strategy x P treatment, random factor: species nested in genus). Significant differences between species and P treatments ($P < 0.001$) in figure (c) are indicated by different letters, from a two-way ANOVA (factors: species and P treatment). Differences between P treatments within a species are highlighted by an asterisk. All tests were conducted on transformed data. Regeneration strategies are indicated by (s) = seeder, (r) = resprouter.

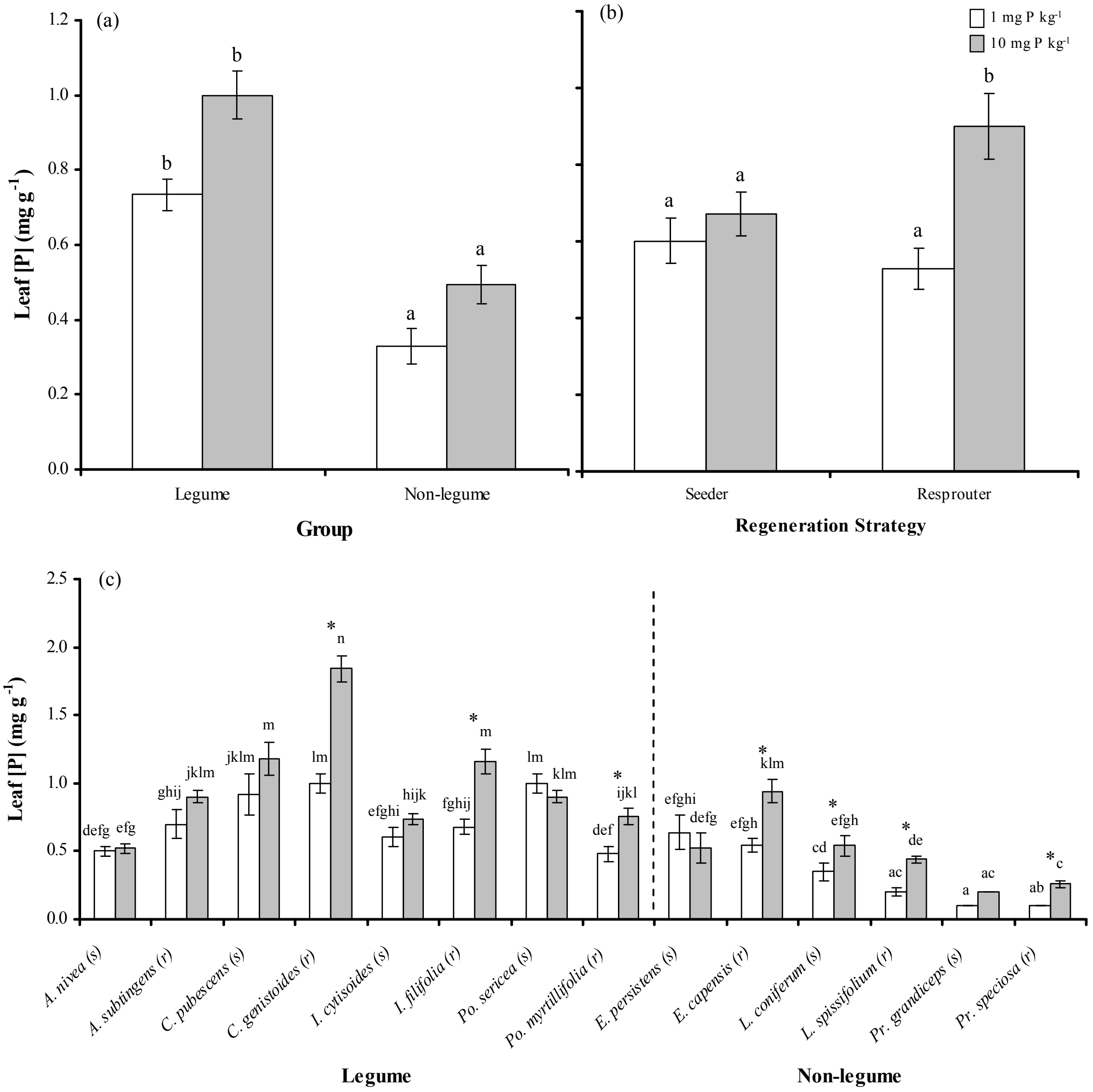

Fig. 2.5 Foliar [P] of (a) legumes and non-legumes, (b) seeders and resprouters, (c) legume and non-legume species supplied 1 or 10 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figures (a) and (b) denote significant differences between groups ($P < 0.05$), regeneration strategies and P treatments ($P < 0.001$) from a linear mixed model (fixed factor: group x regeneration strategy x P treatment, random factor: species nested in genus). Significant differences between species and P treatments ($P < 0.001$) are indicated by different letters. Differences between P treatments within a species are highlighted by an asterisk. All tests were conducted on transformed data.. Regeneration strategies are indicated by (s) = seeder, (r) = resprouter.

Discussion

P-deficits may contribute to the absence of legumes in some ecosystems (Vitousek and Howarth, 1991; Vitousek and Field, 1999; Vitousek *et al*., 2002). Also, in fynbos the decline in legume abundance (Cocks, 1994) and soil P availability during post-fire succession (Hoffman *et al*., 1987) may be linked. The greater responsiveness to P supply of legume than of non-legume growth (Figs. 2.1 and 2.2a) suggests that the legumes studied were more P-limited than the non-legumes. This responsiveness of legume growth to increased P supply is probably a function of their capacity to fix N_2. This releases them from N-limitation, making them sensitive to the next most limiting nutritional constraint, that involving P. However, legumes showed significantly higher foliar [P] when expressed per unit leaf mass (Fig. 2.5a). This more likely reflects the less sclerophyllous nature of legume leaves (specific leaf area 13.4 ± 0.6 versus 6.4 ± 0.4 m^2 kg^{-1}, $P < 0.01$) rather than any intrinsic difference in cellular [P], since leaf P per unit leaf area was similar. Nevertheless, the higher [P] was associated with a higher P content ($P < 0.05$) of the legumes (1.2 ± 0.2 mg P) than of the non-legumes (0.4 ± 0.1 mg P). This means that the legumes took up more P than the non-legumes in this study. This was possible because a proportion of P in the inoculum and Ca/Fe-P was available. This available P is probably analogous to the post-fire nutrient flush when P is plentiful and in an available form (Brown and Mitchell, 1986). However, once the post-fire flush of available P is exhausted, the legumes would need to be able to access the sparingly soluble forms of P in order to persist.

Increased allocation to below-ground biomass is frequently associated with enhanced P-acquisition (Lambers *et al*., 2006) and differences in shoot : root biomass allocation have frequently been linked with resource availability (Brouwer, 1983; Bloom *et al*., 1985; Gedroc *et al*., 1996; McConnaughay and Coleman, 1998, 1999). Surprisingly, the legumes studied here had significantly higher shoot : root biomass ratios than the non-legumes, despite their apparently greater P-constraint. Although all species experienced the same light, moisture and nutrient regimes, the active nodules of legumes implied an ability to supplement their N supply through N_2-fixation (Markham and Zedeveld, 2007), as indicated by higher tissue [N] (Fig. 2.4a). Thus the lower below-ground investment by legumes may have been facilitated or enabled by their N_2-fixing capacity. Although the legumes in this study actually accumulated more P than the non-legumes, reduced root investment might compromise their ability to acquire P from scarce and/or sparingly soluble sources during late post-fire succession.

Plants with root clusters are thought to be better adapted to access the sparingly soluble P forms that predominate in ancient, highly weathered and leached soils than are plants which form mycorrhizal symbioses (Lambers *et al.*, 2008b). Thus the greater prevalence of root clusters in non-legumes compared with legumes, many of which have developed mycorrhizal associations, identifies the latter as less suited to the oligotrophic soils of fynbos environments, especially during late post-fire succession when P availability is low (Brown and Mitchell, 1986). Amongst fynbos legumes, cluster roots appear to be limited to *Aspalathus* and *Cyclopia*, not being documented in any other genera (Allsopp and Stock, 1993; Spriggs, 2004). While the dual strategy (cluster roots and mycorrhizae) employed by *Aspalathus* might seem beneficial in terms of maximizing P-uptake, its utility is compromised by high metabolic costs of cluster roots (Shane *et al.*, 2004; Shane and Lambers, 2005) and mycorrhizae (Lambers *et al.*, 2002). However, the dual strategy may facilitate access to different forms of P or possibly aid P-uptake at different times of the year or at different stages during post-fire succession. Also, since cluster root formation is restricted to periods when the soil is wet whereas mycorrhizae persist into dry periods (Neumann and Martinoia, 2002), the latter may facilitate P-uptake under drier conditions.

Cluster roots confer a significant advantage in terms of P-acquisition partly because of their the ability to release large quantities of carboxylates into the rhizosphere, high carboxylate concentrations enhancing P-solubilization (Gardener *et al.*, 1983; Dinkelaker *et al.*, 1995; Neumann *et al.*, 2000; Lambers *et al.*, 2003, 2006). Thus the 23-fold higher concentration of cluster root rhizosphere carboxylates in non-legumes could make them substantially more effective at P-acquisition than legumes. Furthermore, the capacity to solubilize P depends not only on carboxylate concentrations, but also on their composition. Carboxylates differ in their capacity to solubilize P according to the following sequence of diminishing efficacy: citrate > malate and succinate > acetate and lactate (Jones, 1998; Jones *et al.*, 2003). We found that legumes released predominantly lactate whereas non-legumes released high concentrations of citrate, malate and succinate (Table 2.2). Thus, in addition to releasing more carboxylates, non-legumes also exuded carboxylates having a superior P-solubilization capacity, compared with those released by legumes. However, this result should be seen as preliminary because the method used to collect carboxylates does not accurately reflect the consequences of exudative bursts associated with cluster-root physiology (Shane *et al*., 2004).

Differences in P-acquisition strategy between seeders and resprouters were rather limited, root adaptations and the extent of mycorrhizal colonization being similar. Although we found no difference in mycorrhizal colonization, we cannot exclude the possibility that that mycorrhizal participation in P-acquisition varied since we did not quantify arbuscule abundance as a measure of mycorrhizal activity (McGonigle *et al*., 1990; Brundrett, 2009). There was also limited variation in below-ground biomass investment and cluster root carboxylate concentration between seeders and resprouters (Tables 2.1 and 2.2). However, the legume resprouter *A. subtingens* invested significantly more of its root biomass in cluster roots than did the congeneric seeder *A. nivea* at 1 mg P kg^{-1} (Table 2.1), possibly reflecting a greater P-acquisition capacity. Although shoot P-status has been shown to regulate cluster root formation (Marschner *et al*., 1987; Keerthisinghe *et al*., 1998; Shane *et al*., 2003a, b), this does not explain the greater investment in cluster roots by *A. subtingens* than by *A. nivea*, since these species had similar foliar [P] when supplied with 1 mg P kg^{-1} (Fig. 2.5c). Thus this difference in the cluster root : root ratio appears to be intrinsic to the species concerned. Accordingly, in low P soils, such as those prevalent in late post-fire succession fynbos environments, resprouters may enjoy a distinct advantage over seeders.

Although the difference in cluster-root investment between the two *Aspalathus* species was not evident in other congeneric pairs, all of the resprouters, with the exception of *A. subtingens*, exhibited a significant increase in foliar [P] with increased P supply (Fig. 2.5c). In contrast the majority of seeders did not show increased foliar [P] with increased P supply. For seeders, this was probably a consequence of either an ability to regulate P-uptake or of P-dilution due to increased growth. The exception was *I. cytisoides* in which growth declined, possibly as a result of P toxicity. However, among the resprouters, the increase in foliar [P] with P supply was accompanied by a significant increase in growth in only two species (*Po. myrtillifolia*, *L. spissifolium*; Fig. 2.2c). Thus, resprouters took up more P than they required, possibly storing it for later growth or indicating an inability to down-regulate P-uptake. A weak capacity to down-regulate P-uptake has been associated with species native to low P soils (e.g. *Hakea prostrata*, Shane *et al*., 2004; *Protea compacta*, Shane *et al*., 2008), while species native to higher P soils are better able to down-regulate P-uptake (e.g. *Grevillea crithmifolia*, Shane and Lambers, 2006; *Protea meridianum*, Shane *et al*., 2008). Given that resprouters tend to be longer-lived than seeders in fynbos vegetation (Kruger and Bigalke, 1984; Le Maitre and Midgley, 1992; Schutte *et al*., 1995; Bond and van Wilgen, 1996; Bond and Midgley, 2003) and so have to endure the post-fire decline in P (Brown and Mitchell, 1986), their weak capacity to down-regulate P-uptake may reflect adaptation to low P soils.

In contrast to native fynbos legumes, invasive Australian *Acacia* species appear capable of persisting in fynbos vegetation long after fire, often coming to dominate. This may be possible because species like *Acacia saligna* possess extensive root systems and a greater mycorrhizal network compared with native fynbos legumes (Hoffman and Mitchell, 1986), both of which may provide access to a greater pool of P. Furthermore, in contrast to the pattern reported here (Table 2.1), Witkowski (1991a) reported *A. saligna* as having a greater below-ground biomass investment than the non-legume *Protea repens*.

