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UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

UFMG

ROBERTA DAYRELL DE LIMA CAMPOS

REGENERATION IN OLD CLIMATICALLY-BUFFERED INFERTILE LANDSCAPES

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais – UFMG, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal, no âmbito do Acordo de Cotutela firmado entre a UFMG e a University of Western Australia.

Área de Concentração Fisiologia Vegetal e Ecologia

BELO HORIZONTE – MG
2020

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Regeneration in old climatically-buffered infertile landscapes

Roberta Dayrell de Lima Campos

BSc Biological Sciences, MSc Plant Physiology

This thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia and The Universidade Federal de Minas Gerais

School of Biological Sciences
2020

THESIS DECLARATION

I, Roberta Dayrell de Lima Campos, certify that:

This thesis has been substantially accomplished during enrolment in this degree.

This thesis contains material, which has been submitted for the award of a cotutelle degree in my name, at the Universidade Federal de Minas Gerais.

In the future, no part of this thesis will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and, where applicable, any partner institution responsible for the joint-award of this degree.

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ABSTRACT

Old, climatically-buffered, infertile landscapes (OCBILs) occur in at least 12 out of 35 terrestrial biodiversity hotspots. Despite having a scattered global distribution, plant communities in OCBILs show clear similarities in structural and functional aspects. Soil infertility, especially severe phosphorus (P) deficiency, is a strong environmental filter that leads to a clear predominance of nutrient-conserving, slow-growing strategies among OCBIL plant species. Although associations between P-impoverished habitats and traits of adult individuals are well known, the particularities of regenerative phases, such as seedlings, juveniles and resprouts, are rarely investigated, limiting our understanding of community assembly and species distribution. In this thesis, I studied the ecophysiological strategies of regeneration in two OCBILs, the *campo rupestre* in Brazil and the *kwongan* in Southwest Australia, from a community perspective.

In the first study (Chapter 2), I investigated how ontogenetic shifts in plant ecological strategies in the *campo rupestre* compare with those of a productive nearby habitat. I measured key leaf functional traits and calculated scores for competitor (C), stress-tolerator (S), ruderal (R) strategies of the CSR system for juveniles of conspecific adults of species naturally occurring either in unproductive (*campo rupestre*) or more productive (forest) habitats. Juveniles exhibited a more R-selected strategy in both habitats when compared with adults, but were not necessarily "R" compared to the whole flora spectrum. Strategies of *campo rupestre* juveniles were more S-selected and less R-selected than forest juveniles, suggesting that a functional habitat-dependent specialisation is the main driver of selection for ecological strategy in juveniles, despite the remarkable ontogenetic shifts in ecological strategies.

In order to assess shifts in P-use strategies in post-fire regeneration forms, *i.e.* resprouts (burnt adults) and seedlings, I studied 10 species of three dominant families, Fabaceae, Myrtaceae and Proteaceae, from burnt and unburnt *kwongan* sites (Chapter 3). I assessed differences in leaf total P, inorganic P (Pi) and organic P (Po) concentrations among regeneration forms, and their association with soil P availability, seed P content and other plant ecophysiological traits. Seedlings had greater leaf total phosphorus concentration, [P], than conspecific unburnt adults, mostly due to a greater leaf [Pi]. Proteaceae seedlings had remarkably high concentrations of leaf total P, Pi and organic P fractions, associated with a higher seed P content. Burnt adults exhibited a short-term increase in leaf total [P], but burnt and unburnt adults had similar leaf total [P] one year after a fire, when soil [Pi] was still increased at the burnt site. This study revealed the spectrum of variation in P-use and -allocation strategies within the community, and demonstrated the singular strategies of Proteaceae seedlings from Southwest Australia.

In the third study (Chapter 4), I aimed to characterise main attributes associated with environmental heterogeneity in ferruginous *campo rupestre* (i.e. established in ironstone outcrops) and to test whether seedling survival, growth and abundance, and adult species richness were influenced by specific environmental conditions. I surveyed microhabitats with the presence of each of two dominant shrubs, *Mimosa calodendron* (Fabaceae) and *Lychnophora pinaster* (Asteraceae), and microhabitats lacking both species. Plots associated with the two shrubs exhibited more developed substrates than those lacking these plants, and had greater seedling abundance and adult richness. Larger crown area of the dominant shrubs and understorey plant cover were negatively associated with species richness and seedling abundance, respectively. Growth, but not seedling survival, was predicted by some of the measured attributes. This study showed that regeneration from seed in ferruginous *campo rupestre* is marked by complex spatial heterogeneity, driven by abiotic and biotic factors, which helps to understand the community structure.

Finally, I discussed particularities of OCBILs that preclude landscape-scale seed-based restoration of degraded areas. Issues related to seed sourcing, seed quality/availability, and dormancy-breaking comprise relevant hurdles that limit the use of native seeds for restoration (Chapter 5). I also presented some alternatives for overcoming these challenges, and highlighted the need for investments in restoring those exceptionally-biodiverse landscapes.

Overall, my studies revealed that OCBILs are marked by limited and slow regeneration from seeds, due to restricted seed availability, lack of suitable sites for germination and seedling establishment, and inherently slow growth of seedlings. Although I unveiled general patterns for OCBILs, spatial heterogeneity and different species strategies within communities play an important role in their structuring, contributing to species coexistence and the remarkable biodiversity in these systems. I surmise that the slow regeneration was selected in communities mainly as a response to P-impoverished soils, combined with long-term climatic and geological stability.

Keywords: OCBIL; ontogenetic shift; phosphorus allocation; seedling; spatial heterogeneity

RESUMO

Paisagens antigas, climaticamente tamponadas e pobres em nutrientes (OCBILs, do acrônimo em inglês de *old, climatically-buffered, infertile landscapes*) ocorrem em pelo menos 12 dos 35 *hotspots* de biodiversidade terrestres. Apesar de possuírem distribuição global disjunta, as comunidades de plantas nos diversos OCBILs mostram claras semelhanças em aspectos estruturais e funcionais. A infertilidade de solo, especialmente a acentuada deficiência em fósforo (P), é um forte filtro ambiental que leva à clara predominância de estratégias de conservação de nutrientes e crescimento lento entre as espécies de plantas de OCBILs. Apesar de associações entre habitats empobrecidos em P e atributos de indivíduos adultos serem bem conhecidas, as particularidades das fases regenerativas, tais como plântula, juvenil e rebrota, são raramente investigadas, o que limita nosso entendimento a respeito da organização de comunidades e da distribuição de espécies. Nesta tese, estudei as estratégias ecofisiológicas de regeneração em dois OCBILs, o campo rupestre no Brasil e o *kwongan* no Sudoeste da Austrália, a partir de uma perspectiva de comunidade.

No primeiro estudo (capítulo 2), investiguei como mudanças ontogenéticas em estratégias ecológicas de plantas no campo rupestre se comparam àquelas de paisagens produtivas. Mensurei/estimei atributos funcionais-chave de folhas e calculei as pontuações das estratégias competidora (C), estresse-tolerante (S) e ruderal (R) do sistema CSR para juvenis e adultos coespecíficos de espécies naturalmente presentes em habitats improdutivos (campo rupestre) e de outras presentes nos produtivos (capão de mata). Juvenis exibiram estratégia mais "R" em ambos habitats quando comparados a adultos, mas não foram necessariamente "R" comparados com a flora global. As estratégias de juvenis no campo rupestre foram mais "S" e menos "R" do que aquelas de juvenis na floresta, o que indica que a especialização funcional habitat-dependente é a principal força de seleção para estratégias ecológicas em juvenis, apesar das pronunciadas mudanças ontogenéticas em estratégias ecológicas.

Para avaliar as mudanças de estratégia de uso de P em formas de regeneração pós-queimada, i.e., rebrota (adultos queimados) e plântulas, estudei dez espécies de três famílias dominantes, Fabaceae, Myrtaceae e Proteaceae, em locais queimados e não queimados do *kwongan* (Capítulo 3). Avaliei as diferenças em P foliar total, P foliar inorgânico (Pi) e frações foliares de P orgânico entre formas regenerativas, e a associação entre estas e a disponibilidade de P no solo, a quantidade de P nas sementes e outros atributos ecofisiológicos das plantas. Plântulas apresentaram a maior [P] total nas folhas do que adultos coespecíficos não queimados, principalmente devido a maiores [Pi]. Plântulas da família Proteaceae tiveram concentrações especialmente altas de P total, Pi e frações de P orgânico nas folhas, associadas a um maior conteúdo de P nas sementes. Adultos queimados exibiram aumento de [P] foliar total a curto-prazo, mas adultos queimados e não queimados apresentaram [P] foliar total semelhantes um ano após a queimada, quando [Pi] do solo encontrava-se ainda aumentado no local da queimada. Este estudo revelou o espectro de variação das estratégias de uso e alocação de P na comunidade, e demonstrou a singularidade das estratégias de plântulas da família Proteaceae do sudoeste australiano.

No terceiro estudo (Capítulo 4), tive como objetivo caracterizar os principais atributos associados a heterogeneidade ambiental em campo rupestre ferruginoso (sobre canga) e testar se a sobrevivência, crescimento e abundância de plântulas e a riqueza de espécie de indivíduos adultos são influenciados por condições ambientais. Amostrei microhabitats com a presença de cada um dos dois arbustos dominantes (*Mimosa calodendon* e *Lychnophora pinaster*) e microhabitats desprovido de ambas espécies. Parcelas associadas aos dois arbustos exibiram substratos mais desenvolvidos do que aqueles desprovidos dessas plantas, e maior abundância de plântulas e riqueza de adultos. A área da copa dos arbustos dominantes e a cobertura vegetal abaixo deles estão negativamente associadas à riqueza de espécies e à abundância de plântulas, respectivamente. O crescimento de plântulas, mas não a sobrevivência, foi explicado por meio

dos atributos mensurados. Este estudo revelou que a regeneração de sementes em campo rupestre ferruginoso é marcada pela complexa heterogeneidade espacial influenciada por fatores abióticos e bióticos, que ajuda a compreender a estrutura da comunidade.

Por último, discuti particularidades exibidas por OCBILs que inibem a restauração de áreas degradadas baseada em sementes na escala de paisagem. Questões relacionados ao fornecimento, qualidade e disponibilidade de sementes, e quebra de dormência abrangem dificuldades relevantes que limitam o uso de sementes nativas para restauração. Além disso, apresentei algumas alternativas para transpor tais desafios e destaquei a necessidade de investir na restauração dessas paisagens excepcionalmente biodiversas.

Em termos gerais, meus estudos revelam que OCBILs são marcados pela lenta e limitada regeneração por meio de sementes, devido à disponibilidade restrita de sementes, à escassez de microhabitats adequados para germinação e estabelecimento de plântulas e ao crescimento inerentemente lento dos indivíduos. Apesar de ter encontrado padrões gerais para OCBILs, a heterogeneidade espacial e diferentes estratégias de espécies dentro da comunidade desempenharam um papel importante na sua estruturação, contribuindo para a coexistência de espécies e a excepcional biodiversidade de tais sistemas. Por fim, infiro que a lenta regeneração em comunidades foi selecionada principalmente em resposta a solos empobrecidos em P em conjunto com a estabilidade climática e geológica de longo prazo.

Palavras-chave: alocação de fósforo; heterogeneidade espacial; mudança ontogenética; OCBIL; plântula

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AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains work that has been published and prepared for publication.

Details of the work:
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Chapter 2

Student contribution to work:
R.L.C.D. and F.A.O.S. conceived the idea, with inputs from S.P. R.L.C.D. and A.J.A. designed and conducted fieldwork. P.B.M. identified the species. R.L.C.D., D.N. and S.P. analysed the data. R.L.D.C. led the writing of the manuscript. F.A.O.S., S.P., D.N. and H.L. provided critical feedback to data interpretation and drafting of the manuscript. All authors made intellectual contributions and provided essential feedback.

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R.L.C.D. and H.L. conceived the idea. R.L.C.D., K.R. and H.L designed and conducted fieldwork. R.L.C.D. analysed the data and led the writing of the manuscript. F.A.O.S., K.R. and H.L. provided critical feedback to data interpretation and drafting of the manuscript.

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R.L.C.D. and F.A.O.S. conceived the idea and designed fieldwork. R.L.C.D. and L.F.F. conducted fieldwork. R.L.C.D. analysed the data and led the writing of the manuscript. L.F.F., E.V., H.L. and F.A.O.S. provided critical feedback to data interpretation and drafting of the manuscript.

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ADDITIONAL PUBLICATIONS DURING PhD

Peer reviewed papers:

Dayrell RLC, Garcia QS, Negreiros D, Baskin CC, Baskin JM, Silveira FAO (2017) Phylogeny strongly drives seed dormancy and quality in a climatically-buffered hotspot for plant endemism. *Annals of Botany*, 119:267-277. **(Master thesis).**

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Chapter 1

General Introduction

Terrestrial plant species exhibit a suite of traits that enable or prevent their occurrence under specific sets of environmental conditions (Keddy 1992; Grime 2006). Viable combinations of traits reflect underlying universal trade-offs in functioning that affect fitness, species distribution and community assembly (Lambers & Poorter 1992; Grime *et al.* 1997; Wright, Reich, *et al.* 2004; Chave *et al.* 2009; Reich 2014; Díaz *et al.* 2016), and can be interpreted as "strategies" (Grime & Pierce 2012). Thus, a plant 'strategy' determines the pattern of resource allocation among competing functions within the inherent capacities of an individual that influences fitness across contrasting environmental conditions.

Values of functional traits may change during the life of the plant such as developmental stages (Gatsuk *et al.* 1980; Farnsworth 2004) and may vary in the adult plant, e.g., as a result of major biomass loss after a fire (Christensen 1977; Durán *et al.* 2010). These changes are expected to exhibit a particular combination of traits that enables individuals to pass a set of filters and survive in their natural habitat. Plant ecologists have made great progress in identifying associations between environmental conditions and traits of adult individuals (Garnier *et al.* 2016). There has been, however, less progress in characterising suites of regeneration traits and trait trade-offs, which differentiate plant ecological strategies in the regeneration phases of most ecosystems, and disentangling their contribution to ecological processes, community assembly and species distribution (Grubb 1977; Paine *et al.* 2015; Larson & Funk 2016a). Tropical rainforests and a few temperate ecosystems are notable exceptions in which regeneration processes are better understood (e.g., Kahmen & Poschlod

2004; Poorter & Bongers 2006; Poorter & Markesteijn 2007; Kitajima *et al.* 2013; Larson & Funk 2016b).

Regeneration niche

The regeneration niche can be defined as the species' requirements to replace a mature individual with a new one with a size sufficient for reproduction (Grubb 1977). A chain of events – such as seed dispersal, germination, seedling establishment and growth – creates the patterns of plant recruitment, shaping regenerative processes of each species and driving community assembly (Fig. 1; Schupp & Fuentes 1995). Seedlings and young juveniles are particularly vulnerable to stress factors, and exhibit high mortality across different ecosystems (Stearns 1976; Harms & Dalling 1997; Moles & Westoby 2004). The interaction between traits of these early life stages and their surrounding environment is therefore crucial to determine the fate of new recruits, and has a long-lasting influence in the form and shape of adult plants (Poorter 2007).

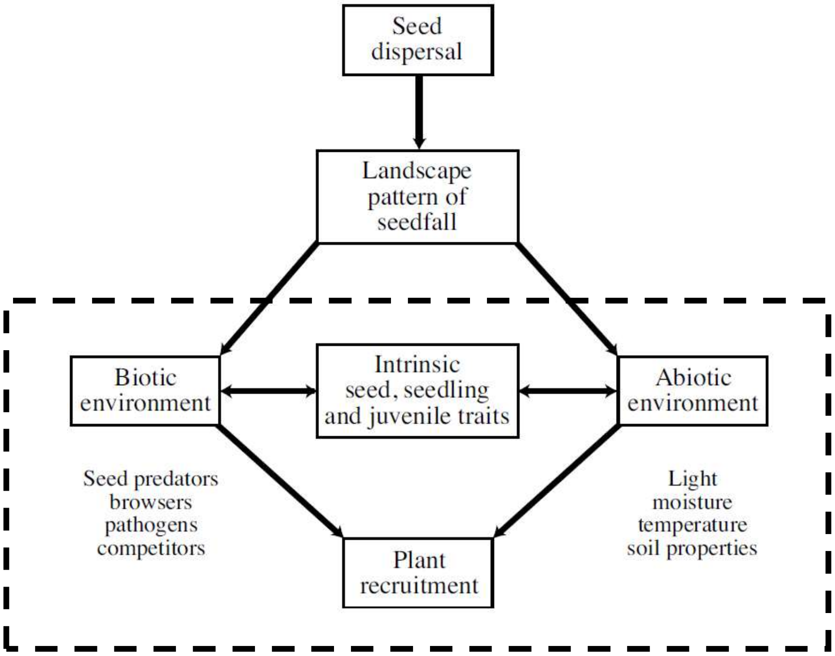

Figure 1. Flow diagram illustrating the main factors determining patterns of plant recruitment.

Seed dispersal determines the placement of seeds in the landscape. Each location possesses certain biotic and abiotic characteristics that influence the probability of the seed to germinate and survive to produce a new adult. Intrinsic seed, seedling and juvenile traits of each species determine the outcome of the interactions between the new recruit and its environments. The dashed box contains the interactions investigated in this thesis. Adapted from Schupp & Fuentes 1995.

Mortality risk of early life stages is especially high in environments where stress (such as drought and shade) is prevalent (Facelli 2008). In these ecosystems, seed dormancy plays a crucial role in synchronizing seedling emergence to periods favourable for seedling establishment (Donohue *et al.* 2010; Baskin & Baskin 2014), the most critical phase in a plant's life cycle (Harper 1977). Large seed size constitutes another feature for successful seedling establishment in stressful environments, as a larger amount of reserves allows for spare resources to be mobilised during periods of negative carbon balance and nutrient scarcity (Barrow 1977; Kidson & Westoby 2000). However, seed dormancy and large seed size are not enough to minimise the risk of seedling death in environments where stress is chronic or favourable conditions are episodic and unpredictable (Facelli 2008). Under these specific circumstances, additional ecological mechanisms are important to enhance the chances of successful seedling establishment (Grime & Hillier 2000).

Seedling and juvenile tolerance to shade and responses to light are well-known to determine gap dynamics and ecological succession in dense forests (Bazzaz 1979). Shade-intolerant species are present in understories of these forests either as seeds or as seedlings; these seedlings survive in these low-light environments but are not able to grow beyond the seedling stage. When a disturbance opens a gap in the canopy, light availability increases, allowing for shade-intolerant species to germinate and/or grow quickly and cast shadow over other slower-growing species. Seedlings of other species are able to tolerate deep shade by means of alternative combinations of traits, which are related to efficiency of carbon gain in low light and to the tolerance to other stress factors (Valladares & Niinemets 2008). These shade-tolerant species continue to grow slowly until they eventually replace the species that depend on high availability of light (Guariguata & Ostertag 2001).

Regeneration by seeds is especially challenging in deserts, where germination opportunities are fewer and less predictable, and early life stages are exposed to extreme air and soil temperatures and water scarcity. Only a fraction of desert species' seeds germinate following large rainfall events, spreading the risk of seedling mortality (Venable 2007; Gremer & Venable 2014). Despite the tight control over seed germination timing (Baskin & Baskin 2014), early life stages will eventually experience dry conditions. The main strategy among seedlings in xeric systems is to allocate many resources to the main root axis in order to quickly produce deep roots (Nicotra *et al.* 2002; Poot & Lambers 2003). Seedling establishment in these ecosystems may also require facilitation. For instance, larger plants may promote seedling emergence and survival, by providing shade and increased water retention by the soil (Facelli & Brock 2000). The formation of patches of vegetation provides microsites that meet the requirements of species for seed germination and seedling establishment and that are more favourable than the environmental conditions in the ecosystem otherwise (Boeken 2008). Moreover, a well-known life history strategy among desert species is the ephemeral one: seeds germinate following a rain event, and plants grow and set seeds very quickly (within a few weeks, sometimes) before water deficit becomes too severe (Mulroy & Rundel 1977).

Soil infertility is a major source of stress for most plants. However, we know little about traits of seedlings and juveniles in nutrient-impoverished ecosystems and their interaction with biotic and abiotic conditions to which they are naturally exposed. The seed nutrient reserves allow seedlings to be partially independent of the soil for their nutrition (Barrow 1977). However, it is unclear whether early stages of development have additional traits beyond the ratio of seed reserves to embryo size to cope with low soil nutrient availability to maximise their chances of survival, and if those are similar or alternative to traits of adults. In this thesis, I explore these topics by studying regeneration in old climatically buffered infertile landscapes – OCBILs.

OCBILs

Primary productivity is usually limited by the availability of nitrogen (N) and phosphorus (P), individually or in combination, depending on the stage of ecosystem development (Wassen *et al.* 2005; Chapman *et al.* 2006; Lambers *et al.* 2008). Primary productivity on young (at early stages of pedogenesis), weakly weathered soils is limited by N, whereas on moderately weathered soils it is co-limited by N and P, and on old, strongly weathered soils it is limited by P (Fig.2; Walker & Syers 1976; Turner & Condron 2013). Other factors can also influence P availability in soils apart from the gradual loss by weathering followed by movement off site or downwards beyond the root zone. For instance, a low-P parent material can result in pedogenically old soils, even with a relatively young formation, whereas old soils can be rejuvenated by disturbances, such as volcanic activity, tectonic uplift, erosion, glaciation and dust deposition (Vitousek *et al.* 2010).