Conclusion

The inability of indigenous legumes to persist during the course of post-fire succession in the fynbos vegetation of the CFR may be, at least partly, a consequence of their lack of root adaptations required to exploit scarce and sparingly soluble sources of P. Those legumes which are able to persist (resprouters) appear better adapted to low P conditions, exhibiting greater investment in cluster roots compared with seeders. Given that more than 75% of fynbos legume species are seeders (Le Maitre and Midgley, 1992), it is unsurprising that legumes are rare in late post-fire succession fynbos environments, in which P availability is low.

Chapter 3

Are leguminous (Psoraleeae) seeders of the Cape Floristic Region associated with greater nutrient availability than resprouters?

Abstract

In fynbos vegetation, legume seeders and resprouters seem to be habitat specific. This habitat specificity may be linked to soil nutrient availability because of the higher nutrient demand associated with the increased growth rate and reproductive effort of seeders compared with resprouters. It was hypothesized that seeders occupy habitats with greater nutrient availability and possess larger, less sclerophyllous leaves than resprouters. Habitat nutrient status and leaf morphologies were compared between seeders and resprouters in the legume genera *Otholobium* and *Psoralea*. Seeder soils had greater nutrient availability than resprouter soils as evidenced by the higher pH, [NO_3^-], exchangeable [Ca], CEC, combined with lower [Fe]. Differences in the latter three were maintained after phylogenetically independent contrasts (PIC) analysis. Only seeders in *Otholobium* had a higher Bray II [P] than their resprouters. Factors contributing to the differences in soil nutrient availability between seeder and resprouter habitats differed between genera. There was no difference in sclerophylly, but seeders possessed smaller leaves than resprouters, with the functional significance of smaller leaves varying from enhanced nutrient acquisition via mass-flow to heat dissipation. High soil nutrient availability and enhanced nutrient acquisition from small leaves correspond with the high nutrient demands of seeders. However, the high P demands of legume seeders may not be sustainable in fynbos and thus conducive to persistence through post-fire succession.

Introduction

In fynbos vegetation of the Cape Floristic Region (CFR), legumes are typically prevalent in the early phases of post-fire succession but tend to be absent from mature vegetation (*ca.* > 10 years post-fire) (Kruger, 1979a; Hoffman *et al.*, 1987; Cocks, 1994). However, not all legumes are unable to persist, with some species known to occur in the later stages of post-fire succession (Hoffman *et al.*, 1987; Cocks, 1994). Little is known about the longevity of legume species in the CFR. However, it seems likely that the persistent species are resprouters as they tend to have longer life-spans than co-occurring seeders in fynbos (Kruger and Bigalke, 1984; Le Maitre and Midgley, 1992; Schutte *et al.*, 1995; Bond and van Wilgen, 1996; Bond and Midgley, 2003). In fynbos vegetation, legume seeders and resprouters seem to be habitat specific as seeders tend to occur in dense isolated populations, while resprouters are scattered among other fynbos species, within the same landscape (Schutte *et al.*, 1995). Similar differences in habitat specificity have been recorded among seeders and resprouters in *Erica* in the CFR (Ojeda, 1998) and *Banksia* in south-western Australia (Lamont and Markey, 1995).

Differences in the distributions of legume seeders and resprouters could be linked to fire regime or the availability of resources (Keeley and Zedler, 1978; Le Maitre and Midgley, 1992; Bond and van Wilgen, 1996; Buhk *et al.*, 2007). In Proteaceae of the CFR, the proportion of seeders in a landscape increases with increased fire frequency (Le Maitre and Midgley, 1992). However, a number of studies has also indicated that distribution of seeders and resprouters is strongly related to resource availability, with seeders occurring more frequently than resprouters in habitats with greater resource (i.e moisture and nutrients) availability (Midgley, 1996; Iwasa and Kubo, 1997; Ojeda, 1998; Bellingham and Sparrow, 2001; Bond and Midgley, 2001, 2003; Garcia and Zamora, 2003). This pattern is not consistent accross the globe or some parts of the CFR as indicated by other studies (e.g Smith *et al.*, 1992; Lamont and Markey, 1995). Nonetheless, among fynbos legumes seeders are reported to occupy habitats with a higher moisture availability compared with resprouters (Schutte *et al.*, 1995). Furthermore, the presence of seeders early on in post-fire succession coincides with a temporary increase in nutrient availability, including N and P, whereas resprouters persist beyond this nutrient flush, into the later stages of post-fire succession (Brown and Mitchell, 1986; Stock and Lewis, 1986). The distributions of fynbos species, including Fabaceae, are strongly linked to edaphic factors such as nutrient availability (Dahlgren, 1963, 1968; Cowling, 1990; Cowling *et al.*, 1992; Cowling and Holmes, 1992; McDonald *et al.*, 1996; Richards *et al.*, 1995, 1997a,b). Hence, soil nutrient availability may also contribute to the habitat specificity of legume seeders and resprouters in fynbos.

The relationship between fynbos species distributions and soil nutrient availability could be the result of contrasting nutritional demands (Richards *et al.*, 1997b). For instance, fynbos Proteaceae generally grow better on their native soils than on dissimilar neighbouring soils (Mustart and Cowling, 1993), implying specific nutrient requirements. Seeders and resprouters potentially have differing nutritional requirements as a result of their differences in allocation of resources to vegetative growth and reproductive effort (Stearns, 1976; Kruger, 1983; Bell, 2001; Bellingham and Sparrow, 2001). For example, in South-western Australia, Pate *et al.* (1990) noted that seeders allocated four times more assimilate to shoots and grew almost three times faster than similarly aged resprouters. In a comparison of reproductive efforts between a congeneric legume seeder and resprouter pair, Hansen *et al.* (1991) found that the seeder produced 32 times more seeds with higher concentrations of N and P compared with the resprouter. In view of its higher growth rate and production of highly nutritious seeds, the seeding strategy is likely to incur a higher nutrient demand relative to the resprouting strategy. This may require seeders to occupy habitats that are more fertile than resprouters (Bloom *et al.*, 1985).

Differences in resource allocation combined with potential differences in habitat nutrient availability may affect the leaf traits of seeders and resprouters, specifically sclerophylly and leaf size. Specific leaf area (SLA) is negatively correlated with the degree of sclerophylly and is frequently used as a measure of sclerophylly (Edwards *et al.*, 2000). Species with high (e.g. seeders) relative growth rates tend to have a high SLA compared with slow (e.g. resprouters) growing species (Poorter and Remkes, 1990; Poorter and de Jong, 1999). Furthermore, species from oligotrophic habitats tend to be more sclerophyllous and have smaller leaves when compared with species from eutrophic habitats (Beadle, 1966; Cowling and Campbell, 1980; Cunningham *et al.*, 1999; Fonseca *et al.*, 2000; McDonald *et al.*, 2003; Niinemets and Kull, 2003; Ordonez *et al.*, 2009; Poorter *et al.*, 2009). This appears to be adaptive, since a higher degree of sclerophylly confers increased leaf longevity (Reich *et al.*, 1997, 1999;

Wright *et al*., 2001, 2004; Poorter *et al*., 2009) and hence enhanced nutrient retention times (Escudero *et al*., 1992; Eckstein *et al*., 1999; Wright and Westoby, 2003). On the other hand, reduced leaf size may promote nutrient acquisition via mass-flow (Yates *et al*., 2010) as small leaves have a greater potential to lose water via transpiration (Parkhurst and Loucks, 1972). Increased sclerophylly and smaller leaves may therefore be particularly advantageous in oligotrophic environments, which leads to the expectation that resprouters should display these traits.

In this study I investigated whether legume seeders and resprouters occupy contrasting habitats, particularly in terms of soil nutrient availability, and whether these differences in habitat are associated with differences in leaf traits. Given the putative link between resource availability and the distribution of seeders and resprouters, it was hypothesized that: (1) legume seeders occupy habitats with greater nutrient availability compared with resprouters, and (2) seeders possess larger leaves and display a lower degree of sclerophylly than resprouters. To test these hypotheses, I characterized the nutritional habitats and leaf attributes of seeder and resprouter species in the legume genera *Otholobium* and *Psoralea*, on the basis of field sampled data.

Materials and Methods

Species and sampling

Otholobium and *Psoralea* are included in the tribe Psoraleeae, with *ca.*53 and 50 species, respectively, in Africa (Goldblatt and Manning, 2000; Stirton, 2005). There is a strong distinction in leaf morphology between the genera. *Otholobium* species tend to possess broad, obovate leaves, whereas the leaves of *Psoralea* species can range from compound-pinnate to scales on young branches (Stirton, 1989; Goldblatt and Manning, 2000). Both genera occur on a variety of soil types, including those derived from granite, sandstone and shale bedrock (Rebelo *et al.*, 2006). There is a strong disparity in the moisture availability of habitats occupied by the two genera. In the CFR, 80% of *Psoralea* species occupy habitats such as seeps, marshes, riverbanks and situations of high altitudes where precipitation is higher (Stirton, 1986; 1989; Goldblatt and Manning, 2000). In contrast, *Otholobium* species predominantly occupy drier habitats with only 11% of species occupying moist habitats similar to those occupied by the majority of *Psoralea* species (Stirton, 1986, 1989).

This studied focused on twelve *Otholobium* species and eleven *Psoralea* species, indigenous to the CFR (Table 3.1). The regeneration strategy of each species was characterized using the available literature (Stirton, 1989; Goldblatt and Manning, 2000) and personal observations (Table 3.1). Sampling was conducted between August and December 2008 at sites where *Otholobium* or *Psoralea* species are known to occur (i.e based on their distribution) and which had not been burnt for more than a year. All sites were located in the western half of the CFR, an area dominated by a winter-rainfall regime (Deacon *et al.*, 1992) (Fig. 3.1). For each species, five leaf and four soil samples were collected per site.

Plant and soil measurements

Leaves from an entire branch from five separate individuals, together with a 1 cm^2 piece of cardboard, were placed on a sheet of paper, photographed (Canon Powershot A530, Canon Inc. Japan) and the leaf area determined with Adobe Photoshop CS version 8.0 (Adobe Systems Inc., USA). In addition, the basal diameter of each branch was measured. The diameter of the largest circle that could be accommodated in the leaf/leaflet perimeter was calculated by analyzing the photographs of the leaves using Photodraw^{TM} 2000 version 2.0 (Microsoft Corporation, USA). Leaves were then dried at 80°C for 48 h and the SLA (m^2 kg^{-1}) and total leaf area per stem area (m^2 m^{-2}) calculated. The dried leaf material was milled in a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) and concentrations of B, Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn measured by dry-ashing pulverized leaf material at 480°C for 8 h and dissolving with a 1:1(v/v) of HCl, according to the method of Kalra (1998). Assessment of the element concentrations in solution was performed using inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista MPX, Mulgrave, Australia). Leaf [N], [C], $\delta^{15}N$, $\delta^{13}C$ were determined using mass spectrometry. For this purpose, 1.9 to 2.1 mg of milled leaf material of each sample was weighed into an 8 x 5 mm tin capsule (Elemental Microanalysis Ltd, Okehampton, UK). The samples were then combusted in a Thermo Flash EA 1112 series elemental analyzer coupled to a Delta Plus XP isotope ratio mass spectrometer via a Thermo Finnigan Conflo III control unit (all from Thermo Electron Corporation, Milan, Italy). Two in-house standards and one IAEA standards were used to calibrate the results.

Soil samples taken from the upper 15 cm of the soil profile at each site using an auger or a shovel where the soil was stony and shallow. Samples were air dried and sieved (1mm mesh) prior to analysis. Soil pH was determined by shaking 2 g of material in 20 mL 1 *M* KCl at 180 rpm for 60 min, centrifuging at 10 000 *g* for 10 min, and measuring the pH of the supernatant. Total soil [N] was determined using mass spectrometry, as described above but using *ca.* 20 mg soil per sample. [NH_4^+] and [NO_3^-] were determined by extracting 2 g of soil in 20 mL of 1 *M* KCl, which was analyzed colorimetrically according to Keeney and Nelson (1982). Total [P] was analyzed according to Bray and Kurtz (1945) using ICP-AES analysis. Available P was determined by extracting 2 g of soil in Bray II solution (Bray and Kurtz, 1945) which was filtered through Whatman No.2 filter paper. The filtrate was analyzed colorimetrically using the Malachite Green method (Motomizu *et al.*, 1983). Exchangeable cations were displaced from 10 g of sample with 25 mL of 0.2*M* ammonium acetate. The samples were filtered through Whatman No. 2 filter paper and made to 200 ml before concentrations of Ca, K, Mg, Na were determined using ICP-AES analysis. Fe was extracted with 0.1 *M* HCl and the extract analyzed using ICP-AES.

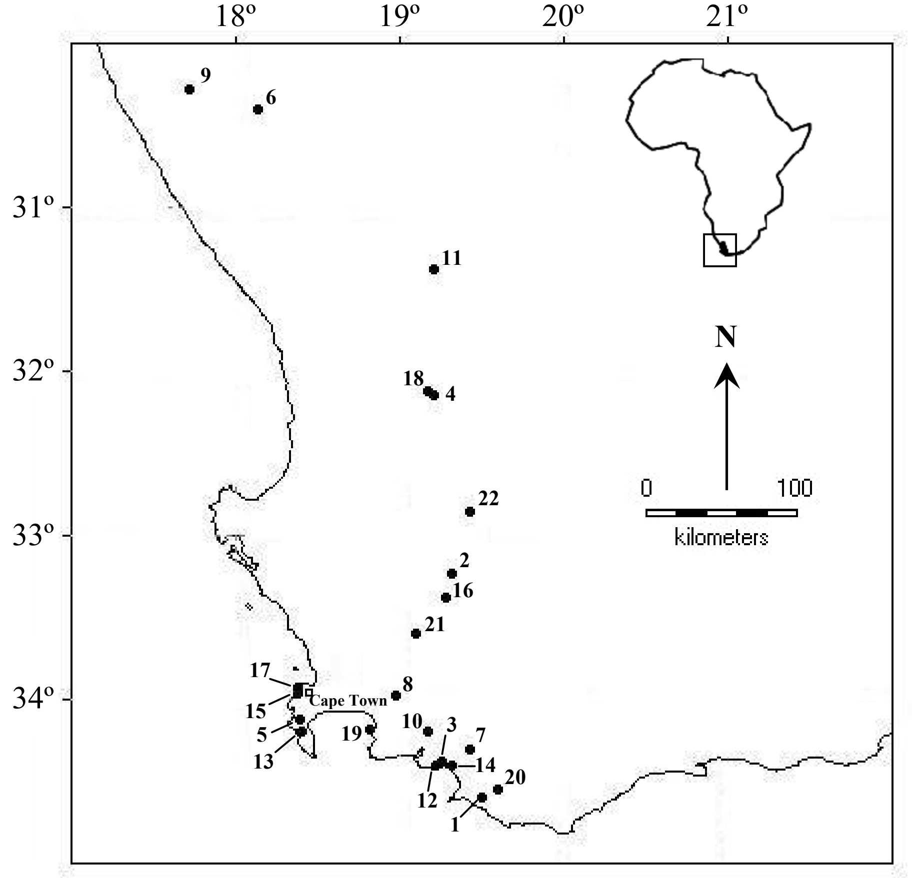

Fig. 3.1 Map showing the distribution of the 22 sites sampled within the CFR. Site numbers correspond to those listed in Table 1.

Table 3.1 The 23 *Otholobium* and *Psoralea* species sampled, with their respective regeneration strategy and site location.

Site	**Species**	**Regeneration Strategy**	**Co-ordinates**	**Voucher Specimen** (Herbarium)
1	*Otholobium bracteolatum*(Eckl. & Zehy.) C.H Strit.	Seeder	34° 36.579' S , 19˚ 30.714' E	Stirton, Musaya, Dludlu, 3205, (BOL)
2	*Otholobium candicans* (Eckl. & Zehy.) C.H Stirt.	Seeder	33° 14.224' S , 19˚ 19.954' E	Stirton, Musaya, Dludlu, 3350, (BOL)
4	*Otholobium flexuosum* C.H Stirt	Seeder	32˚ 07.539' S , 19˚ 10.736' E	Stirton, Musaya, Dludlu, 3276, (BOL)
6	*Otholobium hamatum* (Harv.) C.H Stirt	Seeder	30˚ 24.613' S , 18˚ 08.064' E	Stirton, Musaya, Dludlu, 3306, (BOL)
9	*Otholobium pustulatum* C.H Stirt.	Seeder	30˚ 17.188' S , 17˚43.946' E	Stirton, Musaya, Dludlu, 3286, (BOL)
11	*Otholobium striatum* (Thunb.) C.H Stirt.	Seeder	31˚ 23.234' S , 19˚ 01.600' E	Stirton, Musaya, Dludlu, 3318, (BOL)
3	*Otholobium dreweae* C.H Stirt. ined.	Resprouter	34˚ 23.468' S , 19˚ 15.526' E	Stirton, Musaya, Dludlu, 0010, (BOL)
5	*Otholobium fruticans* (L.) C.H Stirt.	Resprouter	34˚ 01.203' S , 18˚ 24.164' E	Stirton, Musaya, Dludlu, 0018, (BOL)
7	*Otholobium lanceolatum* C.H Stirt. ined.	Resprouter	34˚ 18.145' S , 19˚ 25.101' E	Stirton, Musaya, Dludlu, 0013, (BOL)
8	*Otholobium obliquum* (E.Mey) C.H Stirt	Resprouter	33˚ 59.417' S , 18˚ 58.148' E	Stirton, Musaya, Dludlu, 3198a, (BOL)
10	*Otholobium rotundifolium* (L.f) C.H Stirt.	Resprouter	34˚ 12.883' S , 19˚ 10.214' E	Stirton, Musaya, Dludlu, 3173, (BOL)
12	*Otholobium thomii* (Harv.) C.H Stirt.	Resprouter	34˚ 24.608' S , 19˚ 13.009' E	Stirton, Musaya, Dludlu, 0003, (BOL)
14	*Psoralea affinis* Eckl. & Zehy.	Seeder	34˚ 24.128' S , 19˚ 19.090' E	Stirton, Musaya, Dludlu, 3186, (BOL)
15	*Psoralea aphylla* L.	Seeder	33˚ 58.072' S , 18˚ 22.587' E	Stirton, Musaya, Dludlu, 3400, (BOL)
9	*Psoralea fleta* C.H Stirt. ined.	Seeder	33˚ 23.985' S , 19˚ 17.183' E	Stirton, Musaya, Dludlu, 3341, (BOL)
16	*Psoralea glaucescens* Eckl. & Zehy.	Seeder	30˚ 17.188' S , 17˚ 43.947' E	Stirton, Musaya, Dludlu, 3289, (BOL)
19	*Psoralea pinnata* L.	Seeder	34˚ 11.226' S , 18˚ 49.212' E	Stirton, Musaya, Dludlu, 3165, (BOL)
20	*Psoralea restioides* Eckl. & Zehy.	Seeder	34˚ 33.466' S , 19˚ 36.372' E	Stirton, Musaya, Dludlu, 3217, (BOL)
13	*Psoralea aculeata* L.	Resprouter	34° 04.757' S , 18˚ 23.648' E	Stirton, Musaya, Dludlu, 3405, (BOL)
17	*Psoralea imbricata* (L.) T.M Salter	Resprouter	33˚ 56.186' S , 18˚ 23.367' E	Stirton, Musaya, Dludlu, 3399, (BOL)
18	*Psoralea oreopola* C.H Stirt. ined.	Resprouter	32˚ 09.059' S , 19˚ 01.570' E	Stirton, Musaya, Dludlu, 3271, (BOL)
21	*Psoralea rigidula* C.H Stirt. ined.	Resprouter	33˚ 36.258' S , 19˚ 06.561' E	Stirton, Musaya, Dludlu, 3390, (BOL)
22	*Psoralea verrucosa* Willd. ex Spreng	Resprouter	32˚ 51.769' S, 19˚ 26.601' E	Stirton, Musaya, Dludlu, 3357, (BOL)

Phylogeny reconstruction

A phylogeny of the sampled species was constructed using sequence data from three DNA regions (*trn*L-*trn*F, *rpo*B-*trn*C, ITS) and a morphological character matrix (M. Dludlu unpublished, Botany Department, UCT). The DNA sequence alignment was checked using BioEdit version 7.0 (Hall, 1999) before being combined into a single matrix with the morphological data.

Preliminary parsimony indicated that there was no supported conflict between the individual sequence regions. Thus, phylogenetic relationships were inferred on the basis of a combined data matrix, using Bayesian inference as implemented in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). All trees were rooted on *Hoita* spp.. Separate models of characters were applied to each partition, with maximally complex GTR + I + Γ models being applied to the two sequence partitions in order to avoid under-parameterization (Huelsenbeck and Ronquist, 2004). All parameters were treated as unknown with uniform prior probabilities and were estimated in conjunction with the tree topology. The analysis was conducted using three independent runs; each with four metropolis-coupled Monte Carlo Markov chains (one cold and three heated). Each chain was run for 10^6 generations with sampling done every 100th generation, resulting in 10 000 sample trees per run. Comparison of posterior probabilities (PP) across independent runs, and plots of the –log likelihoods against generation time, were used to confirm that convergence had been observed and to determine the appropriate 'burn-in' period. Trees taken from the 'burn-in' were discarded prior to the calculation of the PP's.

Linear mixed model analysis

Considering the differences in distribution and morphology between the genera, a two-way linear mixed model using restricted maximum likelihood (Wald χ^2 statistic, Genstat 12.1, Lawes Agricultural Trust, 2010; Rothamsted Experimental Station) was conducted to determine whether the differences between seeders and resprouters were consistent across genera. The fixed factor was a two-way interaction between genus and regeneration strategy, and the random factor was species. Where differences between genera and regeneration strategies were found, these were judged to be significant ($\alpha = 0.05$) if the difference between the predicted means exceeded twice the standard error of difference. Prior to analysis, all variables except percentage data, were log transformed. Percentage data were arcsine square root transformed (Sokal and Rohlf, 1995).

Phylogenetically independent contrasts

The linear mixed model treats each species as an independent data point; however, this assumption is violated by the fact that species show varying degrees of phylogenetic relationship, as defined by a hierarchically structured phylogeny (Felsenstein, 1985; Harvey and Pagel, 1991). This results in points being phylogenetically non-independent. Consequently, the relationships between regeneration strategies were also assessed using phylogenetically independent contrasts (PIC's). This approach compares changes in each trait of interest on independent (non-overlapping) evolutionary branches, each of these being defined by pairs of species below a node in a bifurcating phylogeny where the pairs have evolved independently from other pairs (Felsenstein, 1985).

Analysis of the PIC's was conducted using the 'Analysis of Traits' (AOT) module in Phylocom version 3.22 (Webb *et al*., 2008). AOT derives a series of contrasts generated from sister and paraphyletic species comparisons. Under the assumptions of no trait associations, these contrasts are expected to have a mean of zero, and can be tested using a paired sample t-test. To account for phylogenetic uncertainty, PIC analyses were performed on 100 trees drawn from the posterior tree sample generated by the Bayesian inference. The percentage of trees indicating a significant difference between seeders and resprouters was used to gauge support for a significant result. Differences between regeneration strategies were analyzed using PIC's across genera as there were insufficient samples within each genus to permit separate genus-specific analyses. To identify the ancestral conditions and to evaluate the number and position of changes in ancestral seeder and resprouter states, ancestral states were inferred using Fitch (1971) parsimony as implemented in Mesquite version 2.5 (Maddison and Maddison, 2008). Again, this was done over the 100 trees drawn from the posterior tree sample, in order to gauge support for a particular regeneration strategy.

Boosted classification tree analysis

Boosted classification tree (BCT) analysis was used to determine which plant and soil variables best predicted the environmental associations and biological characteristics of seeders and resprouters. Details of BCT analysis are provided in the Appendix. All analyses were carried out in R (version 2.10.1, www.r-project.org, R Development Core Team, 2010) using the 'gbm' library of Ridgeway (2009) and supplemented with functions from Elith *et al*. (2008). Separate models were run for the plant and soil datasets. Optimal tree complexity (tc) and learning rate (lr) for each model were identified using 10-fold cross validation (CV), those values giving the lowest CV predictive deviance being judged the best. For the plant dataset, tc = 4, lr = 0.01 were optimal, while tc = 5, lr = 0.001 were optimal for the soil dataset. In order to improve further the predictive performance of the models, unimportant variables were identified using the simplification script from Elith *et al*. (2008) and removed from the final model. Each model was run 10 times, with the number of trees ranging between 2450–5400 (plant dataset) or 5050–7750 (soil dataset). The CV predictive deviance, expressed as a percentage of the null deviance, and the area under the receiver operator characteristic (AUC) were used to assess model performance. The relative influence of plant and soil predictor variables in separating seeders and resprouters was determined using a script in the 'gbm' library. A one-way ANOVA (Genstat 12.1, Lawes Agricultural Trust, 2010; Rothamsted Experimental Station) was used to determine whether the relative influence differed between predictors. Differences between predictor variables were judged to be significant ($\alpha = 0.05$) if the differences between the predicted means exceeded twice the standard error.

Results

Phylogenetic relationships

The phylogenetic hypothesis derived from the Bayesian analysis was poorly supported, only 9 nodes including the in-group node, have a posterior probability ≥ 0.95 (Fig. 3.2). *Otholobium* and *Psoralea* are monophyletic relative to the out-group and *Psoralea* is monophyletic. The monophyly of *Otholobium* is however uncertain, being achieved in less than 95% of the posterior tree set (Fig. 3.2). The ancestral regeneration strategy was identified as resprouting in 69 and 72 of the 100 trees for the Psoraleeae tribe and *Psoralea* genus respectively. The remaining trees were ambiguous.