The most P-impoverished habitats on Earth occur in old landscapes devoid of disturbances that can rejuvenate the supply of P from rock and of long-term dust inputs, and are collectively known as OCBILs (Fig. 2; Hopper 2009; Laliberté *et al.* 2012; Oliveira *et al.* 2015; Abrahão *et al.* 2019). Although these landscapes can be subject to periodic fires, which mineralises organic P in live and dead organisms, and returns inorganic P to the soil, this has not prevented ecosystem retrogression towards extremely low P availability (Turner & Laliberté 2015). The plant growth limitation imposed by the P-poor soils is associated with a high species richness and endemism in OCBILs, which occur in at least 12 of the 35 biodiversity hotspots on the planet, including the *fynbos* in South Africa, the *kwongan* in

Southwest Australia and the *campo rupestre* in Brazil (Hopper 2009; Hopper *et al.* 2016; Silveira *et al.* 2016).

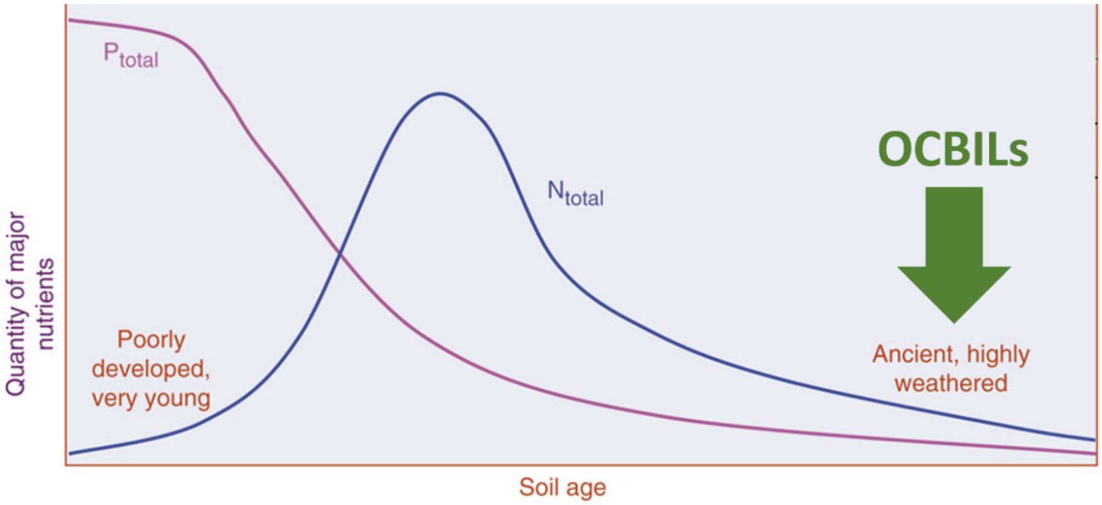

Figure 2. Shifts in the amounts of total soil phosphorus (P; purple) and nitrogen (N; blue) with increasing soil age. 'Poorly developed, very young' soils result from recent disturbances that completely rejuvenated soils, such as volcanic eruptions; 'ancient, highly weathered' soils have been above sea level and not undergone any disturbance for many thousands of years. Green arrow indicates the type of soils found in OCBILs. Adapted from Lambers *et al.* 2008.

OCBILs share a series of ecological and evolutionary similarities, despite their scattered distribution on the globe and harbouring very distinct plant lineages (Hopper 2009; Hopper *et al.* 2016). Most OCBIL species lack specialised mechanisms of seed dispersal (Hopper 2009) which promotes divergence of local populations and allopatric speciation, and results in the large numbers of local endemics and rare species (Echternacht *et al.* 2011; Leles *et al.* 2015). These ancient landscapes are dominated by long-lived perennials, and are resilient to fragmentation (Hopper 2009) and fire events (Bond & Midgley 2001; Le Stradic *et al.* 2015). However, OCBILs are extremely vulnerable to soil removal, and effective strategies of ecological restoration of this ecosystem are extremely challenging and costly, mostly because of issues related to regeneration from seeds (Standish & Hobbs 2010; Le Stradic *et al.* 2014, 2018; Dayrell *et al.* 2017).

The functional ecology of OCBIL species

Plants inhabiting severely P-impoverished soils of OCBILs typically exhibit a nutrient-conserving, slow-growing strategy that comprises high costs per unit leaf area, long leaf lifespan (Specht & Rundel 1990; Lambers & Poorter 1992; Wright et al. 2002; Wright & Westoby 2003), low leaf P concentration and high photosynthetic P-use efficiency (Wright, Groom, et al. 2004; Lambers et al. 2012). The highly efficient P-use is related to different physiological strategies, such as low P allocation to rRNA in leaves (Sulpice et al. 2014), and extensive replacement of phospholipids in young leaves by lipids that do not contain P during leaf development (Lambers et al. 2012; Kuppusamy et al. 2014). These plants also exhibit high levels of P resorption from senescing leaves (Denton *et al.* 2007; de Campos *et al.* 2013; Hayes *et al.* 2014). These traits may co-occur with specialised strategies for P-acquisition (Lambers et al. 2006) or with facilitative root interactions (Muler et al. 2014; Teste et al. 2014), allowing plants to cope with low P in the soil.

A large investment in the nutrient-conserving strategy has been linked to relatively less investment in sexual reproduction (Bazzaz *et al.* 1987; Fujita *et al.* 2014; Gomes *et al.* 2018). Paradoxically, OCBIL plants may allocate a disproportionally high amount of P to seeds as opposed to vegetative structures, a strategy that may enhance chances of seedling establishment on P-impoverished soils (Witkowski & Lamont 1996). However, seed mass and seed nutrient content negatively correlates with seed output (Leishman 2001; Henery & Westoby 2001), suggesting a strong trade-off, especially in nutrient-poor environments. Indeed, soil availability of nutrient resources limits seed set in *Banksia marginata* (Vaughton & Ramsey 1998), and a high P allocation to seeds is associated with a low seed set per individual in *B. laricina* in the *kwongan* (Stock *et al.* 1989). *Campo rupestre* species also have a high proportion of seeds that lack embryos or are otherwise nonviable (Dayrell *et al.* 2017). Thus, the stability provided by the absence of large-scale disturbance events for millions of years allowed the selection of plants that are very successful in persisting in their stable natural habitat, but lack effective strategies for habitat colonisation (Hopper 2009). OCBIL plants are also extremely specialised in their microhabitat and unable to cope with different soil conditions. The increase in nutrients after soil rejuvenation caused by human disturbances is often disadvantageous or even toxic for the plant (Parks *et al.* 2000; Shane & Lambers 2005; Standish *et al.* 2007; Barbosa *et al.* 2010; Williams *et al.* 2019). Combined, the lower investment in sexual reproduction and the high microhabitat specialisation help explain the great vulnerability of OCBILs to soil removal and the challenges for habitat restoration.

Developmental shifts in OCBIL plants

To date, investigations have shown that while adults of OCBIL species have a very low P concentration in leaves, seed P concentrations are often very high (Kuo *et al.* 1982; Denton *et al.* 2007; Groom & Lamont 2010), suggesting that a significant nutrient supply provided by seeds is key to successful seedling recruitment in these habitats (Milberg & Lamont 1997). Studies on seedlings and juveniles of OCBILs have been scarce and mostly focused on specific groups (e.g., Proteaceae in the *kwongan*, Eriocaulaceae in the *campo rupestre*) and/or were restricted to glasshouse experiments. There is also no information on how early developmental stages allocate P compared with adults. High seedling mortality rates have been associated with water deficit during the dry season (Coelho *et al.* 2008; Yates *et al.* 2011), while their survival could be related to a species' ability to access water before the onset of the dry season (Poot & Lambers 2003; Benigno *et al.* 2014) and/or to seed germination and establishment in suitable microhabitats (Lamont *et al.* 1993). In other ecosystems, leaves of plants resprouting after fire exhibit a short-term increase in total P concentration, which could result from an increase in soil P after fire ([P]; Christensen 1977; Reich *et al.* 1990; Durán *et al.* 2010), but no studies attempted to investigate leaf [P] of plants resprouting in OCBILs.

Although studies on regeneration have provided important information about the regeneration phase of OCBIL plants, we still do not know the generality of the findings for most species groups and lack a broader understanding of regeneration processes from a community perspective. Most OCBILs are threatened by human activities (such as mining, agriculture and urban expansion) and demand active restoration (Hopper *et al.* 2016). Previous studies suggest that restoration of OCBILs is extremely challenging and costly, mostly because of issues regarding regeneration from seeds (Standish & Hobbs 2010; Le Stradic *et al.* 2018). The seedling stage is considered as a major bottleneck in the sexual reproduction of long-lived plants (Harper 1977; Fenner 1987). While this statement is true for some OCBIL plant species (Coelho *et al.* 2008; Yates *et al.* 2011), early life stages of some other species in these landscapes have relatively high survival rates under similarly unfavourable conditions (Hallett *et al.* 2014). Understanding the interaction between seedling and juvenile traits and environmental conditions of OCBIL plants can unveil patterns of plant regeneration in these habitats. Such knowledge is important for advances in the construction of new ecological restoration models based on specific theories for OCBILs (Merritt & Dixon 2011), and to increase the predictability of impacts in these environments (Walck *et al.* 2011).

Study Systems

This thesis investigated regeneration processes in OCBILs. The study was carried out in two of the most P-impoverished landscapes in the world, the *campo rupestre* and the *kwongan*. The *kwongan* is the most intensively studied OCBIL (Hopper 2009; Lambers 2014), and the existing knowledge enables investigations aiming to unveil complex patterns of plant function and community assembly. The findings of these studies may guide and optimise investigations into other OCBILs. Notably, convergent specialised root traits of plants have been identified in the *kwongan* and in *campo rupestre* species (Abrahão *et al.* 2014, 2019; Teodoro *et al.* 2019). The parallel investigations of different study systems can thus help management and conservation of the enormous biodiversity in these landscapes.

Campo rupestre

The *campo rupestre* (also known as rupestrian grassland) is a grassy-shrubby landscape that consists of a mosaic of rocky outcrops and sandy, stony, and waterlogged grasslands (Silveira *et al.* 2016). It occurs in severely P-impoverished, shallow, acidic and well-drained soils (Oliveira *et al.* 2015), from different types of substrates: quartzite, sandstone or ironstone (Fig. 3A, B). *Campo rupestre* dominates mountain tops and the highest elevation sites at the *Espinhaço* Range in Southeast Brazil, and other isolated sites in South America. The *Espinhaço* Range is a mountainous region in an ecotonal area between the Atlantic Rainforest (on the east slopes), the Cerrado (on the west slopes), and the Caatinga (on the north slopes; Morellato & Silveira 2018). Transitional patches of gallery and hilltop forest and cerrado occur within the *campo rupestre* matrix (Morellato & Silveira 2018; Coelho *et al.* 2018). The climate in the *Espinhaço* Range is characterised by dry winters (average temperature 14-17 °C) and rainy summers (19-21 °C), and the annual rainfall decreases from roughly 1,600 mm at the southern end to 900 mm at the northern end (Silveira *et al.* 2016; Brito *et al.* 2017).

Campo rupestre harbours at least 5,011 native vascular plant species, nearly 15% of plant diversity in Brazil, despite occupying an area less than 0.8% of the country (Silveira *et al.* 2016). Its remarkable level of endemism reaches nearly 40% (Zappi *et al.* 2015). As an OCBIL, the climatic stability and infertile soils are key factors driving the evolution of this megadiverse flora (Silveira *et al.* 2016; Abrahão *et al.* 2019). In addition to coping with nutrient-impoverished soils, plants in *campo rupestre* need to survive occasional fires (Fig.

3C), water shortage during the dry season and high irradiance (Silveira *et al.* 2016), which results in a clear dominance of a conservative stress-tolerant strategy among species (Negreiros *et al.* 2014).

Figure 3. Typical *campo rupestre* landscape (A) over quartzite (B) over ironstone. (C) *Bulbostylis paradoxa* flowering and other plants resprouting after fire. (D) Degraded area of *campo rupestre* with no spontaneous regeneration following soil removal. Photos: A-C by Roberta Dayrell, D by Fernando Silveira.

Human-induced changes pose a great threat to *campo rupestre.* Major disturbances include open-cast mining, afforestation, invasion by exotic species (such as the African grasses *Urochloa decumbens*, *Melinis minutiflora*), frequent fires, illegal harvesting, road construction and uncontrolled urbanisation (Giulietti *et al.* 1997; Barbosa *et al.* 2010; Fernandes 2016; Miola *et al.* 2019; Salles *et al.* 2019). The landscape is easily disturbed beyond an ecological threshold, after which it cannot recover its original characteristics (Fig. 3D; Le Stradic *et al.* 2018). In a best-case scenario, it would take many decades to centuries for a degraded area to recover with the implementation of huge restoration efforts, and thus conservation of existing areas is crucial to avoid habitat-loss (Buisson *et al.* 2019).

Kwongan

Kwongan encompasses the shrubland-type vegetation occurring mostly within the sandplains in the Southwest Australian Floristic Region (Fig. 4A). The sandplain soils are nutrient-poor, with low availability of nitrogen and particularly P (Wyrwoll *et al.* 2014). The study was conducted in a site at Badgingarra National Park, which exhibits an undulating landscape dominated by lateritic plateau remnants and gravel hills with sand-filled valleys (Sawkins 1981), and thus significant variation in soil properties and fertility on a very fine scale. As opposed to *campo rupestre,* the region has a Mediterranean climate, with warm, dry summers and cool, moist winters, and generally lower annual rainfall (Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). Mean annual rainfall ranges from 450 to 625 mm in the region and plants are naturally exposed to fires (Fig. 4B) and water shortage during the dry season (Mucina *et al.* 2014).

The Southwest Australian Floristic Region has exceptionally high levels of plant richness (more than 7,000 vascular plant species), with high turnover in species composition between neighbouring sites, and its level of endemism is more than 79% (Mucina *et al.* 2014). The landscape has been subjected to major degradation since European settlement (Fig. 4C), and only 25% of the original area of *kwongan* remains, mostly due to extensive land clearing for agriculture. More than 2,000 species of the southwest flora are listed as rare and poorly known and hundreds are threatened (Coates *et al.* 2014). Broad-scale restoration of *kwongan* is extremely challenging, mostly due to seed supply (Standish & Hobbs 2010), but there has been progress in the field (Fig. 4D; e.g. Erickson *et al.* 2017; Pedrini *et al.* 2019).

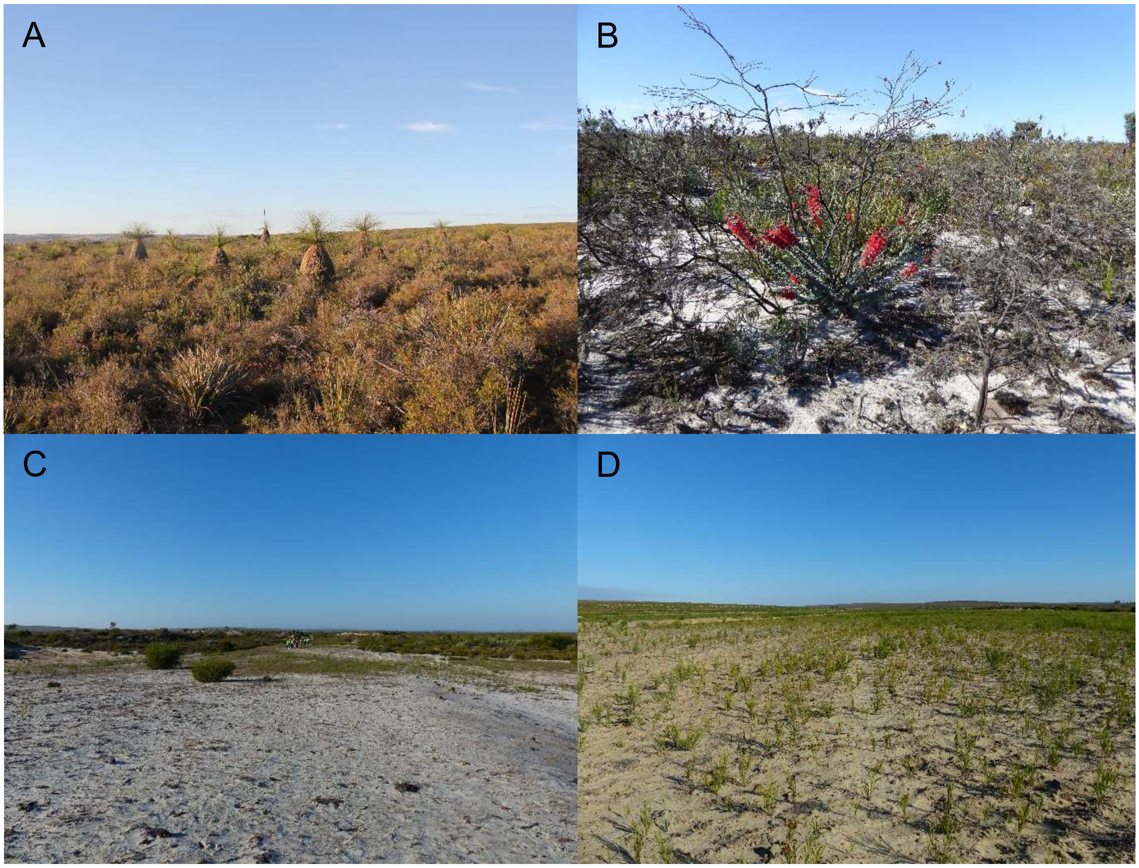

Figure 4. (A) *Kwongan* landscape. (B) *Verticordia grandis* flowering and plants resprouting at the burnt site in Badgingarra National Park (Western Australia). (C) Area of *kwongan* degraded by mineral sands mining at Eneabba (Western Australia). (D) Degraded area following a few years after active restoration. *Acacia* sp. is the most dominant species at this stage in this rehabilitation site. Photos: A, C, D by Roberta Dayrell, B by Marion Cambridge.

Thesis outline

The primary objective of this thesis was to investigate ecophysiological strategies of regeneration in OCBILs by addressing complementary topics in four chapters:

Chapter 2

In the first study, I investigated if the general pattern of regeneration in OCBILs was essentially different from that of a more productive habitat. I aimed to measure ontogenetic shifts of plant ecological strategies quantitatively, and then identified particularities of the regeneration in each habitat. I hypothesised that species exhibit common strategies that favour faster rates of biomass accumulation during early developmental stages to cope with vulnerability, and subsequently shift towards different strategies to optimise resource acquisition and use. Since filtering is a process that occurs continuously across developmental stages, I also expected adults to exhibit a lower variation in ecological strategies than juveniles, and for the extent of the variation to be habitat dependent.

Chapter 3

In this chapter, I investigated viable regeneration strategies within OCBIL plants regarding the use and allocation of P. I studied 10 species of the three dominant families, Fabaceae, Myrtaceae and Proteaceae, co-occurring in recently-burnt and unburnt *kwongan* and investigated the strategies for P use and allocation pertaining to unburnt adults and two regeneration forms: resprouts (on burnt adults) and seedlings. I hypothesised that leaf [P] would be higher in the two regeneration forms than in unburnt adults, driven by greater P availability in the burnt soil and a high seed P content (for seedlings). I also looked for evidence that the higher leaf [P], when present in seedlings and/or burnt adults, is preferentially allocated to the nucleic acid fraction, more than to other fractions (i.e., phospholipids, small metabolites, phosphorylated proteins or inorganic P), since higher rRNA is important for protein synthesis to sustain faster growth rates after fire. I expected to find convergent responses between all species despite the phylogenetic distance, since they are all subjected to strong P-limitation.

Chapter 4

In this study, I switched the focus from inherent plant traits to spatial heterogeneity, aiming to screen for difference in regeneration between microhabitats in an OCBIL. I investigated fine-scale abiotic and biotic drivers of seedling establishment to unveil the role of microhabitat heterogeneity in promoting species coexistence and diversity in a highly threatened ironstone outcrop ecosystem. I hypothesised that seedling survival, growth and abundance and adult richness would be influenced by specific microenvironmental conditions.

Chapter 5

While knowledge on seedlings and juveniles is scarce, many studies have shown that seed availability in OCBILs is low and limits ecological restoration (Hallett *et al.* 2014; Le Stradic *et al.* 2018). In this chapter, I aimed to demonstrate how knowledge on regeneration provides the basis for a more efficient and less costly ecological restoration. I discussed specific hurdles presented by OCBILs that preclude landscape-scale seed-based restoration of degraded areas and presented alternatives and directions for overcoming these challenges.

Chapter 6

The general discussion synthesises the findings of all chapters, highlighting regeneration patterns of OCBILs and discussing different viable strategies of seedlings and juveniles within the community. I propose a theoretical model of how plant recruitment occurs in these biodiverse landscapes and discuss the implications of the findings for habitat conservation and restoration. I also outline areas for future research.