Soil nutrient status

Five of the measured soil nutrient properties indicated significant differences between seeder and resprouter habitats (Table 3.2). Soils inhabited by seeders had a significantly higher pH ($\chi^2 = 6.83$, $P < 0.05$), [NO_3^-] ($\chi^2 = 6.37$, $P < 0.05$), exchangeable [Ca] ($\chi^2 = 14.15$, $P < 0.01$) and CEC ($\chi^2 = 9.16$, $P < 0.01$), while resprouter soils had a significantly higher [Fe] ($\chi^2 = 9.58$, $P < 0.01$). Only the differences in [NO_3^-], exchangeable [Ca], CEC and [Fe] were supported by the PIC analysis, at least for some of the trees (26, 96, 90 and 46% of the trees, respectively). Corroborating these results, the BCT analysis indicated that [Fe] had the greatest influence in determining the occurrence of seeders and resprouters, followed by exchangeable [Ca] (Fig. 3.3a). In the BCT analysis, all properties were significantly ($F_{11, 119} = 5337.73$, $P < 0.001$) different from each other, with the exception of [N] and [Na] (Fig. 3.3a). The following predictors were excluded from the final simplified model as they did not contribute to predicting the occurrence of seeders and resprouters: pH, [C], [K] and exchangeable [K]. The deviance explained by the final model was 52.6 ± 1.3 %, with an AUC score of 0.944 ± 0.021.

Aside from the overall differences between seeders and resprouters, several nutrient properties differed significantly between the soils inhabited by *Otholobium* and *Psoralea* species. *Otholobium* soils had a significantly higher pH ($\chi^2 = 12.34$, $P < 0.01$) and total [P] ($\chi^2 = 5.12$, $P < 0.05$) compared with *Psoralea* soils but their [NH_4^+] ($\chi^2 = 5.44$, $P < 0.05$) was less than half that of found in *Psoralea* soils (Table 3.2). Within *Otholobium*, seeder sites received less mean annual rainfall ($\chi^2 = 5.27$, $P < 0.05$) than resprouter sites but had a higher soil $\delta^{15}N$ ($\chi^2 = 6.79$, $P < 0.05$) and Bray II [P] ($\chi^2 = 12.25$, $P < 0.05$) (Table 2). Furthermore, the soil $\delta^{15}N$ and Bray II [P] of *Otholobium* seeder soils were significantly ($P < 0.05$) higher than the levels associated with both *Psoralea* seeders and resprouters (Table 3.2). No significant differences were evident between the soils occupied by seeders and resprouters in *Psoralea*.

Leaf traits and nutrition

Analysis of morphological traits indicated that seeders were significantlytaller ($\chi^2 = 15.16$, $P < 0.001$) than resprouters, whereas resprouter leaves had a significantly larger dimension ($\chi^2 = 5.63$, $P < 0.05$) than seeders (Table 3.3). The difference in leaf dimension was not supported by the PIC analysis. However, 34% of trees indicated a significant difference in height between seeders and resprouters. Furthermore, height ($F_{11, 119} = 352.72$, $P < 0.001$) had the greatest influence in predicting seeder versus resprouter regeneration strategies in the BCT analysis. The final simplified BCT model excluded eleven leaf nutrient properties, including B, Cu, Fe, K, Mg, Mn, N, P, Zn, $\delta^{13}C$ and $\delta^{15}N$, which explained 91 ± 1.3 % of the deviance, and had an AUC score of 0.977 ± 0.002 (Fig. 3.3b). However, the leaf [N] of seeders was significantly ($\chi^2 = 5.76$, $P < 0.05$) higher than that of resprouters (Table 3.3). This result was not supported by the PIC analysis.

Comparing genera, *Otholobium* species were generally shorter in stature ($\chi^2 = 9.72$, $P < 0.01$) than *Psoralea* species (Table 3.3). *Otholobium* species leaves were four times larger ($\chi^2 = 47.92$, $P < 0.001$) and had a higher SLA ($\chi^2 = 5.06$, $P < 0.05$) compared with *Psoralea* species (Table 3.3). All of the *Otholobium* species sampled had broad obovate leaves/leaflets, while the photosynthetic structures of the *Psoralea* species ranged from compound pinnate leaves (*P. affinis*, *P. pinnata*) to thin branches (*P. aphylla*, *P. glaucescens*, *P. rigidula*) or a combination of both (*P. fleta*, *P. oreopola*, *P. restioides*, *P. verrucosa*). Leaf [B] ($\chi^2 = 12.82$, $P < 0.01$) and [Ca] ($\chi^2 = 15.42$, $P < 0.001$) were significantly higher in *Otholobium* compared with *Psoralea* (Table 3.3). In contrast, leaf [Na] ($\chi^2 = 5.06$, $P < 0.05$) of *Psoralea* was significantly higher than that of *Otholobium* (Table 3.3). Within *Psoralea*, there were no differences in leaf nutrient concentrations between seeders and resprouters. However, *Otholobium* seeders had a significantly higher leaf [P] ($\chi^2 = 4.72$, $P < 0.05$) although, their

N:P ($\chi^2 = 4.37$, $P \leq 0.05$) and C:N ($\chi^2 = 4.30$, $P \leq 0.05$) ratios were significantly lower than that of their resprouters (Table 3.3).

Fig. 3.2 One of the 100 trees drawn from the posterior tree set, used in inference of ancestral regeneration strategy and PIC analysis. Numbers above branches are posterior probabilities. Species regeneration strategies are indicated by shaded squares, while inferred regeneration strategy is represented by branch shading. Ambiguity in inferred ancestral regeneration strategy is indicated by broken branches.

Table 3.2 The soil properties and rainfall of Psoraleeae species habitats sampled, specific to each genus, regeneration strategy and regeneration strategy within each genus. Different letters following the mean ± SE denote significant differences between genera and regeneration strategies ($P \leq 0.05$), determined using a linear mixed model (fixed factor: genus x regeneration strategy, random factor: species). Bold type indicates where a significant interaction between genus and regeneration strategy occurred.

Soil Property	**Genus**		**Regeneration Strategy**		***Otholobium***		***Psoralea***	
	Otholobium	***Psoralea***	**Seeder**	**Resprouter**	**Seeder**	**Resprouter**	**Seeder**	**Resprouter**
Type[‡]					Granite/Shale	Sandstone	Sandstone	Sandstone
pH	5.0 ± 0.1^{b}	4.0 ± 0.2^{a}	4.9 ± 0.1^{b}	4.2 ± 0.1^{a}	5.5 ± 0.2	4.4 ± 0.1	4.2 ± 0.1	3.9 ± 0.1
Total N (mg g^{-1})	1.5 ± 0.1	2.3 ± 0.2	2.3 ± 0.2	1.5 ± 0.1	1.7 ± 0.2	1.3 ± 0.1	2.8 ± 0.3	1.7 ± 0.2
NO_3^- (mg kg^{-1})	10.3 ± 1.8	16.3 ± 2.5	19.4 ± 2.5^{b}	6.3 ± 0.9^{a}	16.3 ± 3.0	4.3 ± 0.8	22.5 ± 3.9	8.8 ± 1.6
NH_4^+ (mg kg^{-1})	4.9 ± 0.3^{a}	12.9 ± 2.4^{b}	7.4 ± 0.7	10.2 ± 2.4	5.4 ± 0.5	4.4 ± 0.4	9.4 ± 1.3	17.1 ± 4.9
δ^{15}N (‰)	2.7 ± 0.3	2.2 ± 0.2	3.1 ± 0.2	1.7 ± 0.2	**4.2 ± 0.3^{b}**	**1.2 ± 0.2^{a}**	**2.1 ± 0.2^{a}**	**2.4 ± 0.4^{a}**
Total P (mg kg^{-1})	23.6 ± 2.9^{b}	10.8 ± 1.2^{a}	17.0 ± 2.3	17.9 ± 2.7	22.7 ± 4.0	24.5 ± 4.2	11.3 ± 1.6	10.1 ± 1.8
Bray II P (mg kg^{-1})	8.7 ± 1.1	7.4 ± 0.9	10.4 ± 1.1	5.6 ± 0.8	**14.0 ± 1.6^{c}**	**3.4 ± 0.2^{a}**	**6.7 ± 1.2^{ab}**	**8.3 ± 1.5^{b}**
K (mg kg^{-1})	138.1 ± 12.0	120.9 ± 16.4	163.3 ± 16.6	93.4 ± 7.5	166.0 ± 21.6	110.2 ± 7.4	160.6 ± 25.7	73.4 ± 12.7
C (mg g^{-1})	25.7 ± 2.0	40.8 ± 3.7	36.7 ± 3.3	28.9 ± 2.6	24.7 ± 3.3	26.8 ± 2.3	48.6 ± 4.7	31.5 ± 5.1
Fe (mg kg^{-1})	9.6 ± 0.8	20.8 ± 4.5	9.4 ± 2.5^{a}	21.0 ± 3.7^{b}	5.5 ± 0.8	13.8 ± 0.9	13.3 ± 4.8	29.7 ± 7.7
Ca (cmol kg^{-1})[†]	3.8 ± 0.4	3.3 ± 0.4	5.0 ± 0.4^{b}	2.1 ± 0.2^{a}	5.5 ± 0.6	2.2 ± 0.3	4.5 ± 0.5	1.9 ± 0.3
K (cmol kg^{-1})[†]	0.35 ± 0.03	0.31 ± 0.04	0.42 ± 0.04	0.24 ± 0.02	0.43 ± 0.06	0.28 ± 0.02	0.41 ± 0.07	0.19 ± 0.03
Mg (cmol kg^{-1})[†]	1.7 ± 0.2	2.1 ± 0.3	2.5 ± 0.3	1.2 ± 0.1	2.0 ± 0.2	1.4 ± 0.2	3.0 ± 0.5	1.0 ± 0.1
Na (cmol kg^{-1})[†]	0.18 ± 0.02	0.34 ± 0.06	0.32 ± 0.05	0.19 ± 0.02	0.15 ± 0.02	0.21 ± 0.03	0.49 ± 0.09	0.17 ± 0.03
CEC (cmol kg^{-1})[§]	7.1 ± 0.5	8.2 ± 0.7	9.6 ± 0.6^{b}	5.4 ± 0.4^{a}	8.7 ± 0.8	5.5 ± 0.5	10.6 ± 0.9	5.3 ± 0.6
Rainfall								
MAR (mm)	601±47	712±40	527±34	793±46	**371 ± 28^{a}**	**832 ± 60^{b}**	**683 ± 42^{b}**	**746 ± 73^{b}**

[‡] Soil type and mean annual rainfall (MAR) according to Rebelo *et al*. (2006). [†] Exchangeable cations. [§] Cation exchange capacity

Table 3.3 The plant traits of Psoraleeae species sampled, specific to each genus, regeneration strategy and regeneration strategy within each genus. Different letters following the mean ± SE denote significant differences between genera and regeneration strategies ($P \leq 0.05$), determined using a linear mixed model (fixed factor: genus x regeneration strategy, random factor: species). Bold type indicates where a significant interaction between genus and regeneration strategy occurred.

Plant Trait	**Genus**		**Regeneration Strategy**		***Otholobium***		***Psoralea***	
	Otholobium	***Psoralea***	**Seeder**	**Resprouter**	**Seeder**	**Resprouter**	**Seeder**	**Resprouter**
Height (m) [‡]	1.1 ± 0.1^{a}	2.5 ± 0.2^{b}	2.5 ± 0.2^{b}	0.9 ± 0.1^{a}	1.8 ± 0.1	0.4 ± 0.1	3.3 ± 0.3	1.6 ± 0.2
SLA (m^{2} kg^{-1})	8.1 ± 0. 5^{b}	5.8 ± 0.4^{a}	7.8 ± 0.5	6.1 ± 0.5	10.2 ± 0.6	6.1 ± 0.4	5.4 ± 0.4	6.2 ± 0.9
Leaf Dimension (mm)	9.5 ± 0.6^{b}	2.4 ± 0.4^{a}	4.3 ± 0.5^{a}	8.1 ± 0.8^{b}	7.3 ± 0.5	11.7 ± 0.9	1.3 ± 0.1	3.8 ± 0.9
Leaf Area/Stem Area (m^{2} m^{-2})	1199 ± 109	930 ± 109	819 ± 71	1345 ± 134	938 ± 127	1459 ±166	699 ± 57	1208 ± 219
N (mg g^{-1})	23.4 ± 0.8	23.5 ± 0.6	25.5 ± 0.6^{b}	21.2 ± 0.7^{a}	27.2 ±0.8	19.7 ± 0.8	23.8 ± 0.7	23.1 ± 1.0
P (mg g^{-1})	0.71 ± 0.05	0.74 ± 0.04	0.83 ± 0.04	0.61 ± 0.04	**0.93 ± 0.06 ^{b}**	**0.49 ± 0.05^{a}**	**0.72 ± 0.06^{ab}**	**0.76 ± 0.06^{ab}**
K (mg g^{-1})	11.8 ± 0.4	9.9 ± 0.5	10.8 ± 0.4	10.9 ± 0.5	11.6 ± 0.5	11.9 ± 0.7	10.0 ± 0.7	9.8 ± 0.7
B (mg kg^{-1})	39 ± 1^{b}	27 ± 1^{a}	33 ± 1	33 ± 2	37 ± 2	40 ± 2	28 ± 1	26 ± 2
C (mg g^{-1})	522 ± 2	515 ± 4	514 ± 2	524 ± 4	513 ± 3	530 ± 3	514 ± 4	517 ± 7
Ca (mg g^{-1})	14.9 ± 0.7^{b}	8.1 ± 0.6^{a}	12.1 ± 0.7	11.2 ± 0.9	14.9 ± 0.8	15.0 ± 1.2	9.4 ± 0.9	6.7 ± 0.7
Cu (mg kg^{-1})	5.0 ± 0.3	6.6 ± 0.7	5.6 ± 0.3	5.9 ± 0.8	5.6 ± 0.6	4.5 ± 0.4	5.7 ± 0.4	7.7 ± 1.5
Fe (mg kg^{-1})	106.9 ± 5.5	160.4 ± 23.5	106.4 ± 6.8	160.9 ± 23.1	117.6 ± 8.0	96.1 ± 7.0	95.2 ±10.8	238.6 ± 45.8
Mg (mg g^{-1})	2.3 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.8 ± 0.2	2.1 ± 0.1
Mn (mg kg^{-1})	90.7 ± 10.3	169.5 ± 21.3	144.6 ± 14.3	110.8 ± 19.7	119.8 ± 16.4	61.7 ± 10.4	169.3 ± 22.7	169.7 ± 38.8
Na (mg kg^{-1})	611 ± 138^{a}	4262 ± 313^{b}	2706 ± 356	1979 ± 305	853 ± 267	370 ± 39	4559 ± 456	3910 ± 417
Zn (mg kg^{-1})	11.1 ± 0.8	17.3 ± 3.8	11.2 ± 0.7	17.3 ± 3.8	8.4 ± 0.6	13.9 ± 1.3	13.9 ± 0.9	21.4 ± 8.3
δ^{13}C (‰)	-28.0 ± 0.1	-27.9 ± 0.2	-28.0 ± 0.1	-27.9 ± 0.1	-27.9 ± 0.2	-28.1 ± 0.2	-28.2 ± 0.2	-27.6 ± 0.2
δ^{15}N (‰)	-2.4 ± 0.2	-2.0 ± 0.3	-2.3 ± 0.2	-2.1 ± 0.3	-1.9 ± 0.4	-2.9 ± 0.2	-2.7 ± 0.1	-1.1 ± 0.7
N:P	42 ± 3	36 ± 2	35 ± 2	43 ± 3	**32 ± 2^{a}**	**51 ± 4^{b}**	**38 ± 3^{ab}**	**33 ± 2^{a}**
C:N	24 ± 1	23 ± 1	21 ± 0	26 ± 1	**19 ± 1^{a}**	**28 ± 1^{b}**	**22 ± 1^{a}**	**23 ± 1^{ab}**