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Chapter 2

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Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Ontogenetic shifts in plant ecological strategies

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Abstract

1. Ontogenetic shifts in plants are ubiquitous, but their ecological and evolutionary significance remain largely unknown. Juveniles have few opportunities to accumulate sufficient biomass to withstand damage, whereas adults allocate resources to reproduction. Thus, age-specific environmental filters are expected to drive selection towards specific resource-investment strategies for each developmental stage.
2. We tested whether species exhibited strategies that favour faster rates of biomass accumulation during early developmental stages to cope with vulnerability, shifting towards habitat-dependent strategies to optimise resource acquisition and use later on. We also hypothesised that juveniles exhibit greater intraspecific variability of strategies than adults, as a result of continuous filtering of traits throughout the plant's development towards adulthood.
3. We measured key leaf traits—leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA)—and calculated scores for competitor (C), stress-tolerator (S), ruderal (R) strategies of the CSR system for juveniles of 54 species and conspecific adults of 27 species naturally occurring either in unproductive (grassland) or productive (forest) habitats.
4. Juveniles exhibited higher SLA and LA and lower LDMC, and thus a more R-strategy in both habitats when compared with adults, but were not necessarily "R" in absolute terms. We also found smaller intraspecific variation for the R-strategy in adults compared with juveniles in both habitats, whereas the variation of the other strategies was habitat dependent.
5. Ontogenetic shifts in ecological strategies appear to have been selected as a response to ontogeny-dependent filters. Thus, strategies that favour less costly leaves and faster growth rates (relatively R-selected) in juveniles shift towards C and/or S strategies in adulthood, depending on habitat productivity. Nevertheless, habitat-dependent specialisation seems to be a major driver of ecological strategy selection in juveniles. Our study reveals ontogeny-dependent strategies, offering a new approach to integrate plant development and functional specialisation.

KEYWORDS

campo rupestre, community assembly, CSR theory, environmental filtering, leaf economics spectrum, leaf traits, regeneration niche, seedling

1 | INTRODUCTION

Variability in many plant traits reflects underlying universal trade-offs in functioning that affect fitness, associated with environmental filtering, species distribution and community assembly (Díaz et al., 2016; Grime & Pierce, 2012; Grime et al., 1997; Lambers & Poorter, 1992; Reich, 2014; Wright et al., 2004). Viable trait combinations evident world-wide include, for example, large seeds on tall plants, but not large seeds on small plants (Díaz et al., 2016). Trait syndromes can thus be interpreted as "strategies" reflecting fitness, often considered equivalent to life histories.

While many definitions of "strategies" exist, Grime and Pierce (2012) emphasised the quantity of essential resources "acquired by an organism during its individual lifetime" and allocation between contrasting functions. However, this "lifetime allocation" view must be reconciled with the finding that resource allocation between functional traits changes during development (Mason, McGaughey, & Donovan, 2013). Also, different developmental stages experience contrasting environmental filters and natural selection pressures (i.e., occupy different niches), which may explain changes in allocation patterns throughout programmed development (i.e., ontogeny; Lasky et al., 2015). Resource allocation depends not only on prevailing environmental conditions, but also on the inherent capacities of the plant—and these change over time as the plant accrues biomass, accumulates internal resources, and augments its acquisitive ability beyond the limited capacities of the initial seedling. Ontogeny likely changes the relative importance of different functional traits throughout the plant life span. Thus, a "strategy" can also be defined as an instantaneous process of resource allocation between competing functions, maximising fitness across contrasting niches during development. In other words, when the strategy is considered as an operating process for resource allocation, the apparent strategy can be expected to shift from seedling to mature stages: viable strategies may exhibit different ontogenetic trajectories for different species according to environment. Similar adult functioning may arise from seedlings with different traits.

To quantify ontogenetic shifts, strategies can be represented by suites of functional traits. Recent analyses of large trait databases demonstrate that small numbers of fundamental leaf traits—in particular specific leaf area (SLA; one-sided area of a fresh leaf divided by dry mass), leaf dry matter content (LDMC; dry mass of a leaf divided by water-saturated fresh mass) and leaf area (LA; one-sided area)—concisely represent the major axes of global plant functioning (resource economics and size; Pierce, Brusa, Vagge, & Cerabolini, 2013; Pierce et al., 2017). Low values of SLA and high values of LDMC are associated with conservative growth, greater investment in leaf protection and increased leaf longevity, whereas the opposite holds for fast-growing species (Lambers & Poorter, 1992). Leaf area largely determines capacity to intercept light, associated with competitive vigour, and constitutes a widely available indicator of the plant/organ size spectrum (Díaz et al., 2016).

Pierce et al. (2017) used global trade-offs in SLA, LDMC and LA to develop a strategy calculator tool (StrateFy) providing numerical quantification (scores) of the three primary strategies (competitor, C; stress-tolerator, S; ruderal, R) of CSR theory (Grime, 1977). Strategies represent adaptation to the intensity of environmental filters involving competition, abiotic limitation to growth (stress) and periodic biomass destruction (disturbance). C-selected "competitors" occupy stable, productive habitats with little disturbance, allocating resources to vegetative growth, large plant and organ size, thereby maximising resource acquisition. S-selected "stress-tolerators" survive in resource-poor or abiotically variable environments with low disturbance by allocating biomass to persistent tissues and mechanisms favouring resource conservation. R-selected "ruderal" species invest in rapid growth and completion of life cycle to avoid frequent lethal disturbance events, surviving in an inactive state as propagules. While much information can be gleaned from measurement of single traits, quantification of adaptive strategies integrates multiple traits, provides a theoretical (explanatory) background, and allows transferability of results and predictive power.

As strategies determine the suites of traits functioning in concert to affect fitness, their quantification provides an overall metric of plant functioning in the same way that "net photosynthetic rate" represents the sum of single traits such as CO_2 uptake and respiration rates. Grime's system has proven useful to represent and compare functional strategies and community assembly across different ecological scales, from that of species (Pierce, Vagge, Brusa, & Cerabolini, 2014), populations (May, Warner, & Wingler, 2017), habitats (Negreiros, Le Stradic, Fernandes, & Rennó, 2014; Rosado & de Mattos, 2017), successional stages (Caccianiga, Luzzaro, Pierce, Ceriani, & Cerabolini, 2006) and biomes (Pierce et al., 2017). The most recent, globally-calibrated, CSR analysis method (Pierce et al., 2017) is supported experimentally (Li & Shipley, 2017). However, all previous investigation has involved adult plants, with no attempt to investigate whether CSR strategies change with ontogeny.

Plant ontogeny may be divided into sequential stages, from seed to senile (Gatsuk, Smirnova, Vorontzova, Zaugolnova, & Zhukova, 1980), that interact differently with environmental factors and are subject to distinct sets of environmental filters (Grubb, 1977). Mounting evidence shows that environmental factors promote habitat specialisation, not only in adulthood, but also in early developmental stages (Metz, 2012; Poorter, 2007; Zalamea et al., 2016), indicating that natural selection plays a major role in the evolution of ontogenetic patterns, despite developmental constraints (Barton & Boege, 2017). Ecological strategies are, therefore, expected to be habitat and stage specific (Grime & Pierce, 2012), but it is not clear how these factors interact.

The susceptibility of seedlings to damage and stress is widely recognised across different vegetation types (Coelho, Capelo, & Figueira, 2008; Moles & Westoby, 2004; Stearns, 1976), being a major driver for the evolution of seed dormancy (Baskin & Baskin, 2014). Although species that are S-selected as adults allocate resources to durable structures, their juveniles may not have sufficient biomass to produce structures that resist harsh environments (Coelho et al., 2008; Hallett, Standish, Jonson, & Hobbs, 2014), and thus a different adaptive strategy could be evident in juveniles. Similarly, while species that are C-selected as adults invest in large leaves, their juveniles may invest either in less costly, short-lived leaves that enable faster growth rates, or in long-lived and well protected leaves that can resist damage caused by herbivores, pathogens and litterfall (Kitajima, Cordero, & Wright, 2013; Poorter & Bongers, 2006). However, adults must cope with the additional costs of reproduction (pollen, flowers, fruits, seeds) and efficiency in acquiring and using limiting resources can prove decisive for fitness (Cunningham, 1997; Stock, Pate, Kuo, & Hansen, 1989). Thus, we expected mean CSR scores to reflect the greater vulnerability of juveniles and the high costs of maintenance and reproduction in adults.

Shifts in the strengths of ecological filters should affect not only the mean strategy, but also strategy variability (Jung, Violle, Mondy, Hoffmann, & Muller, 2010). Intraspecific trait variability should be restricted when habitat filtering is stronger, reflecting niche width (Grime & Pierce, 2012; Violle et al., 2012), and intraspecific variability of CSR scores can therefore be used to assess the width of viable ecological strategies of species (May et al., 2017; Pierce et al., 2013) and assess possible expansions or contractions in niche width during development. Specifically, because adult plants have undergone filtering across previous developmental stages (juvenile included), we expected adults to exhibit a smaller width of ecological strategies than juveniles, and for the extent to be habitat dependent.

Here, we aimed to investigate developmental shifts in plant ecological strategies by assessing the mean and the variability of CSR scores of species at two developmental stages. We compared juvenile and adult stages of species in two adjacent habitats with contrasting levels of productivity: a nutrient-impoverished grassland (unproductive habitat) and a forest (productive habitat). We tested predictions related to the following hypotheses (summarised in Figure 1): (a) the strategy signature of species tends to shift from ability to cope with high levels of disturbances (R-selected) in juveniles towards C- or S-selection in adults (in productive habitats and unproductive habitats, respectively); (b) the juvenile stage exhibits a larger width (greater variation) of CSR strategies than that of adults. We further explored adult and juvenile strategies evident between two adjacent habitats differing in productivity.

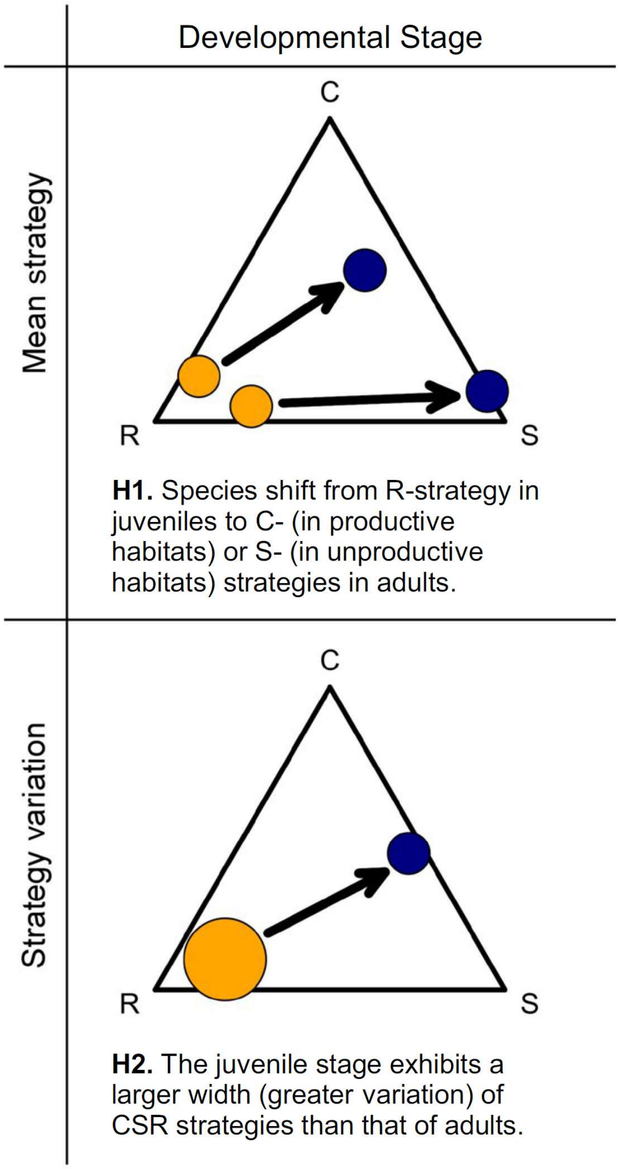

FIGURE 1 Hypotheses (H) represented in ternary plots. Developmental stages: juveniles (orange) and adults (dark blue)

2 | MATERIALS AND METHODS

2.1 | Study area

We measured functional traits in species from two neighbouring natural habitats with highly contrasting vegetation structure and composition at Serra do Cipó (19°17′S, 43°35′W, approximate altitude of 1,200 m a.s.l.), Southeast Brazil. The study site is part of the geographic barrier that represents an ecotone between two global biodiversity hotspots, the *Cerrado* and the Atlantic Rainforest ecoregions. The climatic regime in the region is characterised by dry winters and rainy summers (Brito et al., 2017). We studied the two main mountain top vegetation types: a grassland (known as *campo rupestre*) and a forest (Supporting Information Appendix S1).

The *campo rupestre* is a megadiverse mosaic of grasslands associated with rocky outcrops which occurs on severely P-impoverished, shallow, acidic and well-drained quartzite-derived soils (Oliveira et al., 2015). *Campo rupestre* species often experience strong winds, seasonal fires and water shortage, large fluctuations in daily temperature and high irradiance (Silveira et al., 2016) which results in a clear dominance of the stress-tolerant strategy (Negreiros et al., 2014).

Patches of gallery forest and hilltop forest occur within the *campo rupestre* matrix (Coelho, Carlos, et al., 2018). These forests occur at moist locations, associated with springs and streams or with deeper soil patches that are less well drained than the surrounding grasslands (Coelho et al., 2016; Valente, 2009). Here, plants experience a range of milder environmental conditions compared with the grassland habitat, for example, slightly less nutrient-poor soils and historically lower fire frequency (Coelho, Neves, Perillo, Morellato, & Fernandes, 2018; Valente, 2009).

2.2 | Ontogenetic stages and species selection

We contrasted functional traits and ecological strategies of juvenile and adult stages. Juveniles were defined as structurally simple individuals with an unbranched shoot, mature leaves, no signs of resprouts or clonal origin (to make sure they represented individual genets) and absence of reproductive organs (Gatsuk et al., 1980). Since a few species demonstrated embryonic structures such as persistent cotyledons, our definition comprises both seedling and juvenile states (*sensu* Gatsuk et al., 1980). The average height of juvenile individuals sampled in the grassland was 38 ± 28 mm (mean ± *SD*, range 3–145 mm, *n* = 212) and in the forest 58 ± 27 mm (range 9–153 mm, *n* = 195).

To compare the ontogenetic shifts in leaf traits and CSR strategies among contrasting habitats, we sampled five sites of grassland and five sites of forest to search for juveniles, and selected species whenever we found at least six juvenile individuals to serve as replicates. Since the low density of juveniles strongly limited availability of samples in both habitats, our selection criteria reflected local abundance of juveniles.

In order to compare developmental stages, we also targeted conspecific adults whenever it was possible to sample at least six individuals in the area where juveniles were sampled. Altogether, we measured functional traits in juveniles of 27 species from grassland, and 27 from forest, and adults of 20 species from grassland and seven from forest. *Campo rupestre* species often have very narrow geographic ranges and patchy distributions (Echternacht, Sano, Trovó, & Dubuisson, 2011), and it is possible to find many conspecific adults at the same site. However, the forest species do not present the same pattern, and very few species had at least six individuals within our sampling area, which incurred a smaller sample size for forest adults. Juveniles of just one species were found in both habitats during our sampling efforts (Supporting Information Appendix S2—Table S1).

2.3 | Functional traits and CSR classification

Leaf area, LDMC and SLA measurements were determined according to Pérez-Harguindeguy et al. (2013) from February to early April 2016, mid and late rainy season, when leaves are fully expanded and mature, and juvenile survival is not compromised by the seasonal drought. We collected juveniles (whole individuals) and branches from adults. Samples were immediately saturated using a spray bottle, placed in moist paper bags, sealed in plastic bags and kept in a cooler until placed in a refrigerator for periods of 12 to 24 hr at 4°C (Pérez-Harguindeguy et al., 2013). Measurements were taken from two to eight undamaged, fully expanded leaves (including the petiole) per individual. We scanned leaves and used Leaf Area Measurement (LAM v1.3 by Andrew Askew; www.nucleodiversus.org/uploads/file/leafarea.zip) to determine LA. We used an analytic scale (precision of 0.01 mg) to obtain leaf fresh weight (from turgid leaves) and leaf dry weight (after leaves had dried to constant weight in an oven at 60°C).

Leaf area, SLA and LDMC values were entered into “StrateFy” (Pierce et al., 2017; available at https://doi.org/10.1111/1365-2435.12722) to calculate C-, S- and R-selection scores for each individual. Briefly, Pierce et al. (2017) calibrated the CSR tool using data for 3,068 species obtained from a global database by: (a) performing a principal component analysis (PCA) of the three key leaf traits (LA, LDMC and SLA); (b) regressing trait values against PCA axes; and (c) using these regression equations to produce a Microsoft Excel spreadsheet. This spreadsheet then uses the regression equations to compare any trait values entered against the global trade-off among these traits. Extremely high values of SLA and LDMC are representative of extremes of fast and slow leaf economics, respectively. Leaf area is correlated with seed and whole plant size traits in a spectrum running orthogonal to the economics spectrum, and is also a fundamental determinant of the ability of species to intercept light (Díaz et al., 2016; Pierce et al., 2013). The CSR classification method does not use each trait to directly represent the extent of C, S and R-selection: it is the trade-off between them, integrated and compared against trade-offs evident globally, from which CSR scores are calculated (Pierce et al., 2017). Thus, although only three traits are measured, they represent a wider range of plant functioning. Indeed, co-inertia analysis demonstrates that this method can represent variation in 14 key leaf, reproductive and whole plant functional traits, including leaf nitrogen concentration, seed mass and flowering phenology (Pierce et al., 2017). The method has been tested and supported experimentally (Li & Shipley, 2017).

2.4 | Data analyses

In order to assess ontogenetic shifts and functional specialisation in juveniles, we tested whether developmental stages from both habitats exhibited different leaf trait values (SLA, LDMC and log-transformed LA) and CSR scores using linear mixed-effect models—LMMs (Pinheiro & Bates, 2000). These models contained fixed effects for developmental stages, habitat and their interaction, and random effects for plant species nested within location to avoid pseudo-replication. Significance was estimated by comparing a minimal model with the null model. The minimal adequate models were obtained by a stepwise deletion of nonsignificant parameters and their interaction, starting from a full factorial model ($p > 0.05$). For each model, residuals were visually inspected for heteroscedasticity. In the presence of heteroscedasticity, the variance structure of the data was modelled, with the best variance structure determined by comparing AICc and standardised residual plots (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). The LMMs were performed using the “lme” function, and the variance structure of the data was modelled using the “weights” option within the “nlme” package (Pinheiro et al., 2017). The “effects” package (Fox, 2003) was used for further inspection of means and 95% confidence intervals (CI) to identify significant differences between developmental stages and habitats.

Since a complete control for phylogenetic effects between habitats during species selection was not possible, we examined phylogenetic signal in leaf trait values to account for possible confounding effects of habitat and phylogeny. Briefly, species relationships were reconstructed using "Phylomatic" (http://phylodiversity.net/phylomatic/), topology was corrected and improved manually in "Mesquite" (http://mesquiteproject.org/) based on a number of recent studies of molecular phylogeny. To estimate branch lengths (i.e., time since divergence), we dated nodes according to previous studies (Supporting Information Appendix S3) and positioned undated nodes evenly in the tree with the "bladj" algorithm of Phylocom software (Webb, Ackerly, & Kembel, 2008). We then examined a phylogenetic signal in LDMC and log-transformed SLA and LA separately for adults and juveniles, using the Blomberg's *K* test with 100,000 randomisations (Blomberg, Garland, & Ives, 2003; Münkemüller et al., 2012) in the "picante" package (Kembel et al., 2010).

We tested if the multivariate structure provided by LA, LDMC and SLA could effectively distinguish ontogeny- and habitat-related functional differences. First, trait values of each species at each developmental stage were log-transformed to provide distributions as close as possible to normality prior to PCA. A PCA of LA, LDMC and SLA values was then performed using the "principal" function, and varimax rotation was applied to the first two axes within the "psych" package (Revelle, 2017). We used LMMs as described above to assess differences in rotated components (PC1 and PC2) between groups.