[‡] Heights according to Stirton, 1989 and Goldblatt and Manning, 2000.

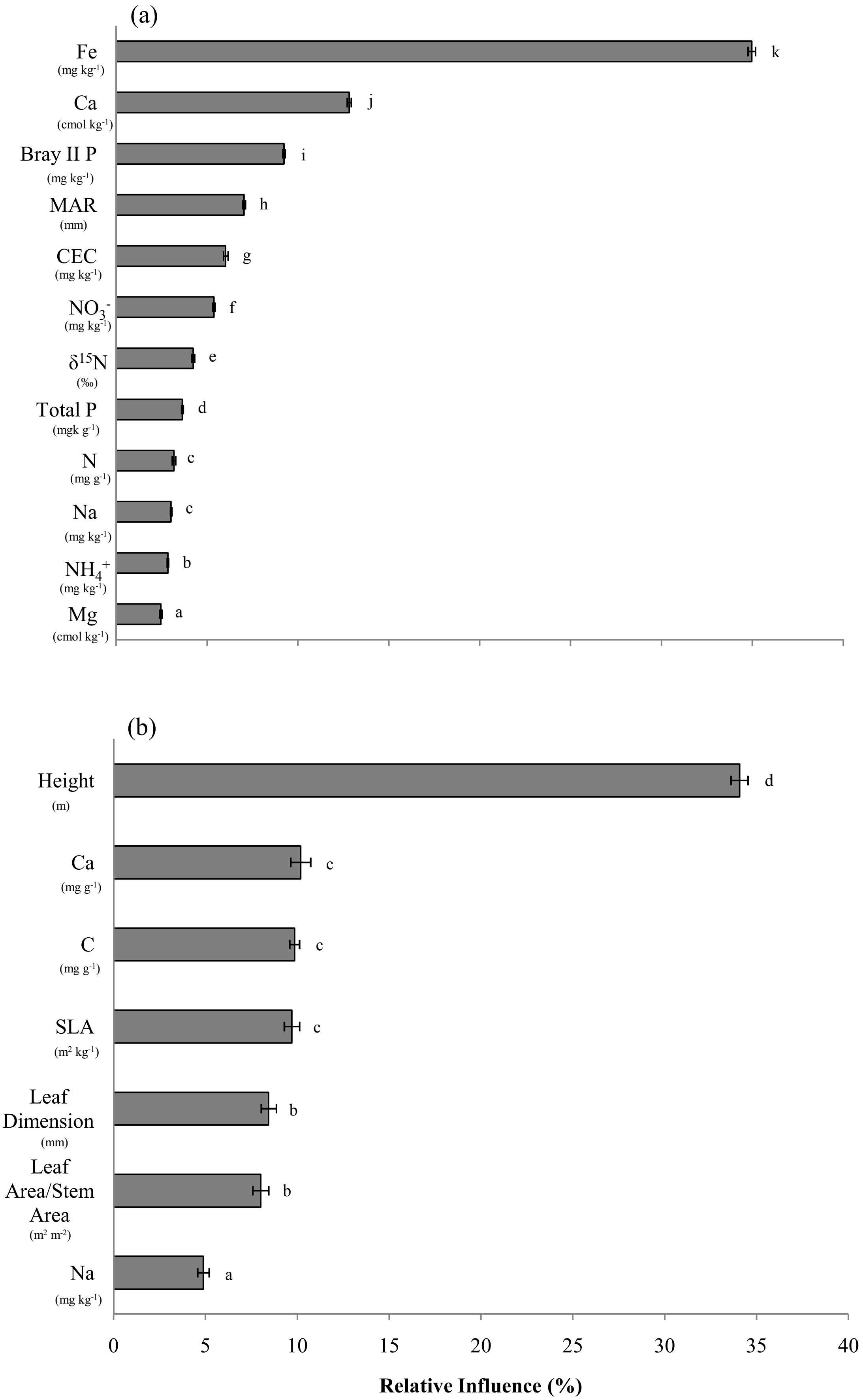

Fig. 3.3 The relative influence (% contribution to the final model) of (a) soil and (b) plant predictor properties separating Psoraleeae seeders and resprouters. Only predictors that were present in all ten of the model runs after simplification are included. Different letters following the mean ± SE denote significant differences between predictors ($P \leq 0.05$) determined from a one-way ANOVA.

Discussion

Psoralea and *Otholobium* species occupy distinct habitats and have different leaf morphologies (Stirton, 1986, 1989; Goldblatt and Manning, 2000). I found that *Otholobium* and *Psoralea* species occur on soils with dissimilar nutrient availabilities as indicated by differences in pH, [NH_4^+] and total [P] (Table 3.2). The higher pH and total [P] of *Otholobium* soils suggests greater nutrient availability, although [NH_4^+] was actually higher in *Psoralea* soils. In addition to the differences in soil nutrient availability, the lower SLA of *Psoralea* species implies that they may be better adapted to oligotrophic habitats, as has been suggested for other genera (e.g. Cunningham *et al*., 1999; Fonseca *et al*., 2000; McDonald *et al*., 2003; Niinemets and Kull, 2003; Ordonez *et al*., 2009; Poorter *et al*., 2009). Furthermore, the smaller leaves of *Psoralea* than that of *Otholobium*, would reduce nutritional investment (Givinish, 1979) and possibly assist in the acquisition of nutrients, such as P, via mass-flow (Yates *et al*., 2010). This would be particularly important in fynbos soils, as they are low in clay content and consequently P-binding capacity (Witkowski and Mitchell, 1987; Tisdale *et al*., 1995)

Seeders and resprouters also occupied distinct edaphic habitats. The higher pH, [NO_3^-], exchangeable [Ca] but lower [Fe] in seeder soils is suggestive of their higher nutrient availability, specifically P, than of resprouter soils (Table 3.2). The differences in pH and [NO_3^-] between seeder and resprouter soils were not well supported by the PIC analysis, this is probably attributable largely to the small number of contrasts (n = 6 to 7), which compromised the statistical power of the analysis. Differences in exchangeable [Ca] and [Fe] between seeder and resprouter soils showed the greatest consistency across all analyses, indicating their importance in distinguishing the habitats of seeders and resprouters. In fynbos soils, Ca and Fe are the elements to which inorganic P is predominantly bound (Witkowski and Mitchell, 1987). Hence it is likely that P is bound to Ca in seeder soils and to Fe in resprouter soils. Using a chemical speciation calculator (Visual MINTEQ v3.0) and the known soil pH,exchangeable [Ca], [Fe] and Bray II [P], there is potentially 1.6 times more soluble P in seeder than resprouter soils. Given that legumes can acquire N through symbiotic N_2-fixation and the generally low P availability of fynbos soils (Witkowski and Mitchell, 1987), the occupation of soils with a high P availability might be critical to sustaining the high nutritional demands of seeders (Pate *et al*., 1990; Hansen *et al*., 1991).

In *Otholobium*, seeders occupied soils with a higher Bray II [P] than resprouters (Table 3.2). This variation in available P could be influenced by a combination of soil parent material and rainfall. The CFR possesses a mosaic of soil types, including limestone-, granite-, sandstone- and shale-derived soils, that vary in fertility (Cowling *et al*., 1997; Rebelo *et al*., 2006). Shale- (4.0 mg P kg^{-1}) and granite-derived (1.7–3.7 mg P kg^{-1}) soils have a greater Bray II [P] than sandstone-derived (0.4–2.2 mg P kg^{-1}) soils (Witkowski and Mitchell, 1987). *Otholobium* seeders predominantly occupied shale- or granite-derived soils, whereas *Otholobium* resprouters occupied sandstone derived-soils (Table 3.2). In addition to the variation in parent material, *Otholobium* seeder habitats receive less rainfall than resprouters, and thus probably experience a lower degree of leaching and nutrient loss. The effect of lower rainfall of *Otholobium* seeder habitats is supported by the fact that the soil $\delta^{15}N$ value associated with seeder habitats was higher than that of resprouters (Table 3.2). Habitats with low rainfall are frequently associated with high $\delta^{15}N$ values compared with habitats with relatively high rainfall (Austin and Vitousek, 1998; Amundson *et al*., 2003). Consequently, variation in parent material and rainfall could be contributing to the differences in soil P availability and other nutritional properties such as CEC and [NO_3^-], between *Otholobium* regeneration strategies.

In contrast to *Otholobium*, there was no difference in available P between seeder and resprouter soils in *Psoralea* (Table 3.2). The failure to detect a comparable difference in *Psoralea* could be a consequence of the very low total [P] of *Psoralea* soils limiting the potential for any differences in available P. Additionally, there was no difference in parent material or rainfall between seeders and resprouters in *Psoralea*. Thus factors other than substrate or rainfall must explain the differences in pH, exchangeable [Ca], CEC and [Fe] observed between *Psoralea* seeder and resprouter soils. In fynbos, soil nutrient availability may be modified by the vegetation (Richards *et al*., 1997a), in a process known as ‘niche construction’(Odling-Smee *et al*., 1996; Laland *et al*., 1999). An increase in soil nutrient availability (positive niche construction) can occur via biological nutrient cycling processes, involving increased carbon deposition (e.g. litterfall) (Hobbie, 1992). Although reported litter production for fynbos vegetation is low (Mitchell *et al*., 1986), *Psoralea* seeders occupy wet habitats (Stirton, 1989; Goldblatt and Manning, 2000), which could reduce fire frequency and thus allow for the buildup of litter. Moreover, seeders which tend to be larger and occur in denser populations than resprouters (Schutte *et al*., 1995, Midgley, 1996, Table 3.3) will have a greater potential for increased organic material input. This is supported by the higher CEC of seeder soils relative to that of resprouter soils (Table 3.2) as soils with a high a organic matter content tend to have a higher CEC (Tisdale *et al*., 1995). Consequently, positive niche construction may be contributing the differences in soil nutrient availability between seeders and resprouters in *Psoralea*.

How do *Psoralea* seeders attain sufficient P to sustain their nutrient demanding strategy? In *Psoralea*, seeders and resprouters occupied soils with similar available [P] and they had similar leaf [P] (Table 3.3). However, using height as proxy for above-ground biomass, the taller seeders must have a greater whole-plant P content than the shorter resprouters (Table 3.3). Thus seeders could be accessing P from sources other than the available P measured in the surface layers (top 15 cm) of the soil. The large seeders are probably deep rooted (e.g Higgins *et al*., 1987; Canadell *et al*., 1996; Schenk and Jackson, 2002) and consequently explore a larger volume of soil. A more extensive root system could also lead to a larger mycorrhizal network that can enhance P-acquisition (Allsopp and Stock, 1993; Smith and Read, 1997). In addition to a large root system, *Psoralea* seeders have relatively small leaves compared with resprouters (Table 3.3), which may enhance nutrient acquisition via mass-flow (Yates *et al*., 2010). The use of mass-flow to acquire nutrients may be encouraged by the fact that *Psoralea* seeders occupy wet habitats. Therefore *Psoralea* seeders may be supplementing their P supply by exploring a larger volume of soil and by trading off water-use efficiency for nutrient acquisition.