Finally, in order to assess ontogeny- and habitat-related shifts in the width of ecological strategies, we developed a novel approach that uses the standard deviation (*SD*) of C-, S- and R- scores of each species at each developmental stage as a measurement of variation in functional strategies within groups of adults or juveniles of each species. A higher *SD* in CSR scores indicates a broader range of strategies, whereas a lower *SD* indicates a smaller strategy width. Paired tests were used to compare developmental stages and unpaired test was used to compare juvenile plants from the two habitats. The

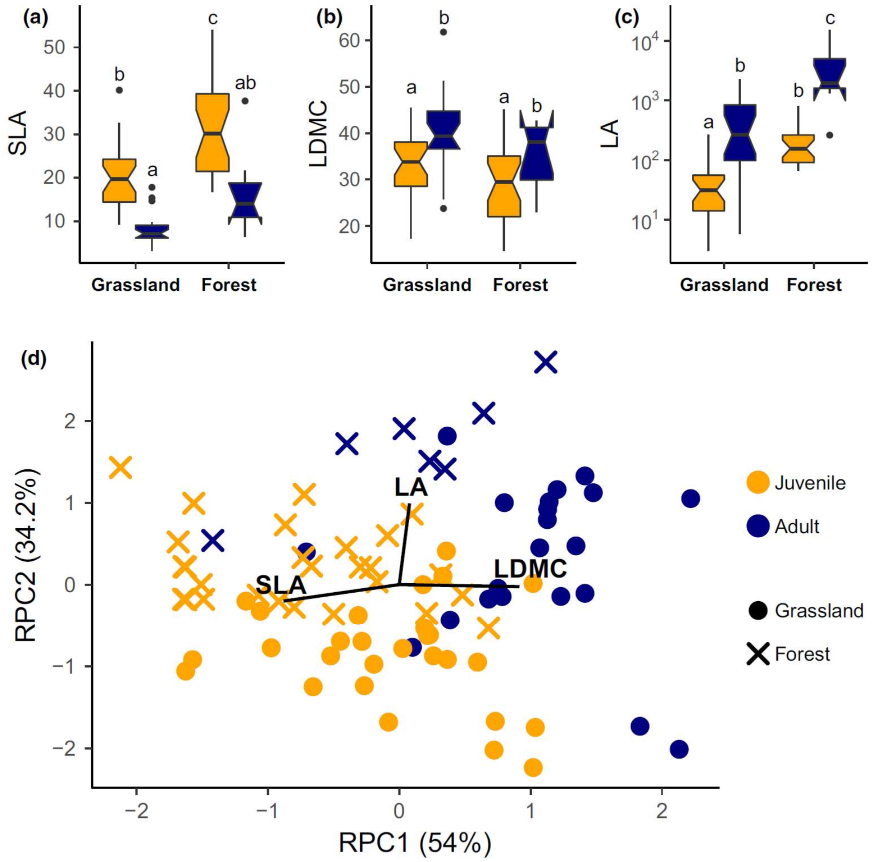

FIGURE 2 Ontogenetic shifts in leaf traits of juveniles and adults from contrasting habitats in a forest-grassland boundary in Southeast Brazil. Comparisons of functional trait values between habitat and developmental stages of: (a) specific leaf area (SLA; mm^2/mg), (b) leaf dry matter content (LDMC; %) and (c) leaf area (LA; mm^2). Boxplots show medians (horizontal lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile range (whiskers) and outliers (filled circles). Within each individual plot, different letters indicate significant differences between trait values based on 95% confidence intervals. (d) Principal component analysis of trait variation for adults and juveniles of plant species from grassland and neighbouring forest. The percentage of variation explained by PCA-rotated component 1 (PC1) and PCA-rotated component 2 (PC2) is shown in parentheses

nonparametric paired Wilcoxon rank sum test was used to assess differences between groups.

All analyses were performed in the R environment (R Development Core Team, 2017) and graphs created with "ggplot2" package (Wickham, 2016). The relative contribution of the three CSR dimensions was represented in ternary plots, with each coordinate varying from 0% to 100%, using the "ggtern" package (Hamilton, 2017). We used the mean of trait values, PCA scores and C-, S- and R-scores for each species at each developmental stage for all figures.

3 | RESULTS

Juveniles from both grassland and forest showed relatively acquisitive leaf economics trait values (higher SLA and lower LDMC; Figure 2a, b), and smaller leaves than adults (Figure 2c; Supporting Information Appendix S2—Table S2). Distinct leaf trait patterns were also observed for contrasting habitats, as forest juveniles had higher SLA and larger leaves than grassland species (Figure 2a, c), but there was no significant difference in LDMC (Figure 2b). The full model including developmental stage, habitat and their interaction best explained LA and SLA values, whereas the final model for LDMC included only developmental stage (Table 1). The multivariate structure provided by our data clearly separated ontogeny- and habitat-related functional differences (Figure 2d; Table 1; Supporting Information Appendix S4— Figure S2), and thus could represent functional variation in our system. We found a weak phylogenetic signal only in the LA of juveniles (*K* = 0.607; *p* = 0.001), and no significant signal for the other two traits or LA of adults (Supporting Information Appendix S4—Table S4).

Juveniles from the grassland were mainly concentrated around the S/SR region of the triangle (mean C:S:R strategy = 2:64:34%; Figure 3a, c, d), whereas adults were mainly S (13:83:4%; Figure 3a, d). Juveniles from the forest exhibited a mean strategy of S/R (9:44:47%; Figure 3b, c, d) and adults exhibited a mean strategy of C/S (34.6: 48.8:16.6%; Figure 3b, d).

Ontogenetic shifts in ecological strategies were significant for all strategies from both habitats, except for the S-selection in forest species (Figure 3a, b, d; Table 1; Supporting Information Appendix S5). In the grassland, juvenile-to-adult shifts were characterised mainly by changes from R- towards C- and S-strategies (Figure 3d). In forest species, ontogenetic shifts were represented

TABLE 1 Results of a linear mixed-effect model testing the effect of developmental stage and habitat type on specific leaf area (SLA), leaf dry matter content (LDMC), log-transformed leaf area (LA), PCA scores—rotated component 1 (PC1) and PCA-rotated component 2 (PC2) and C-, S- and R-scores in plants from Southeast Brazil

Data	Model ID	Model	*K*	Log L	AICc	ΔAICc	*P*
SLA (mm^2/mg)	MM	SLA ~ stage * habitat, random = species	11	−1765.01	3552.49	−655.03	**<0.0001**
	null	SLA ~ 1, random = species	4	−2099.73	4207.52	–	–
LDMC (%)	MM	LDMC ~ stage, random = species	6	−1735.58	3483.30	−239.13	**<0.0001**
	null	LDMC ~ 1, random = species	4	−1857.18	3722.43	–	–
log LA (mm^2)	MM	logLA ~ stage * habitat, random = species	11	−156.71	335.89	−640.43	**<0.0001**
	null	logLA ~ 1, random = species	4	−484.12	976.32	–	–
PC1 (%)	MM	PC1 ~ stage * habitat, random = species	10	−373.51	767.41	415.47	**<0.0001**
	null	LA ~ 1, random = species	4	−587.40	1182.88	–	–
PC2 (%)	MM	PC2 ~ stage * habitat, random = species	10	−336.44	693.27	604.49	**<0.0001**
	null	LA ~ 1, random = species	4	−644.84	1297.76	–	–
C (%)	MM	C ~ stage * habitat, random = species	10	−1718.22	3456.83	−819.65	**<0.0001**
	null	C ~ 1, random = species	4	−2134.20	4276.48	–	–
S (%)	MM	S ~ stage * habitat, random = species	11	−2209.82	4442.11	−316.59	**<0.0001**
	null	S ~ 1, random = species	4	−2375.32	4758.70	–	–
R (%)	MM	R ~ stage +habitat, random = species	7	−2291.08	4626.66	−453.32	**<0.0001**
	null	R ~ 1, random = species	4	−2520.80	5049.60	–	–

Note. Random intercepts per species were specified in the model. Significance was estimated by comparing minimal model (MM) with the null model (null). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model whereby lower AICc values represent the more parsimonious models. Δ AICc is the difference in AICc values between minimal and null models, Log L is the log-likelihood and *K* is the number of parameters in each model. Significant P-values (p < 0.05) are shown in bold.

by changes from R- towards C-selection (Figure 3d). Juveniles from the contrasting habitats also exhibited different ecological strategies, as forest juveniles had a higher C- and R-score, and grassland juveniles showed a higher S-score (Figure 3c, d). The full model including developmental stage, habitat and their interaction best explained C- and S-selection. The final model for R-selection included developmental stage and habitat, but not the effect of their interaction which shows that the magnitude of the ontogenetic shifts away from R-selection was habitat-independent (Table 1).

Ecological strategy width was habitat and ontogeny dependent. In grassland, *SD* in S- and R-scores of juveniles was eight and 25 times greater, respectively, and C-score was a third of that of adults (Figure 4a; Supporting Information Appendix S4—Table S5). The R-strategy *SD* was four times greater for juveniles in the forest compared with that of adults, but *SD* of C- and S-selection was not significantly different (Figure 4b; Supporting Information Appendix S4—Table S5).

4 | DISCUSSION

Despite the recent progress in trait-based plant ecology, plant regeneration traits remain poorly understood (Larson & Funk, 2016), and this limits our knowledge of the assembly of natural and managed communities (Grubb, 1977; Paine et al., 2015). Our study shows ubiquitous ontogenetic shifts in plant ecological strategies amongst species, thereby suggesting the need to integrate plant development with functional specialisation to better predict assembly patterns and responses of plant communities to environmental changes (Larson & Funk, 2016). As we predicted, plants from both habitats exhibited strategies tending towards the R-strategy in the juvenile stage, and shifts of a similar magnitude away from the R-strategy into adulthood, possibly due to the greater vulnerability of early developmental stages to damage (Harms & Dalling, 1997; Moles & Westoby, 2004). Adults also showed smaller intraspecific variability for the R-strategy than juveniles, indicating that adults from both habitats are strongly filtered against this strategy towards more conservative and/or competitive strategies. However, contrary to our expectations, in absolute terms the ecological strategies of juveniles were not strongly R-selected (although juveniles tended to be relatively R-selected when compared with adults, few were “ruderal” per se). R- and S-strategies were equally represented in forest juveniles, whereas the S-strategy prevailed in grassland juveniles. We also found significant differences in mean strategies of juveniles between the two habitats which indicates that habitat filtering takes place during early developmental stages (Metz, 2012; Poorter, 2007). Altogether, our results suggest that despite the remarkable ontogenetic shifts in ecological strategies, a functional habitat-dependent specialisation is the main driver of selection for ecological strategy in juveniles.

According to CSR theory (Grime, 1977) and its underlying premise of trade-offs in resource allocation (Lambers & Poorter, 1992; Lambers, Poorter, & Van Vuuren, 1998), the increased selection away from R in adults relative to juveniles suggests a greater investment in growth rate during early stages of development as a habitat-independent strategy to cope with higher levels of disturbance. A strategy to accumulate biomass faster in juveniles should improve plant fitness if it enhances the chances of an individual reaching a functionally specialised stage that is less susceptible to disturbances such as fire and herbivory (Bond, 2000). However, our results show a large contribution of S-selection to the juvenile mean strategy, suggesting that juveniles from both habitats are slow-growing. We found that stress is the prevailing filter for plants in grassland, regardless of their developmental stage, possibly because even juveniles from unproductive habitats can be resistant to harsh conditions such as water deficit and nutrient deficiency (Milberg & Lamont, 1997; Negreiros, Fernandes, Efremova, Le Stradic, & Neves, 2016; but see Coelho et al., 2008). In the forest, juvenile mean strategy had similar contributions from S- and R-components which seems to represent the mid-point in a spectrum of microhabitat specialists, ranging from gap (poorly-defended fast-growing) to understorey (well-defended slow-growing) species (Kitajima et al., 2013; Poorter & Bongers, 2006). Therefore, during early developmental stages, plants invest in strategies that favour faster rates of biomass accumulation than adults do, but can still remain at the “slow” or conservative extreme of the plant economics spectrum (Reich, 2014) due to habitat filtering.

Recent developments in trait-based community ecology have highlighted the importance of intraspecific variation in understanding community structure and ecosystem functioning (Violle et al., 2012). Ontogenetic changes are regarded as a major source of variation between conspecifics (González-Varo & Traveset, 2016), but it is rarely assessed whether trait variability itself increases or decreases during development. Here, we used the *SD* of CSR scores to test our second hypothesis that variability in ecological strategies is lower in adults as a result of continuous filtering throughout ontogeny. We found that adults exhibited a much smaller *SD* for the R-strategy than their conspecific juveniles. This, together with their overall low mean R-scores, indicates that adults are strongly filtered against the R-strategy during development. As less investment in one particular CSR component implies greater investment in another (Grime & Pierce, 2012), our results suggest a stronger habitat filtering in adulthood towards strategies of resource allocation towards competition and/or stress-tolerance (Negreiros et al., 2014; Pierce et al., 2017), possibly due to the high costs imposed by reproduction activities in this later stage (Oñate & Munné-Bosch, 2009; Thomas, 2011). We also found an intricate pattern for the *SD* of C- and S-scores, which were habitat- and ontogeny-dependent, further indicating that ontogenetic shifts in plant strategies are mainly driven by changes in the intensity of the disturbance filter.

We show that the CSR framework provided additional information useful to assess the functional ecology of the regeneration niche than trait analyses alone. Indeed, juveniles exhibited relatively acquisitive leaf economics and smaller leaves than adults, confirming previous studies (Ishida, Yazaki, & Hoe, 2005; Mason et al., 2013). However, measuring functional traits alone could not have revealed

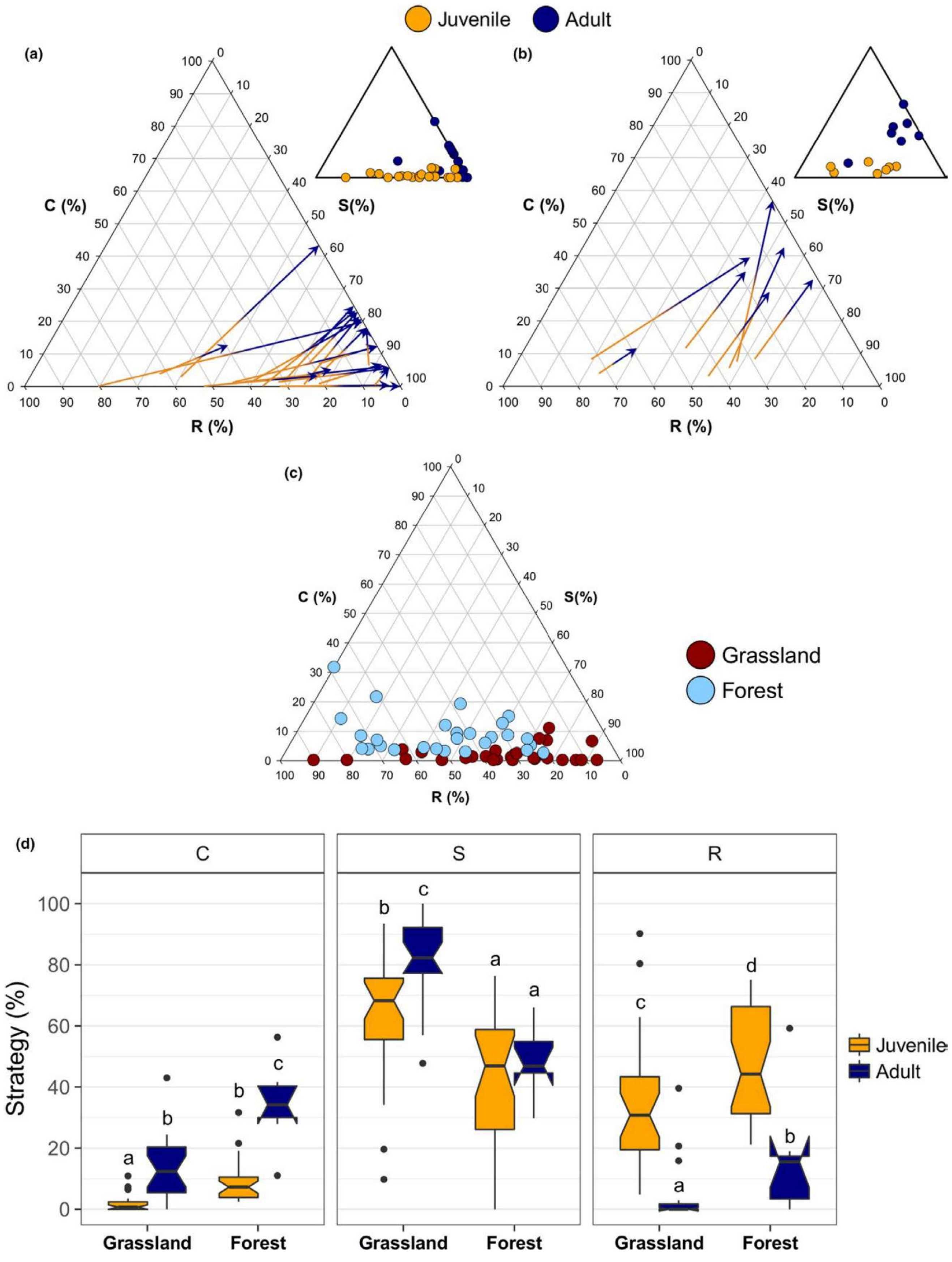
Juvenile
Adult
(a)
(b)
C (%)
S(%)
R (%)
(c)
Grassland
Forest
(d)
C
S
R
Strategy (%)
Grassland
Forest
a
b
c
d

FIGURE 3 Ontogenetic shifts in CSR strategies in grassland and forest habitats in Southeast Brazil. Shifts in the relative proportion of C-, S- and R-selection represented by the direction of arrows in the ternary plot (juvenile species = orange end of arrows; conspecific adults = dark blue end) for plant species from: (a) grassland and (b) forest; small insets in (a) and (b) show the relative proportion of C-, S- and R-selection for each plant species from each habitat. (c) The relative proportion C-, S- and R-selection represented in a ternary plot for juveniles from grassland (red) and forest (light blue). (d) Comparison of C-, S- and R-scores between habitat and developmental stages. Within each individual panel, different letters indicate significant differences between scores based on 95% confidence intervals from linear mixed-effect models (Table 1)

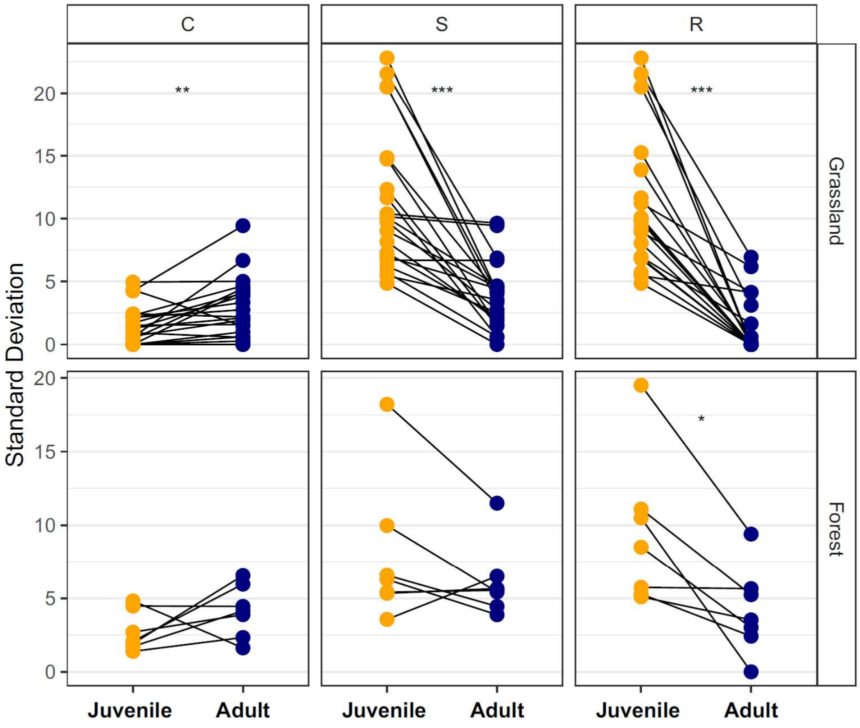

FIGURE 4 Variation in CSR strategies between juveniles and adults. Standard deviation (*SD*) in C-, S- and R-scores compared between juveniles (orange) and adults (dark blue) for grassland and forest habitats separately. The lines connect *SD* values of conspecific juveniles and adults. *Denotes significance at the $p < 0.05$, **$p < 0.01$, ***$p < 0.001$ level (Supporting Information Appendix S4—Table S5)

differences in ontogenetic shifts between habitats, since the exact same pattern—that is, decrease in SLA, and increase in LDMC and LA from juveniles to adults—was observed for both habitats when only trait analyses were performed. Divergent ontogenetic shifts towards different ecological strategies in each habitat only emerged when plants were arranged according to the fundamental trade-offs described by CSR theory. We argue that investigating the ontogenetic shifts in the context of CSR theory (Grime, 1977), combined with a careful analysis of the ecological and physiological significance of SLA, LDMC (Lambers & Poorter, 1992; Lambers et al., 1998) and leaf size (Cornelissen, 1999; Givnish, 1987; Parkhurst & Loucks, 1972), has brought fresh perspectives that were not captured by previous trait analyses, offering an explanation for why the ontogenetic shifts occur, and allowing the processes reported here to be compared with other settings.

Although previous studies have reported phylogenetic nonindependence in leaf traits (Mason, Goolsby, Humphreys, & Donovan, 2016), we only found a weak significant phylogenetic signal for LA of juveniles in our dataset. Since juveniles of 15 (out of 29) grassland species and 19 (out of 21) forest species belong to families sampled in both habitats, and our samples involved 19 plant families, phylogenetic effects are unlikely to play a role in the observed patterns.