Despite the lack of variation in SLA between seeders and resprouters, SLA was found to contribute to the overall separation of seeders and resprouters in the BCT analysis (Fig. 3.3b). This lack of congruence probably occurred because the linear mixed model and PIC analyze each variable separately, whereas the BCT considers all variables simultaneously. Moreover, there was large variability in SLA within seeders and resprouters (Table 1, Appendix). In contrast to SLA, leaf dimension did show a significant variation as the seeders produced smaller leaves than resprouters (Table 3.3). The potential functional significance of smaller leaves could be for enhanced nutrient acquisition as argued by Yates *et al*. (2010). However, the potential for nutrient acquisition via mass-flow is dependent on sufficient soil moisture availability. Given that *Otholobium* seeders tend to occur in arid environments compared with *Psoralea* seeders (Stirton, 1989, Table 3.2), their small leaves are unlikely to enhance nutrient acquisition. However, in addition to nutrient acquisition, small leaves may also assist in heat dissipation (Gates *et al*., 1968; Thuiller *et al*., 2004; Yates *et al*., 2010). A small leaf, with a thin boundary layer has a greater potential for convective heat loss than a large leaf with a thick boundary layer (Nobel, 2005). Hence small leaves in *Otholobium* seeders may function to enhance heat loss in their arid habitats whereas in *Psoralea* seeders they may enhance nutrient acquisition.

Given the contrasting nutritional requirements associated with seeding and resprouting, the prevalence of these strategies is likely to have shifted over time in association with the geological evolution of the CFR environment. The inferred ancestral regeneration strategy of the sampled Psoraleeae species was most likely resprouting. This inference is consistent with the findings for *Thamnochortus*, a genus in Restionaceae, whose most recent common ancestor was a resprouter that inhabited low nutrient sandstone-derived soils (Hardy and Linder, 2005) as do the Psoraleeae resproutrers. This association between the ancestral strategy and low nutrient soils corresponds with the appearance of low nutrient sandstone-derived soils (Oligocene: 33.7–23.8 Ma) before the arrival of more nutrient-rich shale- and limestone-derived soils (Miocene: 23.8–5.3 Ma) (Cowling *et al*., 2009). Thus, the ancestral link between Psoraleeae resprouters and nutrient poor soils, implies that they are probably well adapted to low P soils unlike seeders.

Conclusion

The habitat specificity of Psoraleeae seeders and resprouters in fynbos vegetation of the CFR appears to be linked to nutrient availability. Seeders occupy soils with greater nutrient availability compared with resprouters, as evidenced by the higher pH, [NO_3^-], exchangeable [Ca] and lower [Fe]. A high soil nutrient status combined with the potential enhancement of nutrient acquisition from a reduced leaf size, correlates with the high nutrient demands associated with seeders. However, the high P demands of legume seeders (> 75% of CFR legumes, Le Maitre and Midgley, 1992) may be unsustainable in fynbos soils, and thus may potentially contribute to the general lack of legume persistence during post-fire succession in fynbos vegetation of the CFR.

Chapter 4

General Discussion and Synthesis

Fabaceae (legumes) is a species-rich family that comprises an important component of the overall plant species diversity in the CFR (Goldblatt and Manning, 2000). However, unlike species in Cyperaceae, Ericaceae, Proteaceae and Restionaceae, legumes are generally unable to persist through post-fire succession in the fynbos vegetation which dominates the region (Kruger, 1979a; Hoffman *et al*., 1987; Cocks, 1994). Interestingly, over 75% of legumes in fynbos are seeders, plants which are killed by fire and which regenerate from seed, with the remaining species being resprouters (Le Maitre and Midgley, 1992; Bond and van Wilgen, 1996). In fynbos, seeders tend to be shorter-lived than co-occurring resprouters (Bond and Midgley 2003; Bond and van Wilgen 1996; Kruger and Bigalke 1984; Le Maitre and Midgley 1992; Schutte *et al*. 1995), although seeders in families such as Proteaceae, appear longer-lived than legume seeders, being able to persist for longer periods through post-fire succession. Nonetheless, the majority of fynbos legumes are short-lived seeders that are unable persist through post-fire succession.

Seeders and resprouters are not only distinguished by differences in fire response and longevity but also by patterns of resource allocation. The differences in resource allocation are analogous to r versus k-selection (Pianka, 1970), with seeders tending to invest more biomass above-ground, to grow faster, to reach reproductive maturity sooner and to produce larger amounts of seed than resprouters (Stearns, 1976; Keeley and Zedler, 1978; Kruger, 1983; Pate *et al*., 1985, 1990; Hansen *et al*., 1991; Le Maitre, 1992; Bell, 2001; Bellingham and Sparrow, 2001). These differences in resource allocation could result in contrasting nutritional requirements. For instance, in fynbos, legume seeders (2.5 ± 0.2 m) are more than double the height of resprouters (0.9 ± 0.1 m), and thus probably have not only a greater above-ground biomass but also a greater whole-plant nutrient content (Table 3.3). The differences in seeder-resprouter nutrient content may be amplified in fynbos legumes owing to their higher foliar [N] (legume = 23.5 ± 1.0, non-legume = 5.96 ± 0.47 mg g^{-1}) and [P] (legume = 0.7 ± 0.1, non-legume = 0.32 ± 0.03 mg g^{-1}) than that of non-legumes (Rundel, 1988; Table 3.3). Seed nutrient contents are similar between legume regeneration strategies as demonstrated by the seed P contents of *Otholobium fruticans* (18 μg), a resprouter, and *Otholobium hirtum* (16 μg), a seeder (Allsopp and Stock, 1995). However, higher seed production (Stirton, 1989) will result in the combined seed nutrient allocation of seeders being higher than that of resprouters. Consequently, seeders have a higher whole-plant nutrient content than resprouters, which equates to a higher nutrient demand.

The nutrient demanding strategy of legume seeders requires a high and consistent nutrient supply. Fynbos soils are generally oligotrophic, with particularly low N and P availability (Stock and Lewis, 1986; Witkowski and Mitchell, 1987; Table 3.2). Symbiotic N_2-fixation will enable legume seeders to overcome the low N availability but not low P availability. Furthermore, legumes show a significant increase in growth with P supply (Robson *et al.*, 1981; Jakobsen, 1985; Høgen-Jensen *et al.*, 2002; Figs. 2.1 and 2.2a). Thus seeders will require soils with greater P availability than resprouters. In *Otholobium*, seeders occupy soils with a higher P availability than resprouters (Table 3.2). This difference in P availability is a reflection of the different parent materials from which these soils are derived. *Otholobium* seeders occur on granite/shale-derived soils, which have a higher Bray II [P] than the sandstone-derived soils occupied by resprouters (Witkowski and Mitchell, 1987). However, not all legume seeders occupy soils with a higher P availability than resprouters, as evidenced by *Psoralea* (Table 3.2). In addition, 81% of fynbos vegetation occurs on sandstone-derived soils (Rebelo *et al.*, 2006). Hence, the high nutrient demand of legume seeders may not be generally feasible in fynbos. This is evident in the correspondence between increased legume abundance and P availability post-fire (Kruger, 1979a; Brown and Mitchell, 1986; Hoffman *et al.*, 1987; Cocks, 1994). Therefore the strategy of legume seeders may limit their prevalence to the immediate post-fire period in fynbos. In contrast, resprouters appear to be adapted to low-P soils as their association with low nutrient soils is probably the ancestral condition (Chapter 3) and they have a weak capacity to down-regulate P-uptake (Figs. 2.5a and c).

The sandstone–derived soils that fynbos vegetation occurs on are low in available P, partially because inorganic P is predominantly bound to Ca or Fe, rendering it largely insoluble (Witkowski and Mitchell, 1987). In low-P soils plants, frequently employ root adaptations such as mycorrhizae and root clusters (e.g proteoid and capillaroid) to assist in P-acquisition.

Root clusters release carboxylates and acid-phosphatases (APase) which increase P-solubility (Lambers *et al*., 2003, 2006). Fynbos legumes lack effective root adaptations (low shoot: root, root clusters, effective carboxylates) to access sparingly soluble P sources, relative to non-legumes (Chapter 2). Root clusters are common in Proteaceae and Restionaceae, but limited to *Aspalathus* and *Cyclopia* in Fabaceae (Allsopp and Stock, 1993; Spriggs, 2004; Chapter 2). Moreover, *Aspalathus* root clusters release carboxylates at lower concentrations and lower efficacy of P-solubilization than do Proteaceae (Table 2.2). However, the APase activity of *Aspalathus* cluster roots has not been assessed, which could assist them in accessing organic P (77% total P in sandstone-derived soils, Witkowski and Mitchell, 1987). For example, the crop legume *Lupinus albus* actively secretes APase (Gilbert *et al*., 1999; Wasaki *et al*., 2003), but no data are available on fynbos legumes. APase activity in fynbos Proteaceae (Hunter, 2010) combined with a lack of persistence, suggests fynbos legumes do not secrete APase. Indeed, most legumes appear to rely on mycorrhizae to enhance P-acquisition (Lamont, 1982; Allsopp and Stock, 1993; Chapter 2). Since species with root clusters incur significant advantages for P-acquisition from sparingly soluble sources over species that lack them (Lambers *et al*., 2006, 2008), non-legumes do seem better equipped for low-P soils than legumes.

Despite a lack of very effective root adaptations, mass-flow may assist legumes in P-acquisition given that the largest proportion of total P in sandstone soils, organic P, is fairly mobile (Witkowski and Mitchell, 1987; Cernusak *et al*., 2010). Several *Psoralea* species have small, pinnate leaves and inhabit wet environments (Stirton, 1989; Goldblatt and Manning, 2000; Table 3.3), both of which may encourage transpiration driven mass-flow (Cramer *et al*., 2009; Yates *et al*., 2010). The ability to acquire P via mass-flow may explain why the legume seeder, *Virgilia oroboides*, a resident of stream banks, with pinnate leaves (Goldblatt and Manning, 2000), persists for longer periods (> 40 years, Schutte *et al*., 1995) than most other legume seeders. In *Zea mays*, Barber (1995) found that only 5% of plant P was accumulated via mass-flow. However, work by H-J Hawkins and M.D Cramer (unpublished) found that sandy soils in fynbos, that have a high P mobility, mass-flow could provide > 20% of plant P in Proteaceae. Although the extent to which mass-flow contributes to the P nutrition of legumes remains to be determined, high soil moisture availability and mass-flow may be important for the long term persistence of some legumes.

Given that legumes lack root adaptations which are as effective as those of Proteaceae for accessing sparingly soluble P sources, they need to conserve the P they do acquire. Sclerophylly is a leaf trait which can enhance nutrient conservation. Sclerophyllous leaves have longer life-spans than non-sclerophyllous leaves (Reich *et al.*, 1997, 1999; Wright *et al.*, 2001, 2004; Poorter *et al.*, 2009), increasing their nutrient retention times (Escudero *et al.*, 1992; Eckstein *et al.*, 1999; Wright and Westoby, 2003). Species in oligotrophic habitats, such as those dominated by fynbos, are inclined to have a higher degree of sclerophylly compared with species in eutrophic habitats (Beadle, 1966; Cowling and Campbell, 1980; Cunningham *et al.*, 1999; Fonseca *et al.*, 2000; McDonald *et al.*, 2003; Niinemets and Kull, 2003; Ordonez *et al.*, 2009; Poorter *et al.*, 2009). Sclerophylly is frequently quantified using specific leaf area (SLA), which is negatively correlated with the degree of sclerophylly (Edwards *et al.*, 2000). The SLA of non-legumes (5.3 ± 0.3 m^2 kg^{-1}, Rundel, 1988; Wand, 1995), including Ericaceae, Proteaceae and Restionaceae is significantly lower ($\chi^2 = 8.61$, $P < 0.01$) than that of legumes (7.0 ± 0.7 m^2 kg^{-1}, Chapter 3). Thus legumes exhibit a lower degree of sclerophylly, and therefore a weaker capacity to conserve nutrients compared with non-legumes. This implies that legumes are probably not well adapted to low nutrient soils, which characterize the later stages of post-fire succession.

Although fynbos legumes might not be well adapted to the low nutrient environments that prevail during late post-fire succession, they are well adapted to exploit the early stages of post-fire succession. Firstly, legume seeds have hard coats and long life-spans (e.g > 44 years for *Aspalathus ciliaris*, van Wilgen and Forsyth, 1992; > 30 years for *Virgilia oroboides*, Philips, 1926). This longevity ensures a persistent seedbank, which is important for species whose life-spans are generally shorter than the average disturbance return time (Holmes and Newton, 2004). Secondly, legume seed germination is stimulated by heat. Jeffery *et al.* (1988) and Cocks and Stock (1997) found that peak germination in *Aspalathus*, *Podalyria*, *Psoralea* and *Virgilia*, occurred between 80°C and 100°C. Hence the ideal conditions for legume germination are during fires, where these temperatures are reached (Taylor and Kruger, 1978). Thirdly, the seeding strategy, associated with a fast growth rate and early reproduction (Bell, 2001), allows the majority of legumes to exploit fully the increase in soil P availability (Brown and Mitchell, 1986) and ensure reproductive success before soil P conditions are no longer favourable to their strategy. Therefore, the persistent seedbank, heat stimulated germination and the seeding regeneration strategy, indicate that legumes are well adapted to the early stages of post-fire succession.