In conclusion, we present the first quantitative assessment of development-related shifts in plant ecological strategies. Our results show that early stages of development exhibit a relatively acquisitive strategy that favours faster biomass accumulation, and then shifts towards more conservative and/or competitive strategies at a later stage, depending on habitat. However, juveniles were not necessarily "ruderal" in absolute terms, exhibiting functional habitat-related specialisation, which suggests that habitat conditions play a major role in driving strategies, starting at early stages of development. We found that ontogenetic shifts in the width of ecological strategies were habitat dependent, but that adults were strongly filtered against the R-strategy, towards strategies that optimise resource acquisition and use in their habitats. Our approach unveiled potentially general relationships between strategies, developmental stages and the environment, and provides an opportunity to further understand community assembly and the importance of functional specialisations during different stages of the plant life cycle. Finally, we contend that the ecology and evolution of plant strategies cannot be fully appreciated without integrating the full spectrum of functional ontogeny-dependent traits.

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AUTHORS' CONTRIBUTIONS

R.L.C.D. and F.A.O.S. conceived the idea, with inputs from S.P. R.L.C.D. and A.J.A. designed and conducted fieldwork. P.B.M. identified the species. R.L.C.D., D.N. and S.P. analysed the data. R.L.D.C. led the writing of the manuscript. F.A.O.S., S.P., D.N. and H.L. provided critical feedback to data interpretation and drafting of the manuscript. All authors made intellectual contributions and provided essential feedback.

DATA ACCESSIBILITY

Data from this manuscript are publicly available at Figshare Repository https://doi.org/10.6084/m9.figshare.7097969 (Dayrell et al., 2018).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supporting Information S1. Photographs of grassland-forest boundaries in the study area in Southeast Brazil.

Gallery forest immersed in a matrix of *campo rupestre* grassland.
Photo: Roberta Dayrell.

A hilltop forest fragment immersed in a *campo rupestre* grassland matrix.
Photo: André Arruda.

Supporting Information 2 – Species information and collection details

Table S1. List of species and information concerning the collection date (month-year), habitat (forest – FO; grassland – GL; * denotes species that may occur in both habitats), geographical coordinates of collection sites, number (n) of sampled juvenile and adult individuals. Non-identified (NI). Two populations of *Declieuxia fruticosa* were considered as different units.

Family/ Species	**Collection date**	**Habitat**	**Geographical coordinates**		**Juvenile individuals (n)**	**Adult individuals (n)**
			W	**S**		
Annonaceae						
Guatteria australis A.St.-Hil.	Apr-16	FO	19°16'2"	43°34'56"	8	6
Asteraceae						
Aspilia jolyana G.M.Barroso	Mar-16	GL	19°16'55"	43°35'38"	8	6
Baccharis retusa DC.	Feb-16	GL	19°17'17"	43°34'38"	7	6
Dasyphyllum sprengelianum (Gardner) Cabrera	Apr-16	FO*	19°18'0"	43°36'2"	5	6
Lychnophora passerina (Mart. ex DC.) Gardner	Feb-16	GL	19°17'17"	43°34'38"	7	6
Mikania sessilifolia DC.	Mar-16	GL	19°16'55"	43°35'38"	7	6
Piptocarpha sp.	Feb-16	FO*	19°16'7"	43°34'59"	7	-
Burseraceae						
Protium spruceanum (Benth.) Engl.	Feb-16	FO	19°17'43"	43°34'54"	7	-
Calophyllaceae						
Kielmeyera variabilis Mart. & Zucc.	Feb-16	FO*	19°15'37"	43°30'47"	7	-
	Mar-16	GL*	19°16'55"	43°35'38"	7	-
Elaeocarpaceae						
Sloanea guianensis (Aubl.) Benth.	Feb-16	FO	19°17'43"	43°34'54"	7	-
Erythroxylaceae						
Erythroxylum pelleterianum A.St.-Hil.	Apr-16	FO	19°18'0"	43°36'2"	8	-
Erythroxylum vacciniifolium Mart.	Mar-16	GL*	19°16'55"	43°35'38"	9	6
Erythroxylum sp.	Feb-16	FO	19°16'7"	43°34'59"	8	-
Euphorbiaceae						
Croton timandroides (Didr.) Müll.Arg.	Mar-16	GL	19°16'46"	43°35'55"	8	6
Microstachys ditassoides (Diedrichs) Müll.Arg.	Mar-16	GL	19°16'46"	43°35'55"	8	-
Microstachys glandulosa (Mart. & Zucc.) Esser & M.J.Silva	Feb-16	GL	19°17'29"	43°35'35"	8	-
Microstachys sp.	Mar-16	GL	19°16'46"	43°35'55"	8	-

Family/ Species	**Collection date**	**Habitat**	**Geographical coordinates**		**Juvenile individuals (n)**	**Adult individuals (n)**
			W	**S**		
Fabaceae						
Bauhinia rufa (Bong.) Steud.	Apr-16	FO*	19°18'0"	43°36'2"	8	6
Chamaecrista cathartica (Mart.) H.S.Irwin & Barneby	Feb-16	GL	19°17'29"	43°35'35"	6	6
Chamaecrista desvauxii (Collad.) Killip	Feb-16	GL	19°17'29"	43°35'35"	8	6
Machaerium hirtum (Vell.) Stellfeld	Apr-16	FO*	19°18'0"	43°36'2"	8	-
Lamiaceae						
Hypenia reticulata (Mart. ex Benth.) Harley	Feb-16	GL	19°17'17"	43°34'38"	8	6
Lythraceae						
Cuphea acinos A.St.-Hil.	Mar-16	GL*	19°16'55"	43°35'38"	6	-
Cuphea sperguloides A.St.-Hil.	Mar-16	GL	19°16'46"	43°35'55"	6	6
Diplusodon orbicularis Koehne	Mar-16	GL	19°16'46"	43°35'55"	9	6
Malpighiaceae						
Banisteriopsis campestris (A.Juss.) Little	Mar-16	GL	19°16'46"	43°35'55"	6	6
Banisteriopsis laevifolia (A.Juss.) B.Gates	Feb-16	FO*	19°16'7"	43°34'59"	8	-
Malvaceae						
Ayenia angustifolia A.St.-Hil. & Naudin	Mar-16	GL	19°16'46"	43°35'55"	9	7
Melastomataceae						
Clidemia urceolata DC.	Apr-16	FO*	19°16'2"	43°34'56"	8	-
Leandra aurea (Cham.) Cogn.	Feb-16	FO*	19°17'43"	43°34'54"	6	-
Leandra sp.	Feb-16	FO	19°15'37"	43°30'47"	6	-
Miconia chartacea Triana	Feb-16	FO	19°15'37"	43°30'47"	6	6
Miconia sp. (1)	Feb-16	FO	19°17'43"	43°34'54"	7	-
Miconia sp. (2)	Apr-16	FO	19°16'2"	43°34'56"	7	-
Pleroma heteromalla D. Don (D.Don)	Mar-16	GL	19°16'55"	43°35'38"	6	6
Trembleya laniflora (D.Don) Cogn.	Mar-16	GL	19°16'55"	43°35'38"	6	6
Myrtaceae						
Myrcia splendens (Sw.) DC.	Feb-16	FO*	19°15'37"	43°30'47"	6	-
Siphoneugena kiaerskoviana (Burret) Kausel	Feb-16	FO	19°15'37"	43°30'47"	6	-
Ochnaceae						
Luxemburgia damazioana Beauverd	Mar-16	GL	19°17'29"	43°35'35"	8	6
Phyllanthaceae						
Phyllanthus klotzschianus Müll.Arg.	Mar-16	GL	19°16'46"	43°35'55"	9	-

Family/ Species	**Collection date**	**Habitat**	**Geographical coordinates**		**Juvenile individuals (n)**	**Adult individuals (n)**
			W	**S**		
Rubiaceae						
Borreria capitata (Ruiz & Pav.) DC.	Feb-16	GL*	19°15'34"	43°31'27"	12	6
Coccocypselum sp.	Apr-16	FO*	19°16'2"	43°34'56"	7	-
Declieuxia fruticosa (Willd. ex Roem. & Schult.) Kuntze	Feb-16	GL	19°17'17"	43°34'38"	7	6
	Feb-16	GL	19°17'29"	43°35'35"	6	6
Declieuxia gracilis J.H.Kirkbr.	Mar-16	GL	19°16'46"	43°35'55"	9	6
Psychotria capitata Ruiz & Pav.	Apr-16	FO*	19°18'0"	43°36'2"	8	6
Psychotria cf. *subtriflora* Müll.Arg.	Feb-16	FO	19°15'37"	43°30'47"	7	-
Rudgea sessilis (Vell.) Müll.Arg.	Feb-16	FO	19°17'43"	43°34'54"	7	6
NI 01	Feb-16	FO	19°16'7"	43°34'59"	8	6
Salicaceae						
Casearia sp.	Feb-16	FO*	19°15'37"	43°30'47"	6	-
Verbenaceae						
Lippia stachyoides var. *martiana* (Schauer) Salimena & Múlgura	Feb-16	GL	19°17'29"	43°35'35"	7	6
NI						
NI 02	Feb-16	GL	19°17'29"	43°35'35"	6	-
NI 03	Feb-16	FO	19°16'7"	43°34'59"	7	-
NI 04	Apr-16	FO	19°18'0"	43°36'2"	8	-

Table S2. Summarised values of leaf traits for each stage and species. Columns show species, habitat (forest – FO; grassland – GL), developmental stage (juvenile – JUV; adult – AD), and values for leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA). Trait values are shown as mean (±SD). Non-identified (NI).

Family/ Species	**Habitat**	**Stage**	**LA (mm^2)**	**LDMC (%)**	**SLA (mm^2 mg^{-1})**
Annonaceae					
Guatteria australis	FO	JUV	169.2 (±60.9)	38.0 (±3.3)	30.2 (±4.6)
		AD	5681.0 (±1704.4)	42.5 (±2.1)	11.8 (±1.7)
Asteraceae					
Aspilia jolyana	GL	JUV	26.1 (±13.2)	33.2 (±3.9)	14.5 (±2.0)
		AD	110.0 (±57.3)	36.3 (±3.6)	9.1 (±1.4)
Baccharis retusa	GL	JUV	115.0 (±69.0)	32.2 (±4.9)	13.4 (±2.4)
		AD	268.5 (±37.2)	39.4 (±2.6)	7.1 (±0.9)
Dasyphyllum sprengelianum	FO	JUV	123.8 (±28.9)	19.5 (±3.1)	33.9 (±9.5)
		AD	1951.4 (±849.6)	26.6 (±2.6)	14.0 (±3.3)
Lychnophora passerina	GL	JUV	7.1 (±1.3)	38.0 (±6.8)	12.5 (±3.5)
		AD	5.7 (±0.6)	61.7 (±14.8)	7.8 (±1.5)
Mikania sessilifolia	GL	JUV	14.0 (±4.7)	19.7 (±5.4)	40.2 (±13.3)
		AD	538.5 (±149.0)	36.6 (±3.2)	5.3 (±0.9)
Piptocarpha sp.	FO	JUV	96.6 (±92.5)	21.5 (±1.7)	38.3 (±5.7)
Burseraceae					
Protium spruceanum	FO	JUV	680.2 (±391.6)	38.8 (±2.4)	21.0 (±2.5)
Calophyllaceae					
Kielmeyera variabilis	FO	JUV	457.0 (±106.3)	19.1 (±1.0)	27.6 (±2.4)
Kielmeyera variabilis	GL	JUV	267.0 (±126.9)	36.4 (±3.1)	13.9 (±2.3)
Elaeocarpaceae					
Sloanea guianensis	FO	JUV	751.1 (±484.1)	14.6 (±0.7)	26.7 (±3.4)
Erythroxylaceae					
Erythroxylum pelleterianum	FO	JUV	70.3 (±14.2)	20.9 (±3.9)	42.8 (±6.6)
Erythroxylum vacciniifolium	GL	JUV	33.2 (±12.6)	45.4 (±3.8)	20.8 (±5.2)
		AD	1001.8 (±159.6)	46.9 (±1.5)	6.2 (±0.5)
Erythroxylum sp.	FO	JUV	376.3 (±166.9)	34.2 (±5.4)	20.0 (±4.6)

Family/ Species	Habitat	Stage	LA (mm^2)	LDMC (%)	SLA (mm^2 mg^{-1})
Euphorbiaceae					
Croton timandroides	GL	JUV	14.0 (±6.1)	29.0 (±7.1)	32.6 (±12.2)
		AD	63.1 (±14.8)	37.0 (±2.8)	15.4 (±0.9)
Microstachys ditassoides	GL	JUV	4.1 (±0.8)	39.2 (±4.3)	14.1 (±2.3)
Microstachys glandulosa	GL	JUV	54.3 (±29.0)	27.8 (±3.8)	17.6 (±2.4)
Microstachys sp.	GL	JUV	20.4 (±7.8)	20.4 (±2.9)	19.3 (±2.8)
Fabaceae					
Bauhinia rufa	FO	JUV	553.0 (±199.2)	33.5 (±3.6)	47.3 (±7.2)
		AD	4359.1 (±1183.4)	40.0 (±2.6)	21.6 (±5.3)
Chamaecrista cathartica	GL	JUV	166.0 (±61.0)	37.7 (±2.4)	16.1 (±3.6)
		AD	1067.0 (±215.5)	44.6 (±2.2)	7.6 (±1.0)
Chamaecrista desvauxii	GL	JUV	52.5 (±16.2)	39.7 (±4.2)	22.9 (±5.9)
		AD	112.2 (±14.4)	42.5 (±2.2)	14.7 (±1.8)
Machaerium hirtum	FO	JUV	359.5 (±212.2)	37.4 (±3.9)	36.7 (±5.6)
Lamiaceae					
Hypenia reticulata	GL	JUV	21.2 (±7.5)	30.2 (±2.5)	20.0 (±3.1)
		AD	593.5 (±125.6)	33.7 (±4.8)	6.4 (±0.7)
Lythraceae					
Cuphea acinos	GL	JUV	7.0 (±1.1)	33.0 (±3.6)	23.5 (±6.7)
Cuphea sperguloides	GL	JUV	3.0 (±0.5)	42.8 (±3.7)	12.6 (±1.3)
		AD	6.9 (±2.2)	48.0 (±7.4)	5.9 (±0.7)
Diplusodon orbicularis	GL	JUV	6.7 (±2.0)	42.0 (±3.6)	11.1 (±1.6)
		AD	92.6 (±11.7)	39.3 (±1.1)	6.3 (±0.4)
Malpighiaceae					
Banisteriopsis campestris	GL	JUV	58.6 (±9.3)	38.3 (±2.0)	21.2 (±4.4)
		AD	1270.8 (±714.9)	42.8 (±2.3)	5.2 (±1.4)
Banisteriopsis laevifolia	FO	JUV	72.7 (±36.3)	35.9 (±4.2)	17.5 (±2.8)
Malvaceae					
Ayenia angustifolia	GL	JUV	33.8 (±21.7)	34.3 (±5.7)	20.5 (±6.8)
		AD	114.9 (±36.5)	45.0 (±3.7)	6.9 (±0.9)

Family/ Species	Habitat	Stage	LA (mm^2)	LDMC (%)	SLA ($mm^2\ mg^{-1}$)
Melastomataceae					
Clidemia urceolata	FO	JUV	87.0 (±42.7)	24.9 (±3.1)	54.0 (±12.4)
Leandra aurea	FO	JUV	807.1 (±466.2)	28.6 (±2.2)	26.3 (±5.3)
Leandra sp.	FO	JUV	194.8 (±94.8)	26.8 (±1.3)	24.8 (±3.4)
Miconia chartacea	FO	JUV	180.6 (±111.5)	31.4 (±2.4)	21.8 (±5.7)
		AD	14986.3 (±4670.6)	42.7 (±1.8)	6.4 (±0.5)
Miconia sp. (1)	FO	JUV	131.1 (±34.5)	43.1 (±3.4)	19.1 (±1.9)
Miconia sp. (2)	FO	JUV	80.4 (±56.1)	29.7 (±1.8)	36.7 (±5.0)
Pleroma heteromalla	GL	JUV	57.9 (±69.0)	24.0 (±2.0)	30.1 (±6.9)
		AD	2282.7 (±1120.0)	30.2 (±3.1)	7.5 (±0.2)
Trembleya laniflora	GL	JUV	140.9 (±73.2)	41.9 (±7.5)	9.3 (±4.3)
		AD	840.1 (±387.9)	51.2 (±2.3)	3.2 (±0.4)
Myrtaceae					
Myrcia splendens	FO	JUV	183.8 (±59.9)	39.5 (±5.0)	18.3 (±4.4)
Siphoneugena kiaerskoviana	FO	JUV	66.2 (±19.7)	45.1 (±3.4)	17.6 (±1.7)
Ochnaceae					
Luxemburgia damazioana	GL	JUV	44.1 (±35.8)	35.3 (±5.0)	31.6 (±16.1)
		AD	839.9 (±227.3)	44.7 (±3.9)	8.3 (±0.8)
Phyllanthaceae					
Phyllanthus klotzschianus	GL	JUV	28.6 (±6.8)	38.0 (±4.3)	17.7 (±3.6)
Rubiaceae					
Borreria capitata	GL	JUV	63.5 (±63.2)	21.7 (±2.0)	26.6 (±3.7)
		AD	182.0 (±76.5)	23.7 (±2.9)	17.8 (±1.5)
Coccocypselum sp.	FO	JUV	154.1 (±77.7)	22.4 (±4.1)	48.0 (±10.2)
Declieuxia fruticosa (1)	GL	JUV	43.5 (±25.4)	34.7 (±7.9)	16.2 (±10.1)
		AD	470.1 (±77.9)	38.8 (±5.5)	6.2 (±1.3)
Declieuxia fruticosa (2)	GL	JUV	26.0 (±17.7)	29.1 (±3.0)	26.9 (±7.1)
		AD	97.5 (±33.7)	37.9 (±2.8)	9.8 (±1.1)
Declieuxia gracilis	GL	JUV	11.0 (±4.2)	25.8 (±3.2)	15.5 (±5.0)
		AD	22.3 (±4.7)	25.7 (±2.1)	9.3 (±1.2)
Psychotria capitata	FO	JUV	72.7 (±46.5)	32.3 (±1.6)	31.6 (±4.8)

Family/ Species	**Habitat**	**Stage**	**LA (mm^2)**	**LDMC (%)**	**SLA ($mm^2\ mg^{-1}$)**
		AD	1951.2 (±778.6)	38.1 (±3.7)	15.9 (±1.1)
Psychotria cf. subtriflora	FO	JUV	184.2 (±47.4)	17.6 (±2.9)	27.4 (±4.9)
Rudgea sessilis	FO	JUV	156.9 (±107.6)	29.2 (±2.7)	16.7 (±2.3)
		AD	1306.0 (±142.7)	33.0 (±1.8)	9.9 (±0.9)
NI 01	FO	JUV	70.0 (±20.8)	21.1 (±2.3)	42.7 (±8.4)
		AD	262.6 (±78.8)	22.9 (±1.5)	37.7 (±3.1)
Salicaceae					
Casearia sp.	FO	JUV	97.4 (±23.3)	27.2 (±3.1)	40.4 (±4.3)
Verbenaceae					
Lippia stachyoides var. *martiana*	GL	JUV	34.2 (±32.4)	28.7 (±3.4)	23.4 (±3.8)
		AD	280.0 (±93.2)	41.2 (±1.3)	5.7 (±0.3)
NI					
NI 02	GL	JUV	14.5 (±2.7)	17.3 (±2.5)	27.2 (±6.4)
NI 03	FO	JUV	193.5 (±76.9)	30.6 (±1.4)	31.8 (±3.6)
NI 04	FO	JUV	92.9 (±18.6)	29.4 (±1.5)	41 (±3.7)

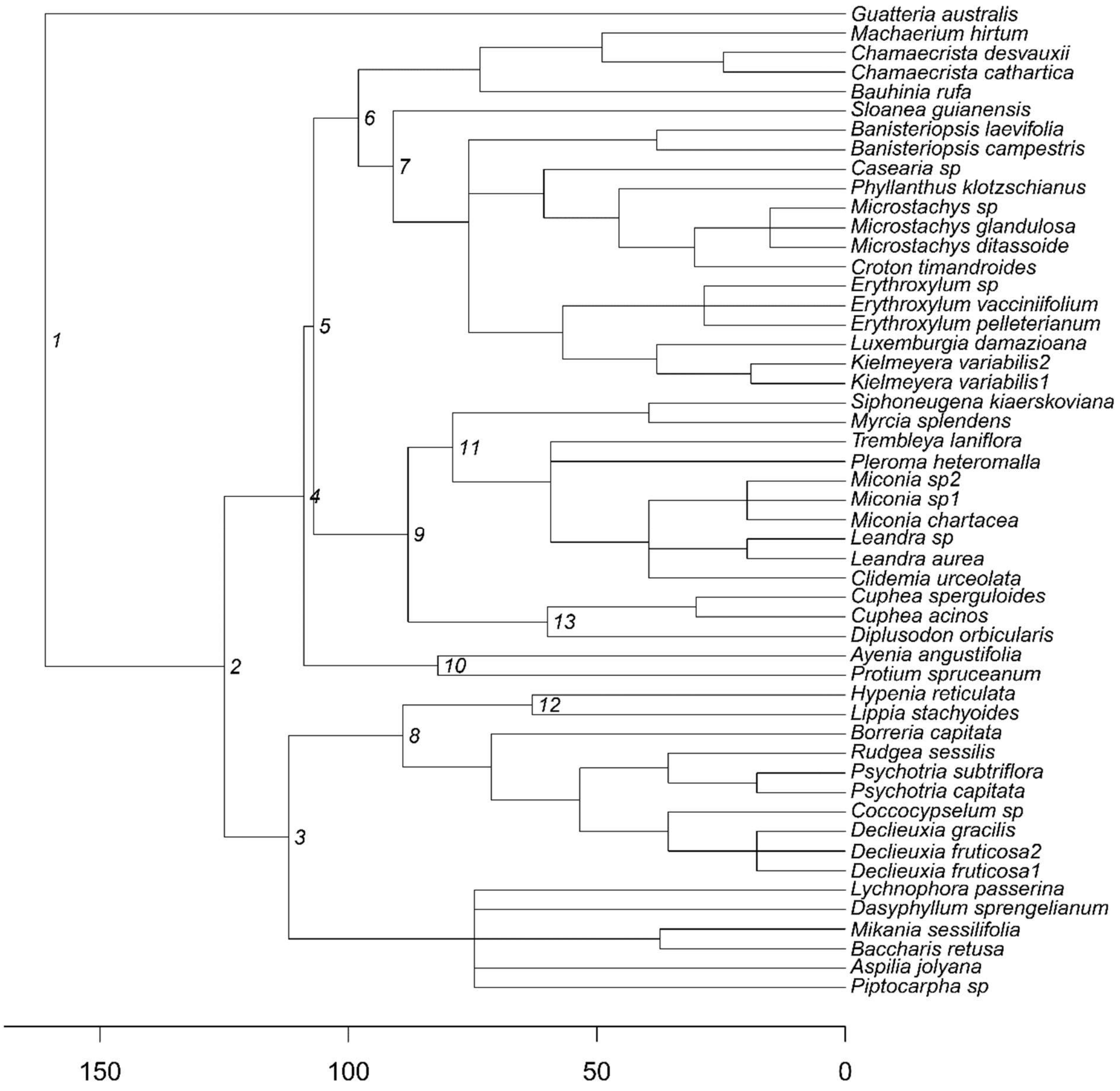
Supporting Information 3

Details on the resolution of polytomies and determination of node ages of the phylogenetic tree with 49 species from *campo rupestre* and neighbouring forest, sampled at *Serra do Cipó* south-eastern Brazil. The initial topology of the phylogenetic tree was obtained using the Phylomatic software (Webb & Donoghue, 2005) with the database of stored tree R20120829 for plants. To improve the resolution of several nodes and to obtain more precise estimates of branch lengths, estimated ages of 13 nodes were obtained in several studies listed in Table S3. Thereafter, non-dated nodes were positioned evenly between dated nodes using *bladj* algorithm of Phylocom software (Webb et al., 2008). Figure S1 shows dated nodes and the resulting phylogenetic tree.