A paradoxical conundrum of parts of the CFR, is the presence of alien invasive legumes such as *Acacia cyclops*, *Acacia longifolia, Acacia mearnsii* and *Acacia saligna* which, in contrast to native legumes, can dominate fynbos vegetation (Richardson *et al*., 1992). Alien *Acacia* species have high nutrient demands as they are large plants (height: 3-7 m, Goldblatt and Manning, 2000), having high growth rates (Milton, 1981; Witkowski, 1991a) and producing large numbers of seed (1100 – 7000 seeds m^{-2} $year^{-1}$; Milton and Hall, 1981). Furthermore, the SLA of *A. saligna* (6.4 m^2 kg^{-1}; Kraaij and Cramer, 1999) and *A. longifolia* (7.3 m^2 kg^{-1}; Kraaij and Cramer, 1999) are similar to those of native legumes (7.0 m^2 kg^{-1}; Table 3.3), implying that they have a weaker capacity to conserve nutrients. Owing to their lower degree of sclerophylly, alien *Acacia* species produce large quantities of nutrient rich litter (Milton, 1981, Witkowski, 1991b). It is intriguing to consider, therefore, how alien legumes are able to satisfy their P demands and be successful in fynbos, unlike native legumes?

To satisfy their P requirements, some alien *Acacia* species may occupy sites with relatively high P availability. For example, *A. cyclops* dominates the strandveld, whose soils show the highest levels of available P in the fynbos biome (Witkowski and Mitchell, 1987; Witkowski, 1994). However, a species such as *A. saligna* is prevalent in Sandplain fynbos of the Cape lowlands, which has low soil P availability (Witkowski and Mitchell, 1987; Witkowski, 1994). *A. saligna* has a more extensive root system than native legumes, which increases nutrient uptake by enlarging the soil volume explored and by increasing the number of sites for mycorrhizal colonization (Hoffman and Mitchell, 1986). Furthermore, Witkowski (1994) found that the root penetration of *A. saligna* in sand was three times faster than that of *A. cyclops* over a 30 day period, which should assist them in tapping into the water table. Greater water availability likely contributes to enhanced P-acquisition via mass-flow (Cramer *et al*., 2009). Accordingly, the ability of alien *Acacia* species to satisfy their P demands via the occupation of relatively high P soils and the use of deep, more extensive root systems and mycorrhizal networks strategies probably contributes to their success in fynbos.

Conclusion

Low soil P availability probably limits legume persistence through post-fire succession in fynbos vegetation of the CFR. The majority of legumes in the fynbos are short-lived seeders, which have a high nutrient demand. Given that legumes are able to fix N_2, high soil P availability is required to satisfy their nutritional demands. However, except for immediately post-fire, P availability in fynbos soils is low. Moreover, legumes are not well adapted to low P soils because they are unable to access sparingly soluble P sources effectively and their leaves are weakly sclerophyllous. Consequently the high nutrient demands of legume seeders in fynbos cannot be sustained long-term in the post-fire environment. Instead, legumes appear well adapted to exploit the conditions that exist immediately post fire.

Future Research

Although this study contributes to the knowledge of legume persistence in fynbos vegetation, certain areas of fynbos legume biology require further investigation. These areas include:

Legume seeder-resprouter nutrition

- Comparison of growth rates, biomass, annual seed output, foliar and seed nutrition between seeders and resprouters
- Comparisons using several genera and between sister taxa so as to ensure phylogenetic independence

P-acquisition strategies

- Acid-phosphatase activity in cluster root and non-cluster root exudates of legumes relative to non-legumes
- Determine the potential for mass-flow by measuring water loss of legumes with differing leaf sizes and habitat moisture availability
- Quantify the potential contribution mass-flow has to the P nutrition of legumes and non-legumes

Native vs alien legumes

- Contrast the P biology of native and alien legume species, with respect to nutritional requirements, acquisition strategies and availability within habitats.

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Appendix

Boosted tree analysis

Boosted tree analysis is a form of non-linear modelling that is able to detect and predict environmental patterns (Hastie *et al*., 2001; Friedman, 2002; De'ath, 2007; Elith *et al*., 2008). For example boosted tree models can be used to identify environmental variables (e.g climate, soil type) that best explain the distribution of a vegetation type and then use the set of environmental variables to predict the future or unknown distribution of the vegetation type. The advantages of boosted tree models is that they are able handle different types of predictor variables, accommodate missing data and outliers, don't require the transformation of data, fit complex non-linear relationships and automatically fit interactions between predictor variables (Friedman and Meulman, 2003; Elith *et al*., 2008).

Boosted tree analysis incorporates classification and regression trees. In boosted classification trees (BCT) the response variable is categorical whereas in boosted regression trees (BRT) the response variable is numeric, the explanatory/predictor variables in both cases can be categorical and/or numeric (De'ath and Fabricius, 2000). BCT/BRT models comprise of two components, decision trees and boosting. Decision trees are constructed by repeatedly splitting the data into two homogeneous groups to minimize predictive deviance, where the splitting point is defined by a simple rule based on a single explanatory variable (e.g rainfall, soil pH) (De'ath and Fabricus, 2000; Elith *et al*., 2008). The splitting procedure is then recursively applied to each homogenous group until an overlarge tree is grown, which is then pruned by indentifying the weakest links through cross-validation (CV) (De'ath and Fabricus, 2000; Hastie *et al*., 2001; Elith *et al*., 2008). Boosting involves the construction of many trees, where each tree is built on the previous tree but focuses on the variation that was not explained by the previous tree. The trees are then summarized into an optimized tree that reduces in predictive deviance (Hastie *et* al., 2001; Friedman, 2002; Elith *et al*., 2008).

There are two important parameters associated with BCT/BRT modelling, tree complexity (tc) and the learning/shrinkage rate (lr), both of which need to be optimized in order to reduce predictive deviance and determine the final number of trees. Tc controls the number of splits in a tree and determines whether there are any interactions between variables. Lr controls the contribution each tree has to the growing model and therefore the rate at which predictive performance increases with the total number of trees (Elith *et al.*, 2008). To help identify the optimal tc and lr, 10-fold CV is employed. 10-fold CV divides the dataset up into 10 subsets of equal size, drops out one subset and builds a tree, which is used to predict the responses from the omitted subset. This process is repeated for each subset, with the sum of deviances between subsets and the withheld data used to evaluate the predictive deviance of the model (De'ath and Fabricus, 2000; Elith *et al.*, 2008).

The performance of BCT/BRT models are assessed using predictive deviance and the discrimination as measured by the area under the receiver operator characteristic (AUC) (Hanley and Mc Neil, 1982). Predictive deviance can be derived from the training dataset (i.e the data on which the model is built), 10-fold CV or an independent dataset. The training dataset is never used because of its tendency to over-fit (Elith *et al.*, 2008). The chosen predictive deviance is expressed as a percentage of the null deviance, with the greater the deviance explained, the greater the predictability of the model. The AUC values give a measure of discrimination between observed presences and absences of the response variable. A value of 1 indicates that the model can perfectly discriminate between presences and absences, while a value of 0.5 indicates that the model cannot predict presences or absences any better than a simple random allocation of probabilities (Leathwick *et al.*, 2008).

To determine which predictor variables have the greatest influence in predicting the response variable, the relative influence of each predictor, expressed as a percentage, is calculated. The relative influence of a predictor is determined by the number of times a predictor is selected for splitting, weighted by the square improvement to the model as a result of each split and averaged over all trees (Friedman and Meulman, 2003). The higher the number, the greater the influence in predicting the response variable.

Table 1 The soil properties and rainfall (mean ± SE, n= 4) of the Psoraleeae species sampled, with their respective regeneration strategy.

Site	**Species**	**Regeneration Strategy**	**MAR** (mm)	**pH**	**Total N (mg g^{-1})**	**NH4^{+}**	**NO3 $^{-}$**	**Total P**	**Bray II P**	**Fe**
						(mg kg^{-1})				
1	*O. bracteolatum*	seeder	530	6.9±0.1	0.8±0.0	3.7±0.1	2.6±0.7	36.2±7.3	16.9±2.5	7.2±2.0
2	*O. candicans*	seeder	570	5.2±0.1	2.5±0.1	6.3±0.2	9.2±2.8	20.0±7.5	20.3±4.7	1.5±0.1
4	*O. flexuosum*	seeder	250	4.6±0.3	0.9±0.2	6.9±1.8	6.9±1.6	12.4±1.0	11.0±0.9	8.0±2.2
6	*O. hamatum*	seeder	235	5.1±0.2	1.3±0.1	5.7±0.4	17.5±5.1	9.1±0.1	6.4±1.3	5.0±1.0
9	*O. pustulatum*	seeder	355	5.0±0.2	2.4±0.2	5.2±0.4	42.2±2.6	20.1±1.1	9.8±1.7	5.4±2.2
11	*O. striatum*	seeder	285	6.2±0.2	2.5±1.0	4.8±0.6	19.1±5.4	38.4±19.2	19.7 ±5.1	5.6±2.0
14	*P. affinis*	seeder	585	3.3±0.1	1.4±0.1	7.4±0.2	1.6±0.7	6.4±0.3	2.5±0.0	5.9±0.4
15	*P. aphylla*	seeder	960	4.7±0.1	3.8±0.6	16.2±5.3	25.1±8.4	17.5±1.7	9.0±1.6	11.6±3.2
9	*P. fleta*	seeder	750	4.2±0.2	2.2±0.6	7.4±2.8	5.6±1.9	9.5±5.5	8.6±5.8	8.6±2.3
16	*P. glaucescens*	seeder	355	5.0±0.2	2.4±0.2	5.2±0.4	42.2±2.6	20.1±1.1	9.8±1.7	5.4±2.2
19	*P. pinnata*	seeder	865	4.3±0.2	4.4±0.9	13.1±2.2	40.8±5.4	11.0±3.2	7.6±2.5	43.6±26.1
20	*P. restioides*	seeder	585	3.8±0.3	2.6±0.3	7.3±3.3	20.0±12.2	3.5±0.9	2.6±0.2	5.0±1.7
3	*O. dreweae*	resprouter	585	4.6±0.1	1.8±0.2	3.0±1.1	1.7±0.4	23.2±3.4	3.6±0.6	11.8±1.0
5	*O. fruticans*	resprouter	960	4.6±0.1	1.6±0.2	4.7±1.0	8.0±0.1	53.8±4.4	3.2±0.5	12.6±2.6
7	*O. lanceolatum*	resprouter	545	4.7±0.0	1.7±0.1	4.9±1.3	2.6±1.2	48.0±3.6	3.5±0.7	11.8±1.4
8	*O. obliquum*	resprouter	985	4.1±0.1	1.3±0.1	5.5±0.2	6.2±2.2	6.2±0.3	3.2±0.3	17.9±1.7
10	*O. rotundifolium*	resprouter	1330	4.1±0.1	0.9±0.1	5.4±0.5	3.3±3.3	3.4±0.3	3.8±0.2	18.2±2.4
12	*O. thomii*	resprouter	585	4.4±0.1	0.9±0.1	3.1±0.2	3.8±1.4	12.1±0.9	3.0±0.5	10.4±1.1
13	*P. aculeata*	resprouter	780	3.2±0.2	2.9±0.4	13.8±3.6	3.3±0.8	6.7±0.3	4.0±0.6	14.8±7.4
17	*P. imbricata*	resprouter	960	5.0±0.0	2.2±0.2	5.1±0.4	7.4±1.1	19.8±2.6	15.4±3.0	19.5±3.7
18	*P. oreopola*	resprouter	395	4.1±0.1	0.6±0.1	4.2±0.3	10.8±1.1	5.6±1.2	3.4±0.7	17.0±1.5
21	*P. rigidula*	resprouter	1200	3.4±0.1	1.2±0.1	7.2±1.4	4.8±0.9	3.0±0.4	4.3±1.7	13.5±1.6
22	*P. verrucosa*	resprouter	395	3.7±0.1	1.6±0.2	55.0±11.0	17.7±5.4	15.3±4.8	14.5±3.5	83.5±24.8

Table 1 cont.

Site	**Species**	**Regeneration Strategy**	**C (mg g^{-1})**	**Ca**	**K**	**Mg**	**Na**	**CEC**
						(cmol kg^{-1})		
1	*O. bracteolatum*	seeder	9.3±1.6	5.9±1.3	0.17±0.08	0.4±0.1	0.05±0.00	6.5±1.4
2	*O. candicans*	seeder	45.3±2.3	6.3±0.7	0.27±0.02	2.8±0.2	0.13±0.00	10.5±0.8
4	*O. flexuosum*	seeder	10.3±2.6	2.0±0.4	0.39±0.11	1.1±0.3	0.09±0.01	4.3±0.4
6	*O. hamatum*	seeder	21.4±1.9	4.2±0.6	0.38±0.02	2.0±0.4	0.13±0.04	7.3±0.9
9	*O. pustulatum*	seeder	33.3±3.8	6.1±0.6	0.86±0.09	3.0±0.5	0.24±0.02	11.2±0.9
11	*O. striatum*	seeder	28.4±12.2	8.2±2.9	0.49±0.13	3.1±0.5	0.29±0.11	12.1±3.2
14	*P. affinis*	seeder	32.4±2.4	1.7±0.1	0.15±0.04	0.9±0.1	0.17±0.03	6.0±0.3
15	*P. aphylla*	seeder	54.4±6.6	4.4±0.6	0.77±0.08	7.0±0.9	1.15±0.23	15.2±1.6
9	*P. fleta*	seeder	37.1±8.2	3.8±1.5	0.09±0.04	0.9±0.3	0.11±0.04	6.3±1.8
16	*P. glaucescens*	seeder	33.3±3.8	6.1±0.6	0.86±0.09	3.0±0.5	0.24±0.02	11.2±0.9
19	*P. pinnata*	seeder	70.5±17.2	4.7±1.5	0.33±0.07	3.9±1.4	0.83±0.15	12.3±3.0
20	*P. restioides*	seeder	64.5±8.8	5.9±1.5	0.27±0.04	2.4±0.4	0.45±0.01	12.5±1.7
3	*O. dreweae*	resprouter	44.6±6.5	3.5±0.5	0.31±0.02	2.0±0.1	0.24±0.02	7.7±0.8
5	*O. fruticans*	resprouter	27.5±3.7	3.6±0.6	0.40±0.03	1.5±0.2	0.16±0.02	7.1±0.7
7	*O. lanceolatum*	resprouter	30.8±2.1	2.1±0.1	0.27±0.01	2.6±0.3	0.46±0.07	6.9±0.5
8	*O. obliquum*	resprouter	21.6±1.0	0.7±0.1	0.23±0.07	0.3±0.0	0.06±0.00	2.6±0.5
10	*O. rotundifolium*	resprouter	17.7±1.3	1.5±0.3	0.20±0.03	0.7±0.1	0.13±0.02	3.7±0.5
12	*O. thomii*	resprouter	18.9±2.1	1.8±0.2	0.30±0.02	1.3±0.2	0.20±0.02	4.9±0.5
13	*P. aculeata*	resprouter	64.7±10.8	2.6±0.3	0.26±0.04	1.7±0.2	0.24±0.05	9.2±0.8
17	*P. imbricata*	resprouter	41.1±4.9	3.7±0.3	0.42±0.01	1.8±0.2	0.33±0.03	7.4±0.5
18	*P. oreopola*	resprouter	8.4±0.7	1.0±0.1	0.05±0.01	0.4±0.1	0.07±0.01	2.2±0.2
21	*P. rigidula*	resprouter	24.3±2.4	1.5±0.2	0.08±0.01	0.7±0.1	0.07±0.01	4.4±0.3
22	*P. verrucosa*	resprouter	18.9±5.9	0.6±0.2	0.12±0.03	0.5±0.1	0.14±0.06	3.2±0.5

Table 1 cont.

Site	**Species**	**Regeneratior Strategy**	**δ^{15}N (‰)**
1	*O. bracteolatum*	seeder	3.8±0.2
2	*O. candicans*	seeder	2.7±0.1
4	*O. flexuosum*	seeder	6.3±0.6
6	*O. hamatum*	seeder	4.3±0.2
9	*O. pustulatum*	seeder	3.2±0.2
11	*O. striatum*	seeder	4.6±0.1
14	*P. affinis*	seeder	1.6±0.2
15	*P. aphylla*	seeder	2.2±0.1
9	*P. fleta*	seeder	1.4±0.3
16	*P. glaucescens*	seeder	3.2±0.2
19	*P. pinnata*	seeder	2.4±0.9
20	*P. restioides*	seeder	1.4±0.0
3	*O. dreweae*	resprouter	0.03±0.1
5	*O. fruticans*	resprouter	2.2±0.4
7	*O. lanceolatum*	resprouter	2.6±0.1
8	*O. obliquum*	resprouter	1.0±0.1
10	*O. rotundifolium*	resprouter	-0.2±0.6
12	*O. thomii*	resprouter	1.5±0.2
13	*P. aculeata*	resprouter	1.6±0.3
17	*P. imbricata*	resprouter	2.7±0.3
18	*P. oreopola*	resprouter	5.2±0.1
21	*P. rigidula*	resprouter	-0.1±0.3
22	*P. verrucosa*	resprouter	2.6±0.3

Table 2 The foliar nutrition and plant traits (mean ± SE, n = 5) of the Psoraleeae species sampled, with their respective regeneration strategies.

Site	Species	Regeneration Strategy	Height (m)	SLA (m^2 kg^{-1})	Leaf Dimension (mm)	N	P	K	C	Ca	Mg
						(mg g^{-1})					
1	*O. bracteolatum*	seeder	2	10.1±0.9	9.12±0.42	20.2±0.8	0.82±0.07	8.6±0.9	516±6	18.9±1.5	1.8±0.1
2	*O. candicans*	seeder	1	7.3±0.1	3.60±0.28	32.0±0.4	1.04±0.05	9.7±0.8	514±4	13.1±1.2	2.8±0.5
4	*O. flexuosum*	seeder	2.5	15.3±1.9	8.28±0.56	30.2±2.0	1.04±0.09	13.1±0.6	500±5	16.3±2.5	2.8±0.3
6	*O. hamatum*	seeder	1.5	8.2±0.5	5.36±0.39	26.5±0.6	0.66±0.04	13.1±0.9	516±4	16.4±1.5	2.0±0.3
9	*O. pustulatum*	seeder	1	8.8±0.9	5.60±0.21	26.4±1.0	0.58±0.04	11.3±0.3	521±13	10.9±0.7	2.4±0.1
11	*O. striatum*	seeder	2.5	11.5±0.6	11.92±0.67	28.0±1.3	1.44±0.09	13.8±1.5	514±5	13.6±1.8	2.3±0.1
14	*P. affinis*	seeder	3	7.7±0.2	1.14±0.06	25.8±2.4	0.60±0.11	8.0±1.4	536±3	9.0±1.3	2.8±0.4
15	*P. aphylla*	seeder	4	2.9±0.1	1.84±0.05	23.2±1.0	0.94±0.17	12.1±2.2	505±5	6.9±0.4	3.6±0.4
9	*P. fleta*	seeder	6	5.1±0.4	1.10±0.02	27.1±0.9	1.06±0.14	8.0±0.5	531±5	9.5±0.6	2.7±0.1
16	*P. glaucescens*	seeder	2	3.5±0.3	1.30±0.05	21.2±0.8	0.56±0.05	11.4±0.8	529±5	5.3±0.6	2.2±0.2
19	*P. pinnata*	seeder	4	7.5±0.5	1.08±0.03	25.7±1.5	0.64±0.09	6.0±0.4	500±2	6.9±0.7	3.8±0.3
20	*P. restioides*	seeder	0.5	5.7±0.5	1.07±0.03	19.9±0.6	0.54±0.06	14.3±0.8	483±-6	18.7±0.8	1.5±0.1
8	*O. dreweae*	resprouter	0.15	7.0±0.4	17.48±1.07	26.5±1.1	0.96±0.04	15.8±1.0	517±2	10.8 ±1.3	3.5±0.3
12	*O. fruticans*	resprouter	0.4	7.2±1.4	7.92±0.51	23.3±0.5	0.74±0.02	13.2±1.1	542±4	13.0±1.5	2.1±0.2
10	*O. lanceolatum*	resprouter	0.06	4.1±0.1	6.32±0.39	19.9±0.6	0.40±0.03	8.4±0.6	535±3	14.7±0.6	3.3±0.4
3	*O. obliquum*	resprouter	1	8.7±0.6	8.52±0.44	19.3±0.7	0.40±0.03	12.5±0.7	544±6	11.2±2.1	2.0±0.4
5	*O. rotundifolium*	resprouter	0.2	6.0±0.1	12.88±1.21	15.0±0.3	0.24±0.02	14.3±1.3	520±3	12.6±0.6	1.2±0.3
7	*O. thomii*	resprouter	0.4	3.4±0.2	17.28±0.79	14.1±0.3	0.18±0.02	7.5±1.0	520±3	27.6±2.6	1.8±0.2
13	*P. aculeata*	resprouter	2	4.3±0.2	1.75±0.10	25.4±0.3	0.96±0.05	12.3±1.2	559±3	4.3±0.6	1.6±0.2
17	*P. imbricata*	resprouter	0.5	14.4±0.8	12.32±0.48	21.8±0.7	0.52±0.06	10.0±0.6	469±12	12.3±0.6	3.0±0.1
18	*P. oreopola*	resprouter	3	4.0±0.3	1.01±0.02	22.2±0.6	0.80±0.09	5.2±0.5	506±4	8.0±0.5	2.4±0.2
21	*P. rigidula*	resprouter	0.5	3.4±1.2	1.22±0.03	15.8±0.3	0.44±0.02	8.4±0.5	506±2	3.8±0.4	1.9±0.2
22	*P. verrucosa*	resprouter	2	4.8±0.2	2.90±0.19	30.3±1.8	1.08±0.17	12.8±1.6	545±6	4.8±0.6	1.8±0.2

Table 2 cont.

Site	Species	Regeneration Strategy	Na	Mn	Fe	Cu	Zn	B	N:P	C:N	δ^{13}C (‰)	δ^{15}N (‰)
			(mg kg^{-1})									
1	*O. bracteolatum*	seeder	3958±451	25±3	56±5	1.0±0.3	3.0±0.0	43±3	26±3	26±1	-27.5±0.3	-3.7±0.1
2	*O. candicans*	seeder	217±13	141±9	121±5	6.0±0.5	11.6±1.2	35±1	31±1	16±0	-29.4±0.1	-3.5±0.2
4	*O. flexuosum*	seeder	345±31	289±24	189±13	6.6±0.9	9.2±1.2	36±2	29±1	17±1	-27.5±0.1	-2.7±0.8
6	*O. hamatum*	seeder	172±9	66±9	115±17	9.4±1.3	10.0±0.7	29±3	41±2	20±1	-27.3±0.4	-2.4±0.8
9	*O. pustulatum*	seeder	122±5	107±10	114±8	6.0±0.6	7.2±0.4	49±5	46±3	20±0	-27.7±0.2	-3.1±0.2
11	*O. striatum*	seeder	301±59	91±19	110±2	4.4±0.7	9.2±1.0	33±1	20±1	19±1	-27.8±0.4	-1.3±0.3
14	*P. affinis*	seeder	5433±525	215±45	77±5	3.8±0.4	16.6±3.5	32±3	50±10	22±2	-28.8±0.3	-2.3±0.1
15	*P. aphylla*	seeder	6905±741	71±12	63±5	6.2±0.7	13.4±2.9	24±1	28±4	22±1	-27.4±0.1	-2.7±0.1
9	*P. fleta*	seeder	3251±197	305±37	77±2	7.2±1.0	13.0±1.1	28±2	27±2	20±1	-27.4±0.3	-2.6±0.1
16	*P. glaucescens*	seeder	1293±57	72±21	72±3	6.2±0.4	11.0±0.8	21±2	39±3	25±1	-27.0±0.5	-2.6±0.1
19	*P. pinnata*	seeder	7602±648	294±38	121±8	7.4±0.5	11.6±0.4	35±1	44±6	20±1	-29.1±0.2	-2.8±0.2
20	*P. restioides*	seeder	2869±278	59±10	162±55	3.4±0.2	18.0±1.9	29±1	39±5	24±1	-29.5±0.2	-3.2±0.1
8	*O. dreweae*	resprouter	346±29	12±2	94±9	4.0±0.3	16.6±1.3	25±2	28±1	20±1	-28.5±0.2	-1.6±0.1
12	*O. fruticans*	resprouter	797±24	67±8	133±25	7.6±0.9	19.4±1.9	51±1	32±1	23±0	-29.7±0.3	-2.7±0.2
10	*O. lanceolatum*	resprouter	306±37	60±6	76±9	3.6±0.9	5.8±0.5	30±1	51±5	27±1	-27.2±0.2	-3.9±0.3
3	*O. obliquum*	resprouter	293±82	176±6	73±5	4.4±0.4	12.2±2.9	47±3	49±3	28±1	-27.6±0.3	-3.1±0.3
5	*O. rotundifolium*	resprouter	233±27	25±4	137±4	3.0±0.5	9.2±1.7	40±5	65±6	35±1	-27.2±0.3	-3.5±0.1
7	*O. thomii*	resprouter	242±21	30±6	64±5	4.2±1.1	20.2±3.3	48±6	83±11	37±1	-28.5±0.1	-2.8±0.2
13	*P. aculeata*	resprouter	5647±325	30±2	57±2	5.0±0.7	5.6±0.4	30±2	27±2	22±0	-27.1±0.2	-2.7±0.0
17	*P. imbricata*	resprouter	1969±118	42±3	520±118	8.6±0.2	15.4±2.1	35±2	44±4	22±0	-29.1±0.1	-2.3±0.2
18	*P. oreopola*	resprouter	6559±354	146±26	181±63	4.2±0.4	48.0±42.3	25±2	29±2	23±1	-26.9±0.2	-3.0±0.2
21	*P. rigidula*	resprouter	2258±361	145±50	45±3	13.2±7.5	23.6±3.9	20±2	36±2	32±1	-28.4±0.1	-2.8±0.2
22	*P. verrucosa*	resprouter	3117±737	485±89	398±35	7.4±0.8	14.4±1.3	17±1	30±4	18±1	-26.7±0.4	5.4±0.4