Table S3: Estimated age (in million years before present) of nodes numbered in Figure S1.

node	estimated age	Reference
1	161	Wikström et al. (2001)
2	125	Forest & Chase (2009a)
3	112	Wikström et al. (2001)
4	109	Forest & Chase (2009b)
5	107	Forest & Chase (2009b)
6	98	Forest & Chase (2009b)
7	91	Forest & Chase (2009b)
8	89	Wikström et al. (2001)
9	88	Forest & Chase (2009b)
10	82	Forest & Chase (2009c)
11	79	Forest & Chase (2009b)
12	63	Bremer (2009)
13	60	Sytsma et al. (2004)

Figure S1: Phylogenetic tree with the 49 species from *campos rupestres*, south-eastern Brazil. Dated nodes are numbered from 1 to 13. A time scale (in million years before present) is shown below the tree.



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Supporting Information 4

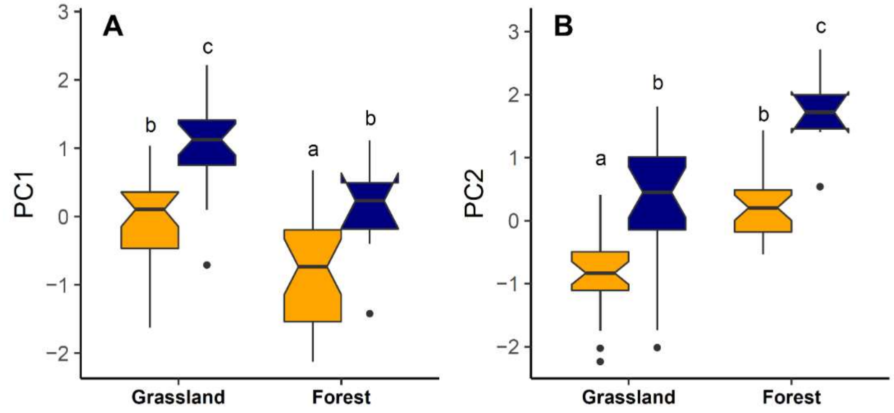


Figure S2. Comparisons between principal components analysis (PCA) scores for adults (dark blue) and juveniles (orange) of plant species from grassland and forest. A) Rotated component 1 (PC1) and B) rotated component 2 (PC2). Within each individual plot, different letters indicate significant differences based on 95% confidence intervals from linear mixed-effect models (Table 1).

Table S4. Phylogenetic signal in leaf functional traits of the sampled adult and juvenile plants according to Bloomberg's K-test. Significant *P*-value ($P < 0.05$) marked in bold indicate that closely related species have trait values that are more similar than expected by chance.

	Leaf traits	K	*P*
Adults	LA	0.648	0.1891
	LDMC	0.573	0.3082
	SLA	0.650	0.1257
Juveniles	LA	0.607	**0.0011**
	LDMC	0.379	0.4050
	SLA	0.393	0.3181

Table S5. Results of Wilcoxon rank sum test comparing the variation of CSR strategies between habitats and life stages, using species standard deviation of CSR values as inputs. A) Paired comparisons of individual variability among developmental stages analysed separately for the two habitats (grassland, n=21 pairs of juvenile/adult; forest, n=7 pairs of juvenile/adult). Significant *P*-values (*P* < 0.05) are marked in bold.

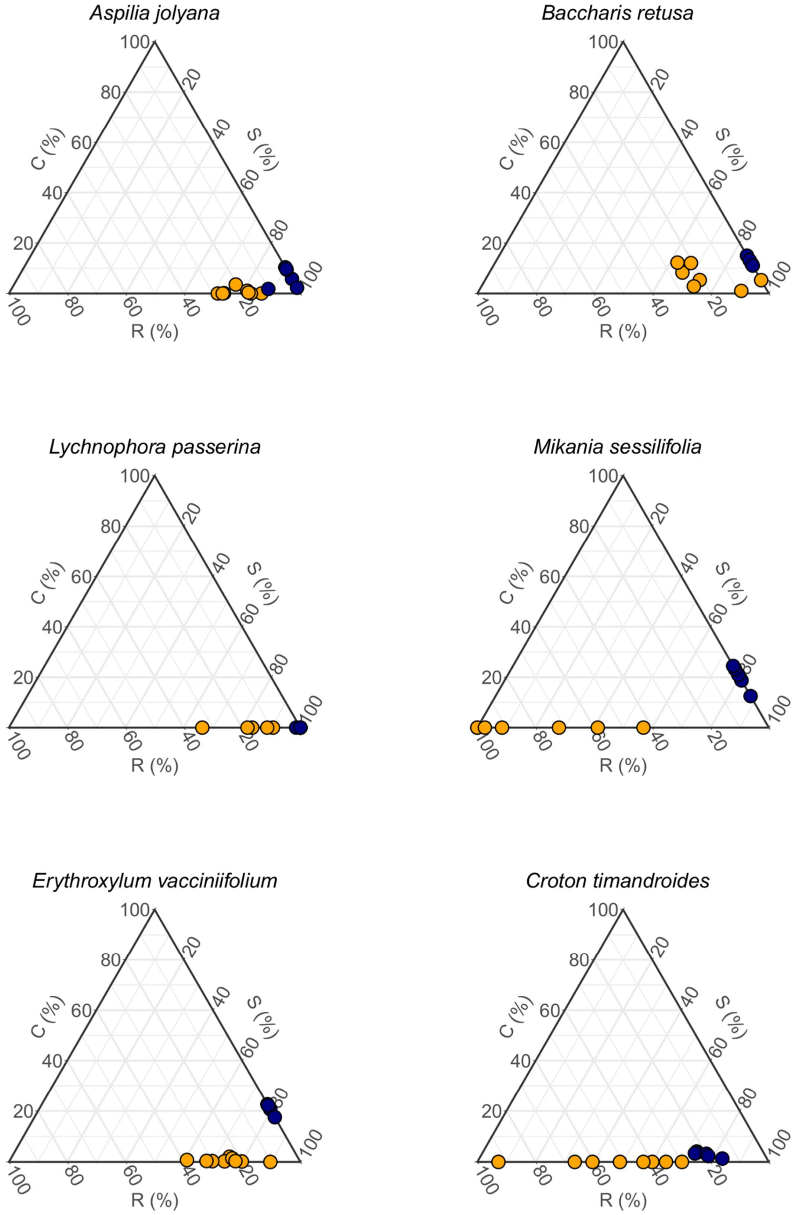
		Standard Deviation			
		Juvenile	Adult	W	*P*
Grassland	C	1.5 (±1.5)	2.9 (±2.4)	167	**0.0040**
	S	11.4 (±5.7)	3.8 (±2.6)	231	**< 0.0001**
	R	11.5 (±5.7)	1.3 (±2.2)	231	**< 0.0001**
Forest	C	2.7 (±1.4)	4.1 (±3.0)	6	0.2188
	S	7.9 (±4.9)	6.1 (±2.5)	20	0.375
	R	9.4 (±5.1)	4.2 (±1.8)	28	**0.0156**

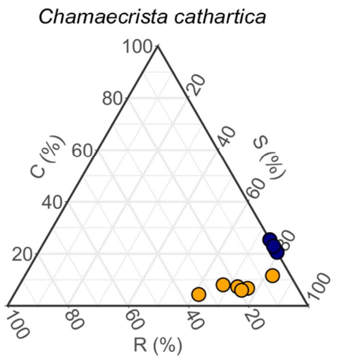
Dayrell RLC, Arruda AJ, Pierce S, Negreiros D, Meyer PB, Lambers H, Silveira FAO (2018).
Ontogenetic shifts in plant ecological strategies. *Functional Ecology.*

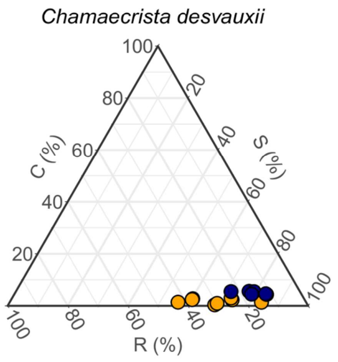
Supporting Information 5

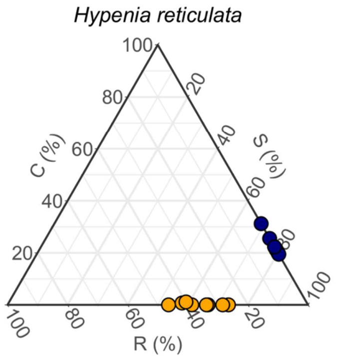
Ternary plots showing the location of juveniles and adults in the CSR triangle for individual samples (filled circles) in each population (juveniles - orange, adults - dark blue). Non-identified (NI).

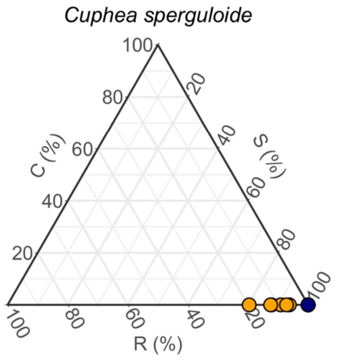
Campo rupestre grassland

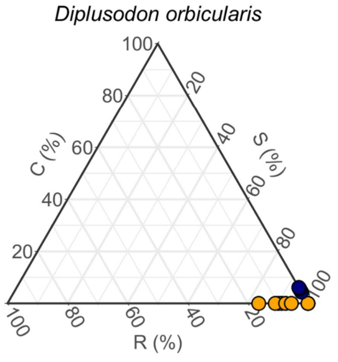


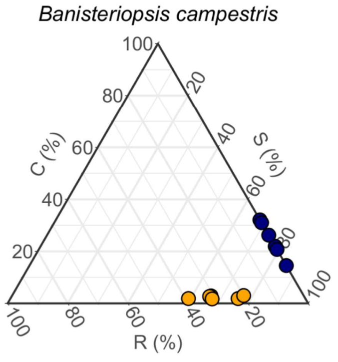

Chamaecrista cathartica
C (%)
S (%)
R (%)
100
80
60
40
20

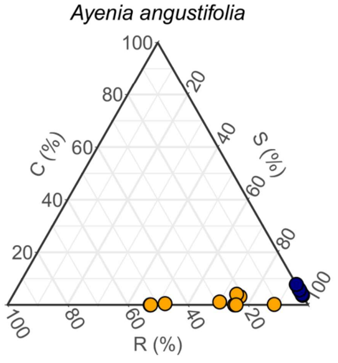

Chamaecrista desvauxii
C (%)
S (%)
R (%)
100
80
60
40
20

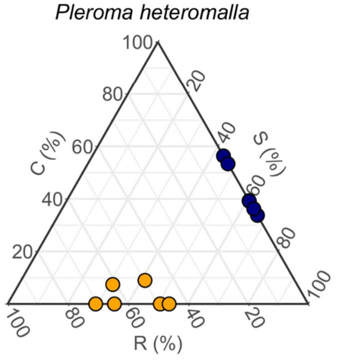

Hypenia reticulata
C (%)
S (%)
R (%)
100
80
60
40
20


Cuphea sperguloide
C (%)
S (%)
R (%)
100
80
60
40
20


Diplusodon orbicularis
C (%)
S (%)
R (%)
100
80
60
40
20


Banisteriopsis campestris
C (%)
S (%)
R (%)
100
80
60
40
20


Ayenia angustifolia
C (%)
S (%)
R (%)
100
80
60
40
20


Pleroma heteromalla
C (%)
S (%)
R (%)
100
80
60
40
20

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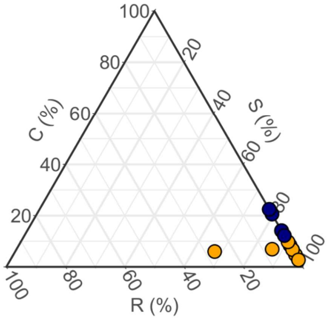
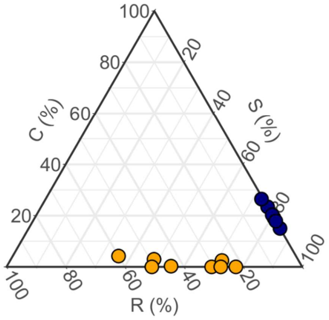
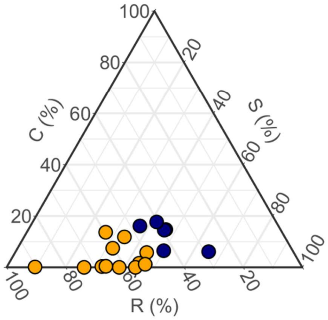
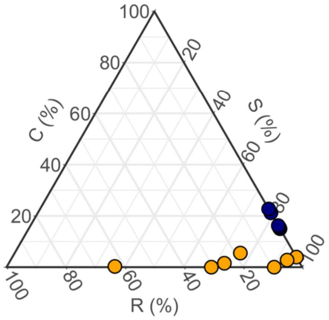

Trembleya laniflora


Luxemburgia damazioana


Borreria capitata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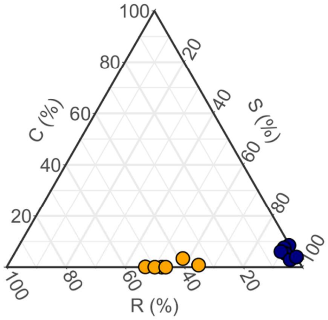
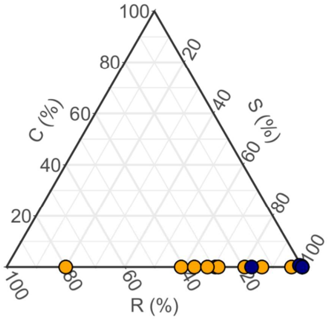
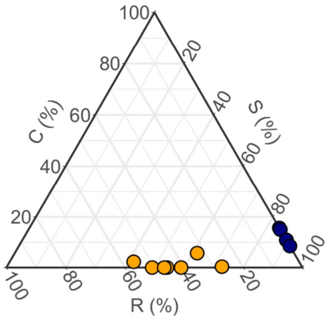
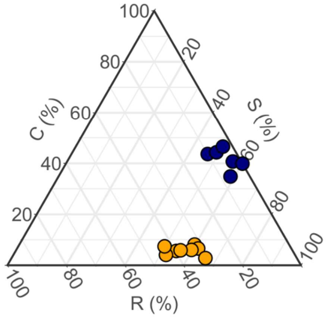

Declieuxia fruticosa (2)

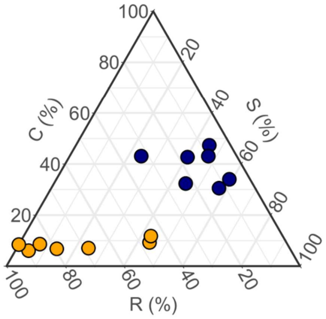

Declieuxia gracilis

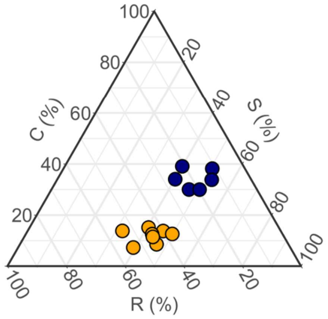

Lippia stachyoide

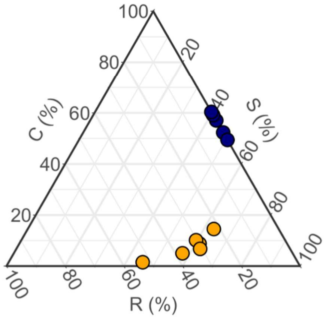
Dayrell RLC, Arruda AJ, Pierce S, Negreiros D, Meyer PB, Lambers H, Silveira FAO (2018). Ontogenetic shifts in plant ecological strategies. *Functional Ecology*.

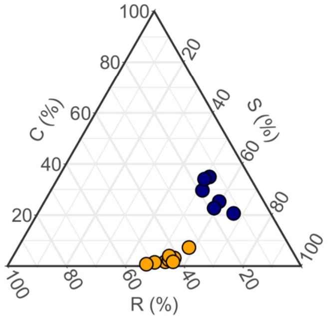
Forest

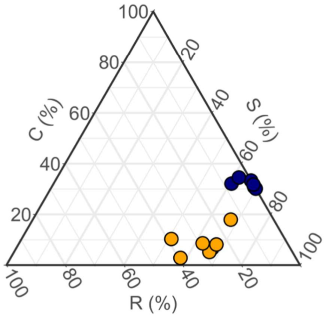

Guatteria australis

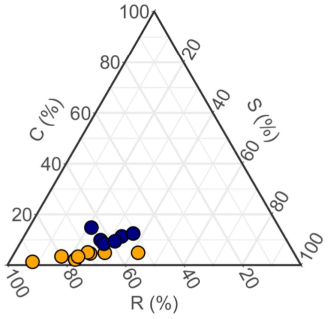

Dasyphyllum sprengelianum


Bauhinia rufa


Miconia chartacea


Psychotria capitata


Rudgea sessilis


Rubiaceae (NI)

Chapter 3

Shifts in leaf phosphorus allocation patterns during post-fire regeneration responses in nutrient-poor soils

ABSTRACT

Adult plants from P-impoverished habitats exhibit multiple strategies to optimise P use and cope with P limitation. Strategies for P acquisition and use concerning different regeneration forms, such as resprouts (burnt adults) and seedlings, are important to understand community assembly, but remain poorly known. Here, we investigated shifts in P-use strategies in post-fire regeneration forms in the P-impoverished *kwongan* (Southwest Australia). We specifically assessed differences in leaf total P, inorganic P (Pi) and organic P fractions (nucleic acids, phospholipids, small metabolites and a residual fraction) among regeneration forms, and their association with soil P availability, seed P content and other plant traits. We focused on 10 species of three dominant families (Fabaceae, Myrtaceae and Proteaceae) from burnt and unburnt sites at Badgingarra National Park. Seedlings had greater leaf total [P] than conspecific unburnt adults, while burnt adults exhibited a short-term and less pronounced increase in leaf total [P]. One year after fire passage, soil [Pi] was still increased at the burnt site, but burnt and unburnt adults had similar leaf total [P]. The greater leaf total [P] in seedlings was mostly due to a greater leaf [Pi] than in unburnt adults, but also due to higher levels in at least some of the organic P fractions. Proteaceae seedlings had remarkably pronounced concentrations of leaf total P, Pi and organic P fractions (especially lipid P), associated with a higher seed P content. The assessment of different species from three dominant families of the *kwongan*, reveals the

spectrum of variation in P-use and -allocation strategies in the community, and demonstrates the singular strategies of Proteaceae seedlings from Southwest Australia.

INTRODUCTION

Terrestrial plants exhibit great variation in traits, resource allocation and growth rate (Chapin 1980; Lambers & Poorter 1992; Reich *et al.* 1992) that enable or prevent their growth and reproduction under specific sets of environmental conditions, ultimately driving community assembly (Keddy 1992; Grime 2006). Phosphorus (P) availability is one of the main filters that limit plant growth in many ecosystems, including many biodiversity hotspots (Wassen *et al.* 2005; Chapman *et al.* 2006; Lambers, Raven, *et al.* 2008). Plants inhabiting severely P-impoverished soils, such as those from the *kwongan* in Southwest Australia, typically exhibit a conservative strategy that comprises a costly leaf area, long leaf lifespan, slow growth rates (Specht & Rundel 1990; Lambers & Poorter 1992; Wright & Westoby 2003), low leaf P concentrations, high photosynthetic P-use efficiency (Wright, Groom, *et al.* 2004; Lambers *et al.* 2012) and proficient P remobilisation from senescing leaves (Denton *et al.* 2007; Hayes *et al.* 2014), allowing plants to survive and reproduce in systems with low P availability.

Phosphorus is an essential component of many cellular structures and metabolic processes, and plants exhibit multiple strategies to optimise P use and cope with P limitation. To explore specific strategies, the total P content in leaves can be divided into free inorganic phosphate (Pi) and four organic P fractions: nucleic acid P, lipid P, metabolic P and residual P (Chapin & Bieleski 1982; Hidaka & Kitayama 2013). Leaf Pi consists of two pools: the metabolically active cytoplasmic Pi pool, whose concentration is strictly regulated, and vacuolar Pi, which is used to store Pi in excess of current demands and is the most variable Pi fraction (Veneklaas *et al.* 2012). Most of the nucleic acid P fraction in a plant consists of ribosomal RNA (rRNA; Bieleski 1968; Kanda *et al.* 1994), and investment in ribosomes is essential to support protein synthesis demands of growth (Elser *et al.* 2010). The lipid P fraction comprises mainly phospholipids of cell membranes, and the metabolic P fraction consists mostly of intermediates of carbon metabolism and small nucleotides (Veneklaas *et al.* 2012; Hidaka & Kitayama 2013). The residual P fraction mainly contains phosphorylated proteins (Hidaka & Kitayama 2013), which are involved in regulating cellular processes (Ranjeva & Boudet 1987).

Physiological strategies to use P highly efficiently and optimise P allocation to different fractions are well known for Proteaceae, and explain why so many members of the family are able to thrive on the P poorest soils of Southwest Australia (Lambers *et al.* 2010). For instance, they exhibit low P allocation to rRNA (typically the largest organic P pool in plants), which results in low rates of protein synthesis, further leading to less need for P in other pools and slow growth. At the same time, these plants are able to maintain high concentrations of phosphorylated metabolites on a protein basis which allows the enzymes to operate effectively, which explains their high photosynthetic P-use efficiency (Sulpice *et al.* 2014). Some Proteaceae also extensively replace phospholipids in young leaves with lipids that do not contain P during leaf development, while maintaining relatively fast rates of photosynthesis (Lambers *et al.* 2012; Kuppusamy *et al.* 2014). In crop species, this same process of lipid replacement is a response to P starvation (Dörmann & Benning 2002), which inevitably leads to a decline in the rate of photosynthesis (Brooks *et al.* 1988; Rao & Terry 1989). Species of some other families, such as Fabaceae and Myrtaceae, also inhabit the same P-impoverished soils (Zemunik *et al.* 2016). They exhibit convergence with Proteaceae in leaf traits related to high P-use efficiency (Pereira *et al.* 2019), but not the same strategies for distributing P among different P fractions within the leaves (Yan *et al.* 2019), and most of their physiological strategies of coping with P scarcity remain poorly understood.

The *kwongan* is a fire-prone ecosystem, and its extant plant species are able to persist by two post-fire regeneration responses: resprouting and/or recruitment of new seedlings (Miller & Dixon 2014). Traits and resource-allocation patterns in individuals emerging from these two responses may differ from those from unburnt adults due to the inherent differences in plant size (Christensen 1977; Weiner 2004; Poorter *et al.* 2015), differences in lignified sclerenchymatic tissue during leaf development depending on functional priorities – such as growth, defence, storage, reproduction (Farnsworth 2004; Ishida *et al.* 2005), and greater soil P availability on burnt sites (Lamont *et al.* 1993). This variation in traits and resource-allocation patterns throughout the life of individual plants (e.g., seedling, adult, adult resprouting after fire) is important to explain community assembly and species distribution (Grubb 1977; Larson & Funk 2016). So far, studies regarding species from fire-prone ecosystems have shown that burnt individuals resprouting after fire exhibit different [P] in leaves from unburnt ones (Christensen 1977; Reich *et al.* 1990; Radho-Toly *et al.* 2001; Durán *et al.* 2010). We also know that while adult individuals have a very low leaf [P], seed [P] is often disproportionately high (Kuo *et al.* 1982; Denton *et al.* 2007; Groom & Lamont 2010), suggesting that a significant nutrient supply provided by seeds is key to successful seedling recruitment in these habitats (Milberg & Lamont 1997). However, it is not clear how the high P supply is used by seedlings or by plants resprouting after fire passage.

Here, we aimed to investigate and assess shifts in P-use strategies during post-fire regeneration responses in severely P-impoverished soils. We investigated P-allocation patterns of unburnt and burnt adults and seedlings naturally occurring in their P-impoverished habitats at the community-level. Our hypotheses were: 1) leaf [P] is higher in seedlings than in unburnt adults; 2) leaf [P] is higher in burnt than in unburnt adults; 3) seed P content is positively associated with leaf [P] in seedlings; 4) greater P availability in soil after fire is associated with greater P allocation to leaves of seedlings and burnt adults; and 5) the higher leaf [P], when present in seedlings and/or burnt adults, is preferentially allocated to the nucleic acid fraction, more so than to the other fractions, since more rRNA is important for protein synthesis to sustain faster growth rates after fire. We also assessed whether P-acquisition and P-allocation strategies were associated with plant lineages.

MATERIALS AND METHODS

Study area, species selection and study design

We conducted our study in a *kwongan* shrubland at Badgingarra National Park (Cadda Rd, 30°23'S, 115°24'E, and altitude of approximately 230 m a.s.l.), Southwest Australia, c. 180 km north of Perth. The park is located west of the Dandaragan Scarp, in an undulating landscape dominated by lateritic plateau remnants and gravel hills with sand-filled valleys (Sawkins 1981). The area is within the Southwest Australian Floristic Region, where P availability is particularly low (McArthur *et al.* 2004) and the most species-rich and dominant families are Myrtaceae, Fabaceae and Proteaceae (Mucina *et al.* 2014). The region has a Mediterranean climate, with warm, dry summers and cool, moist winters. Mean annual rainfall (measured between 1962 and 2018) is 539 mm, of which about 75% falls in winter, between May and September (Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/).

We sampled plants from two adjacent sites with contrasting fire history: (1) a burnt site, subjected to a prescribed fire in October 2016, and (2) an unburnt site, which was last burnt in 1995 (fire history provided by the Department of Biodiversity, Conservation and Attractions, Government of Western Australia). We selected ten species that occurred at both unburnt and burnt sites and belonged to the three most species-rich families: Fabaceae (*Daviesia chapmanii*, *Jacksonia floribunda*, *Jacksonia nutans*), Myrtaceae (*Eucalyptus todtiana*, *Verticordia* *grandis*, *Melaleuca leuropoma*), Proteaceae (*Banksia candolleana*, *Hakea flabellifolia*, *Petrophile macrostachya*, *Stirlingia latifolia*).

Samples were collected in two field campaigns: at the beginning of the first rainy season after fire (June 2017) and at the beginning of the following dry season (October and November 2017). For each species, we aimed to identify individuals that represented three different post-fire regeneration responses, hereafter referred to as 'regeneration forms': 1) 'seedling', emerged from seeds in the burnt site after fire, 2) 'burnt adult', resprouting plants in the recently burnt site, and 3) 'unburnt adult', individuals growing in a site which has not been burnt for more than 20 years and were used as controls. Unburnt and burnt adult plants were sampled in the first field campaign. Seedlings emerged in the burnt site at the onset of the dry season. Therefore, at the second field campaign, we collected plant material from unburnt and burnt adults, and also from conspecific seedlings whenever it was possible to find at least four replicates. Individual plants served as replicates for adults. For seedlings, individual plants served as replicates for leaf dry-matter content (LDMC) in most cases, and nutrient analyses included a pool of individuals that added sufficient plant material to perform the assays (number replicates shown in Table S1).

Leaf sampling and leaf dry-matter content

At the beginning of the first rainy season after fire, we collected branches with the youngest fully-expanded leaves from unburnt and burnt adults. Samples were immediately hydrated using a spray bottle, placed in moist paper bags, sealed in plastic bags, kept in a cooler until placed in a refrigerator for periods of 12 to 24 h at 4°C, and leaves were patted dry to obtain leaf fresh weight (Pérez-Harguindeguy *et al.* 2013). Leaves were then dried to constant weight in an oven at 70°C and used to determine leaf dry weight, total P and manganese (Mn) concentrations. All LDMC measurements were determined on at least two undamaged, fully-developed leaves (including the petiole) per individual (Pérez-Harguindeguy *et al.* 2013). We used an analytic scale (precision of 0.01 mg) to obtain leaf fresh weight (from rehydrated leaves) and leaf dry weight (from dry leaves).

At the beginning of the first dry season after fire, we sampled all three regeneration forms. Leaves from unburnt and burnt adults were collected in the field, and immediately frozen in liquid N_2 to prevent organic P from being converted into inorganic P (Pi). We collected whole seedlings and associated soil, placed them in pots, and kept them under shaded conditions in water-saturated soil for 24 h to rehydrate the leaves. Leaves from seedlings were sampled, used to obtain leaf fresh weight and also immediately frozen in liquid N_2. All leaf samples were stored at -80°C for 2-3 months until freeze-dried for seven days (VirTis Benchtop "K", New York, USA) to be used to measure P fractions. Seedling leaf dry weight was determined from freeze-dried samples.

Leaf total P and Mn concentrations at the beginning of the rainy season

Some plants from low-P habitats release carboxylates in the rhizosphere as a strategy to mobilise soil P, and end up also mobilising a range of micronutrients, including Mn. Based on this, high leaf [Mn] has been proposed as a proxy for the root exudation of significant amounts of carboxylates (Lambers, Hayes, *et al.* 2015; Pang *et al.* 2018) and was used here to screen for belowground changes in root functioning related to P-mobilisation strategies among individuals in burnt and unburnt sites. We determined differences in leaf [P] and [Mn] across unburnt and burnt adults at the beginning of the first dry season after fire. Dried leaf samples used for LDMC measurements were ground to a fine powder in a ball-mill grinder (Geno Grinder, Spex SamplePrep, Metuchen, NJ, USA) using plastic vials and yttrium-stabilised zirconium ceramic beads. Subsamples (200 mg) were then digested in concentrated HNO_3:$HClO_4$ (10:1) under heat, and analysed for [P] and [Mn] using inductively coupled plasma optical-emission spectrometry (ICP-OES; School of Agriculture and Environment, University of Western Australia, Perth, Australia).

Leaf total P concentrations and P-fractions at the start of dry season

To assess differences in P-allocation to the various P fractions among the three regeneration forms collected at the start of dry season, we divided the total leaf P pool into Pi and four organic fractions: nucleic acid P (i.e., RNA and DNA), lipid P (i.e. phospholipids), metabolic P (small P-containing metabolites such as sugar phosphates and ATP), and a residual P (phosphoproteins and unidentified molecules). The procedure that separates the four organic fractions also extracts Pi along with the metabolic P fraction. To obtain the real concentration for metabolic P fraction, [Pi] needs to be subtracted from this ‘metabolic P plus Pi’ fraction. However, some unknown organic compound(s) in the leaves of Myrtaceae and *B. candolleana* adults caused an interference in the colorimetric assays to determine leaf [Pi], preventing us to calculate the metabolic P fraction for these species. Therefore, we chose to present data relative to ‘metabolic P plus Pi’ fraction to enable comparisons of all species across all regenerative forms.

The freeze-dried samples were ground as described above, and divided into three subsamples. The fractionation procedure to determine P in the four organic fractions was the same as described by Yan *et al.* (2019), adapted from Hidaka and Kitayama (2013). Briefly, a subsample of the dried ground leaf material (50 mg) was homogenised and extracted three times in 1 mL of cold chloroform:methanol:formic acid (CMF; 12:6:1 v/v/v) and three times with 1.26 mL of cold chloroform: methanol:water (CMW; 1:2:0.8 v/v/v) in a 2 mL tube. The supernatant was collected, transferred to a chilled 10 mL tube, 1.9 mL chloroform-washed water was added and centrifuged, causing the extract to separate into two layers. The aqueous upper layer (fraction 1) and the green bottom layer (lipid P fraction) were transferred to different acid-washed 25 mL conical flasks. The pellet remaining in the 2 mL tube was then extracted with 1 mL of cold 85% (v/v) methanol. The methanol extract was added to the conical flask containing fraction 1. The 2 mL tube was placed under speed vacuum for ~15 min to remove methanol and chloroform from the pellet. The dry pellet was extracted twice with cold 1 mL 5% (w/v) trichloroacetic acid (TCA) solution for one hour by constant shaking in a cold room (4 °C). The 5% TCA extract was added to the conical flask containing fraction 1 (metabolic P plus Pi fraction). The pellet remaining in the 2 mL tube was extracted three times with 1 mL 2.5% (w/v) TCA at 95°C on a hot plate for 1 h, by shaking at 20 min intervals. The 2.5% TCA extract was added to a clean acid washed conical flask (nucleic acid P). The residue remaining from the hot TCA extraction is the residue P fraction. All liquid-solid separations were accomplished by centrifugation at 1180 g at 4°C. Samples were kept on ice at all times until hot TCA extraction, to prevent the conversion of organic P forms to Pi.

Another subsample (20 mg) of the dried ground leaf material was used to determine total [P]. This subsample and each of the P fractions were acid-digested in concentrated HNO_3:$HClO_4$:HCl (6:1:2) under heat before quantifying [P]. The sum of [P] in the fractions was used to assess recovery, which was always greater than 85% of the total [P] determined directly from the leaf. Leaf [Pi] was determined by disrupting a subsample (20 mg) of dried ground leaf material in 0.5 mL 1% (v/v) acetic acid by mechanical shaking (Precellys 24 Tissue Homogenizer, Bertin Instruments, Montigny-le-Bretonneux, France) at 1500 rpm for 15 s, repeated twice more with a 5 s pause between shakings. The homogenate was centrifuged twice at 21,000 × g for 15 min at 4 °C to remove debris. The samples were kept on ice at all times to prevent organic P being converted into Pi. Phosphorus concentrations in Pi extracts and in digested samples were determined colorimetrically using the malachite green method (Motomizu *et al.* 1983).

Soil characterisation

To assess fire-induced differences in soil properties that remained at the time of seedling emergence, we established a 150 m transect at unburnt and burnt sites and collected one soil sample every 15 m (10 soil samples for each site) at a depth of 0–20 cm, using a 20-mm diameter sand auger. Samples were sealed in plastic bags and kept in a cooler until placed in a refrigerator at the same day of collection. We first obtained soil fresh weight (~15 g of soil from sealed bags after removing large organic debris) and soil dry weight (after soil had dried to constant weight in an oven at 105°C). Remaining samples were sieved (<2 mm) to remove large organic debris, homogenised and air-dried prior to analyses. Total P, Pi and organic P concentrations were determined using the ignition method (Saunders & Williams 1955). Readily exchangeable phosphate (resin P) was measured by extraction with anion-exchange membranes (Turner & Romero 2009). The [P] in all the extracts was determined using ICP-OES. Electrical conductivity (EC) was measured in a 1:2 soil-to-solution ratio in water using an EC meter. Soil pH was determined using a glass electrode in a 1:2 soil-to-solution ratio in both deionised water and 10 mM $CaCl_2$.

Seed total P

To assess the importance of seed P content in driving P allocation to leaves in seedlings, seeds of *J. floribunda* and *J. nutans* were also collected at Badgingarra National Park, while seeds of *M. leuropoma*, *B. candolleana* and *S. latifolia* were obtained from a commercial seed supplier (Nindethana Seed Services, Albany, Western Australia). We could not obtain seeds of *D. chapmanii* when carrying out the analyses for this study. Four lots of seeds were analysed to determine seed mass, [P] and P content for each species. Each lot of seeds was counted and oven dried (70°C, 72 h), before being weighed and ground, as previously described. Samples were then digested in concentrated HNO_3:$HClO_4$ (10:1) and [P] was determined using ICP-OES.

Leaf gas exchange

To assess whether adult burnt plants differed from the unburnt in leaf functioning, we measured gas exchange on plants from each of the two sites for each of the four Proteaceae in October 2017. We focused on Proteaceae because of their well-documented specialised traits to cope with P deficiency and fire in Southwest Australia (Lambers *et al.* 2013; Lambers, Finnegan, *et al.* 2015). Proteaceae are also known for their rapid rates of photosynthesis, despite their extremely low leaf [P] (Denton *et al.* 2007; Lambers *et al.* 2012). The measurements were made on one mature, fully expanded, undamaged and sun-exposed leaf from each of 10 replicate plants for each regeneration form, using an LI-6400 portable gas-exchange system (Li-Cor, Lincoln, NE, USA).

All gas-exchange measurements were performed at ambient temperature (16–21°C), with a constant [CO_2] of 400 μmol mol^{-1} air. Photosynthetically active radiation was set at 1500 μmol quanta m^{-2} s^{-1} (LI-6400-02B red-blue light source; Li-Cor). Leaves were harvested to determine leaf area using a leaf area meter following the gas-exchange measurements, which was used to calculate area-based net photosynthetic rates at saturating light intensity ($A_{\max}$) and stomatal conductance (g).

Statistics

Within each species, differences in LDMC, concentrations of leaf total P, total Mn, Pi and each P fraction, $A_{\max}$ and g, across regeneration forms, were tested using generalised least squares models (GLS; Pinheiro & Bates, 2000). Within each regeneration form, differences in concentration of leaf total P, total Mn, Pi and each P fraction, across species, were tested using GLS (Pinheiro & Bates 2000). Differences in concentrations of leaf total P, Pi and each P fraction, across regeneration forms, families and their interaction, were tested using linear mixed-effects models (LMM), with species set as the random effect (Pinheiro & Bates 2000). Differences in seed [P] across species were also assessed using GLS (Pinheiro & Bates 2000). Finally, differences in soil attributes, and soil total P, Po, Pi and resin P concentrations, across sites (unburnt and burnt), were tested using GLS (Pinheiro & Bates 2000).

Significance of models was estimated by comparing a minimal model with the null model. In LMMs, the minimal models were obtained by a stepwise deletion of non-significant parameters and their interaction, starting from a full factorial model. In the minimal model, only significant factors remained. For each model, residuals were visually inspected for heteroscedasticity. In the presence of heteroscedasticity, the variance structure of the data was modelled, with the best variance structure determined by comparing AICc and standardised residual plots (Zuur *et al.* 2009). The GLSs and LMMs were performed using the 'gls' and 'lme' functions, respectively, and the variance structure of the data was modelled using the 'weights' option within the 'nlme' package (Pinheiro *et al.* 2017). Comparisons between test regeneration forms within each model were determined by the post-hoc Tukey–Kramer honest test (McCulloch & Neuhaus 2005) by using 'lsmeans' package (Lenth 2016). Statistical analyses were performed with the R software platform (R Development Core Team 2017). Significance level was set at $P<0.05$ for all analyses.

RESULTS

1. Leaf dry-matter content of unburnt and burnt adults, and seedlings

Leaf dry-matter content was generally greater for unburnt adults, followed by the burnt ones and lowest in seedlings (Table 1; Supplementary material Table S2). Values of LDMC in burnt adult ranged between 76 and 98% of those of unburnt adults, while seedling values ranged from 53 to 94%. *Banksia candolleana* was the only exception, where seedling LDMC values were the same as that of adults. There was no significant difference in LDMC between unburnt and burnt adults of *H. flabellifolia* and *D. chapmanii*.

Table 1. Leaf dry-matter content (LDMC; mg g^{-1}) of adults from an unburnt site, resprouting adults from a burnt site sampled at the beginning of the wet season and of seedlings sampled at the beginning of the dry season. For adults, fresh mass was obtained from leaves that were fully rehydrated before measurement. For seedlings, fresh mass was obtained from leaves that were immediately cut off plants in moist soil under shaded conditions for one day after field harvest. Different letters indicate significant differences among regeneration forms within each individual species based on generalised least squares models followed by Tukey–Kramer honest test. Values are shown as means ± SE (relative change to unburnt adult; %). NA indicates lack of data.

Family / species	**Unburnt**	**Burnt**		**Seedling**	
	(mg g^{-1})	(mg g^{-1})	%	(mg g^{-1})	%
Fabaceae					
Daviesia chapmanii	374^{b} ± 8	363^{b} ± 7	(98)	249^{a} ± 19	(67)
Jacksonia floribunda	452^{c} ± 3	369^{b} ± 10	(82)	265^{a} ± 6	(59)
Jacksonia nutans	449^{c} ± 5	340^{b} ± 11	(76)	273^{a} ± 12	(61)
Myrtaceae					
Melaleuca leuropoma	539^{c} ± 6	413^{b} ± 8	(77)	283^{a} ± 13	(53)
Eucalyptus todtiana	475^{b} ± 7	434^{a} ± 7	(92)	NA	
Verticordia grandis	422^{b} ± 9	344^{a} ± 12	(82)	NA	
Proteaceae					
Banksia candolleana	528^{b} ± 15	428^{a} ± 8	(82)	492^{ab} ± 47	(94)
Stirlingia latifolia	401^{c} ± 8	363^{b} ± 5	(91)	277^{a} ± 21	(70)
Hakea flabellifolia	465^{a} ± 5	440^{a} ± 22	(95)	NA	
Petrophile macrostachya	496^{b} ± 4	460^{a} ± 14	(93)	NA	

2. Leaf total P and Mn concentrations at the beginning of the rainy season

Nine out of the 10 species had significantly higher leaf total [P] for burnt adults than for their unburnt counterparts at the beginning of the rainy season (Fig. 1; Supplementary material Table S3). The median [P] for burnt plants compared with unburnt plants was 11% higher for *E. todtiana*, ~40% for *J. floribunda* and *P. macrostachya*, ~50% for *J. nutans* and *M. leuropoma*, ~70% for *B. candolleana* and *H. flabellifolia* and around 100% for *V. grandis* and *S. latifolia*. The difference was also significant when the leaf [P] was expressed per unit leaf fresh weight for six species, including all four Proteaceae, *M. leuropoma* and *V. grandis*, but not for any of the Fabaceae (Supplementary material Fig. S1, Table S4). Only two species showed differences in leaf [Mn] between burnt and unburnt plants, and these differences were significant on both a dry and a fresh weight basis. Unburnt adults of *P. macrostachya* exhibited a three-fold greater leaf [Mn] than burnt ones, while those of *M. leuropoma* had 40% less (Supplementary material Fig. S1, Tables S2 and S3).

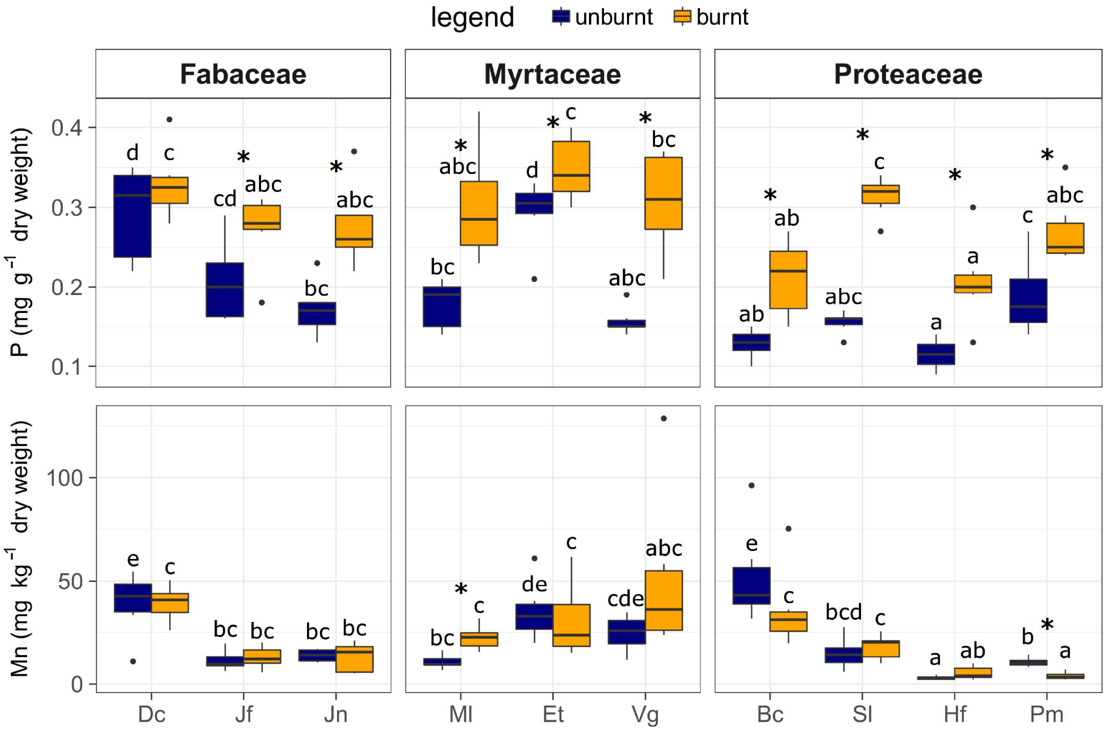


Figure 1. Leaf total phosphorus (P) and manganese (Mn) concentrations on a dry weight basis of adults from an unburnt site (unburnt) and resprouting adults from a burnt site (burnt) for 10 species collected at the beginning of the rainy season at Badgingarra National Park, Southwest Australia (Supplementary material Table S3). Box plots show medians (horizontal lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile ranges (whiskers) and outliers (filled circles). Different letters indicate significant differences among species within each regeneration form, * expresses significant differences between unburnt and burnt adults within each individual species based on generalised least squares models followed by the Tukey–Kramer honest test. *Daviesia chapmanii* (Dc), *Jacksonia floribunda* (Jf), *Jacksonia nutans* (Jn), *Melaleuca leuropoma* (Ml), *Eucalyptus todtiana* (Et), *Verticordia grandis* (Vg), *Banksia candolleana* (Bc), *Stirlingia latifolia* (Sl), *Hakea flabellifolia* (Hf), *Petrophile macrostachya* (Pm). Regeneration forms were unburnt and burnt adult plants.

3. Leaf total P concentration at the start of the dry season

At the start of the dry season, only two out of the 10 sampled species, *B. candolleana* and *M. leuropoma*, exhibited higher leaf [P] for burnt adults (~40% greater) than for their unburnt counterparts (Fig. 2A; Supplementary material Table S5). Seedlings had higher leaf [P] than the conspecific unburnt adults for all six sampled species, but the magnitude of the difference greatly varied among species (Fig. 2A; Supplementary material Table S5). *Banksia candolleana* and *S. latifolia* seedlings showed the largest difference in leaf [P] relative to unburnt adults, approximately eight- and four-fold, respectively. The smallest difference in leaf [P] was observed in seedlings of *J. nutans* (28%), and an intermediate difference in *J. floribunda* (72%), *D. chapmanii* (two-fold) and *M. leuropoma* (2.5-fold). Seedlings also showed higher leaf [P] than the burnt adults, except for *D. chapmanii* and *J. nutans* (Fig. 2A).

The effect of regeneration form on leaf [P] was significant, and also depended on the interaction between site and family, but not on family by itself (P<0.0001; Supplementary material Table S6). This reflects the greater leaf [P] of seedlings than of the other regeneration forms and the very high [P] for Proteaceae seedlings (Fig. 2B). Proteaceae adults also tended to show lower leaf [P] than adults of other two families, but this was not significant (Fig. 2B).

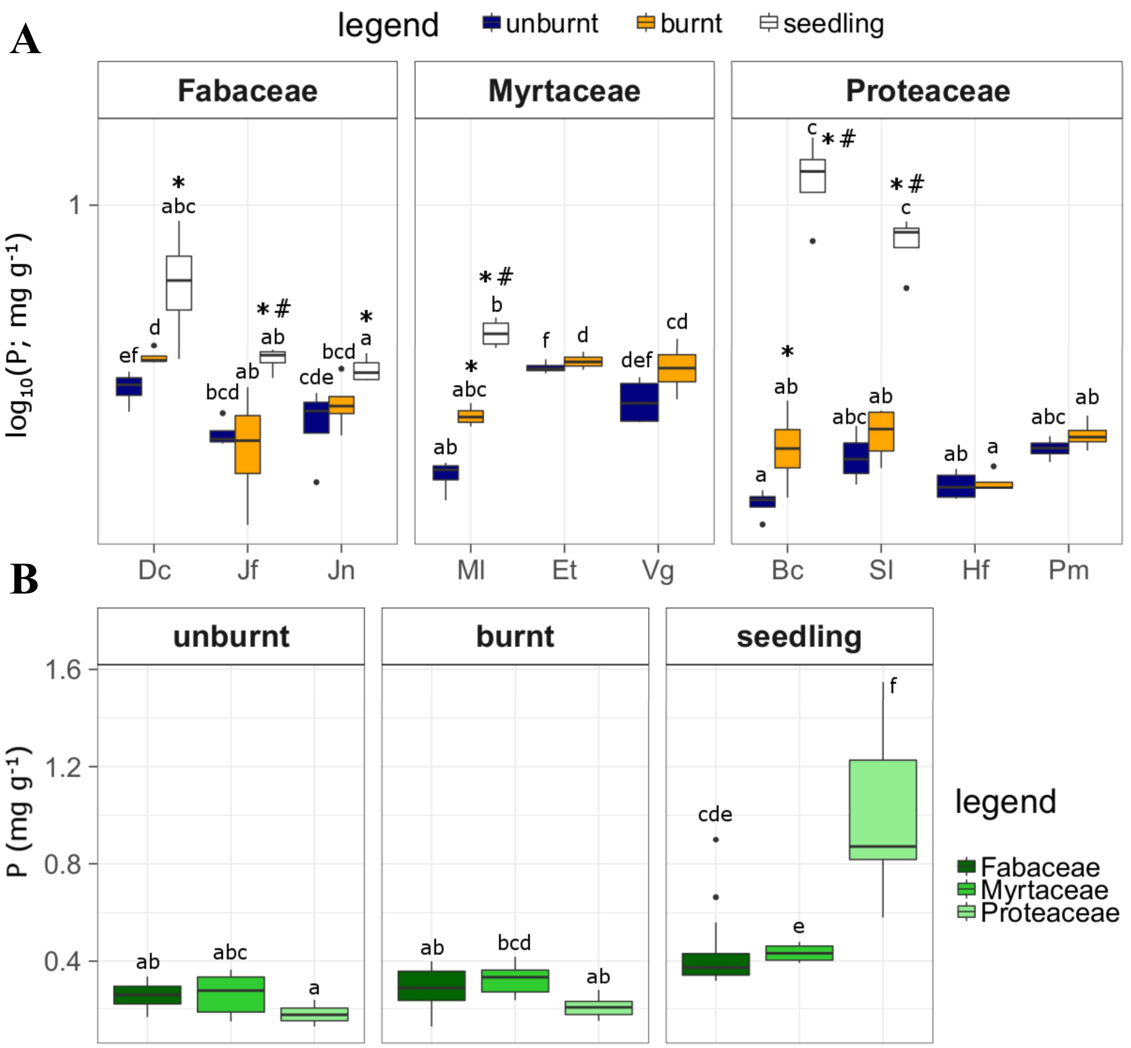


Figure 2. Leaf total phosphorus (P) concentrations of unburnt and burnt adults and seedlings at the beginning of the rainy season for A) each individual species and B) the three families collected in October and November 2017 at Badgingarra National Park, Southwest Australia (Supplementary material Tables S4, S5). Box plots show medians (horizontal lines) with quartiles (box margins), the lowest and highest values within 1.5-interquartile ranges (whiskers) and outliers (filled circles). Within panel A, different letters indicate significant differences among species within each regeneration form, and symbols express significant differences among regeneration forms within each individual species (* relative to unburnt; # relative to burnt) based on generalised least squares models followed by the Tukey–Kramer honest significant difference test. Within panel B, different letters indicate significant differences based linear mixed-effects models followed by the Tukey–Kramer honest test. *Daviesia chapmanii* (Dc), *Jacksonia floribunda* (Jf), *Jacksonia nutans* (Jn), *Melaleuca leuropoma* (Ml), *Eucalyptus todtiana* (Et), *Verticordia grandis* (Vg), *Banksia candolleana* (Bc), *Stirlingia latifolia* (Sl), *Hakea flabellifolia* (Hf), *Petrophile macrostachya* (Pm). Sampled families were Fabaceae, Myrtaceae and Proteaceae, and regeneration forms were unburnt and burnt adults and seedlings.

4. Soil characteristics

One year after the plants were burnt, total soil [P] was remarkably low at both sites, but significantly higher at the burnt site (mean ± SE; 5.5 ± 0.4 mg kg^{-1}) than at the unburnt site (4.3 ± 0.3 mg kg^{-1}; Fig. 3A; Supplementary material Table S7). More than 70% of soil P was in organic form (Po), and there was no difference in soil [Po] between sites. Soil [Pi] was significantly higher at the burnt site, and accounted for the higher total [P] (Fig. 3A). Although the variable 'site' had an effect on resin [P] in our model (*P*=0.0017; Supplementary material Table S7), the difference between burnt and unburnt sites was not significant in the post-hoc test (Fig. 3B). Notably, the variation at the burnt site was much larger than that at the unburnt site (unburnt = 0.53 ± 0.04, burnt = 0.69 ± 0.13 mg kg^{-1}). Soil electrical conductivity was greater at the burnt site, but very low at both sites, and no differences were observed in other parameters (Table 2; Supplementary material Table S7).

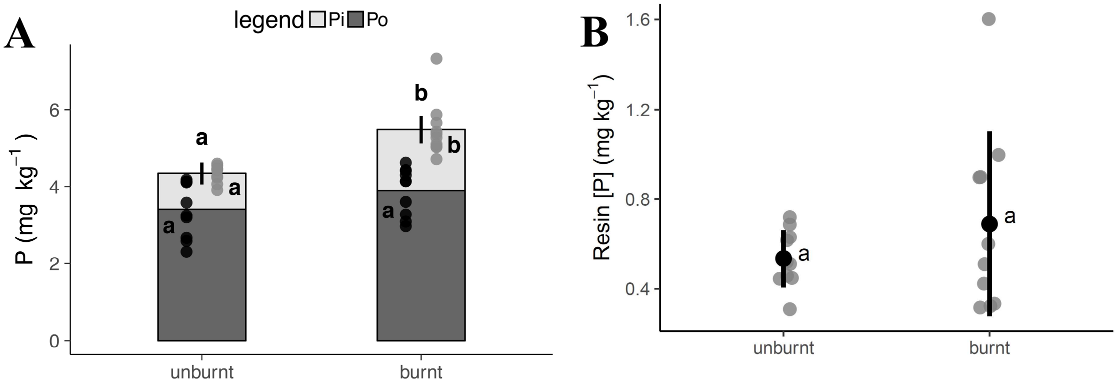


Figure 3. Soil phosphorus (P) concentration measured at two adjacent sites, of which one was unburnt and the other burnt, collected at the beginning of the dry season (one year after fire) at Badgingarra National Park, Southwest Australia. A) Inorganic P (Pi, lighter bar and dots), organic P (Po, darker bar and dots) and total soil P (the sum of Pi and Po). B) Resin P (Supplementary material Table S7). Bars (in plot A) and black and bigger dots (in plot B) represent means, lighter and smaller dots are individual samples, error bars are SD, and different lower-case letters represent significant differences between sites based on generalised least squares models followed by the Tukey–Kramer honest test.

Table 2. Soil chemical and physical properties at two adjacent sites, of which one was unburnt and the other burnt, collected at the beginning of the dry season (one year after fire) at Badgingarra National Park, Southwest Australia. (Means ± SE, n = 10). Bold numbers indicate significant difference between sites (Supporting Information Table S7).

	unburnt	burnt
Soil moisture	2.7 ± 0.19	3.3 ± 0.26
EC (µS cm^{-1})	**16 ± 1.0**	**19 ± 1.0**
pH ($CaCl_2$)	4.5 ± 0.03	4.6 ± 0.05
pH (H_2O)	5.8 ± 0.02	5.8 ± 0.02

EC: electrical conductivity

5. Seed total P

Seed P content exhibited great variation among the five species that were assessed (Table 3, Supplementary material Table S8), and was highly correlated with seed mass (R^2 = 0.997). *Banksia candolleana* was the only species exhibiting higher seed [P] than all other species, and had a remarkably higher seed P content also driven by its highest seed mass. Seed mass rather than seed [P] determined seed P content of the other species. *Stirlingia latifolia* had the second-highest seed P content, followed by *J. floribunda* and *J. nutans*. *Melaleuca leuropoma* seeds had the lowest P content, and its low seed mass differed from that of *B. candolleana* by four orders of magnitude.

Table 3. Seed phosphorus (P) content (µg $seed^{-1}$), seed P concentration (mg g^{-1}) and seed mass (mg) of five species from Badgingarra National Park, Southwest Australia. (Means ± SE, n = 4-6). Different letters represent significant differences among species based on generalised least squares models followed by the Tukey–Kramer honest test (Supporting Information Table S8).

Family / species	µg P $seed^{-1}$				mg P g^{-1}				Seed mass (mg)			
Fabaceae												
Jacksonia floribunda	17.4	±	1	b	3.8	±	0.16	a	4.65	±	0.2	c
Jacksonia nutans	5.5	±	2	a	2.4	±	0.84	a	2.21	±	0.12	b
Myrtaceae												
Melaleuca leuropoma	0.2	±	0.003	a	4.0	±	0.06	a	0.05	±	0.005	a
Proteaceae												
Banksia candolleana	1941.4	±	86	d	13.6	±	0.37	b	143.4	±	5.6	e
Stirlingia latifolia	46.7	±	2	c	3.8	±	0.1	a	12.2	±	0.15	d

6. Leaf P-fractions

The leaf P pool was partitioned into five major fractions nucleic acid P, lipid P, metabolite P plus Pi, residual P and Pi. The higher leaf total [P] in seedlings than in their conspecific adults did not result from proportionally higher values in all P fractions, but mainly from a higher [Pi] (Figure 5). There was a remarkable difference for the Proteaceae species, with [Pi] representing about 30% of the total [P] in unburnt adults, while it was 60% in seedlings. In Fabaceae and Myrtaceae, [Pi] was ~30% of the total [P] in unburnt adults and ~38% in seedlings. The residual P fraction was also significantly greater for seedlings of all species, except for *D. chapmanii.* In seedlings of *B. candolleana*, residual [P] was seven-fold greater than that in unburnt adults, while the seedlings from other species exhibited two- to 2.5-fold higher values for this fraction. Seedlings of all species, except *D. chapmanii and J. nutans*, exhibited greater nucleic P fraction, with concentrations ~1.3 to 4-fold greater than those in conspecific unburnt adults.

Metabolic P fraction also contained Pi, which represented most of this fraction. *Jacksonia nutans* was the only species that showed a significant difference in metabolic P between seedlings and unburnt adults, in which the seedlings had a significantly lower metabolic [P] ($P < 0.05$; comparison not shown). Due to technical difficulties in measuring [Pi] for adults of the Myrtaceae and *B. candolleana*, we were not able to calculate the metabolic P fraction for these species. However, values obtained for seedlings of *B. candolleana* and *M. leuropoma* clearly showed that the much greater metabolic P plus Pi fraction was mostly due to higher [Pi].

Seedlings of *B. candolleana*, *S. latifolia* and *M. leuropoma* also had significantly greater [P] in all other fractions relative to the adult regeneration forms (Fig. 5). The [P] was remarkably high in all fractions of *B. candolleana* seedlings (6, 4 and 12-fold greater for lipid, nucleic acid, and metabolic P plus Pi, respectively). Seedlings of *S. latifolia* also had a three-fold greater lipid [P] than unburnt adults did.

There were few and less pronounced differences in P fractions between the two adult regeneration forms from the burnt and unburnt sites (Fig. 5). Residual [P] was ~1.6-fold greater in burnt adults of all Myrtaceae species and *B. candolleana* than in the unburnt conspecifics. Burnt adults of *M. leuropoma* also had ~1.3-fold greater lipid and nucleic acid [P] and the ones of *D. chapmanii* 1.3-fold greater [Pi] than their respective unburnt conspecifics.

Similar to leaf total [P], the effect of regeneration form on P fractions was significant, and depended on the interaction between site and family, but in most cases, not on the family alone (Supplementary material Table S6). However, [Pi] directly depended on the family alone, but this analysis excluded Myrtaceae. Seedlings of all families had significantly greater [P] in all fractions than the unburnt adults, except for the lipid fraction in Fabaceae, and differences were more pronounced among regeneration forms within the Proteaceae (Supplementary material Fig, S2). Only Myrtaceae exhibited a significant difference in P fractions between adults, with slightly greater [P] of the lipid and residual fractions in the burnt than in the unburnt ones (Supplementary material Fig. S2).

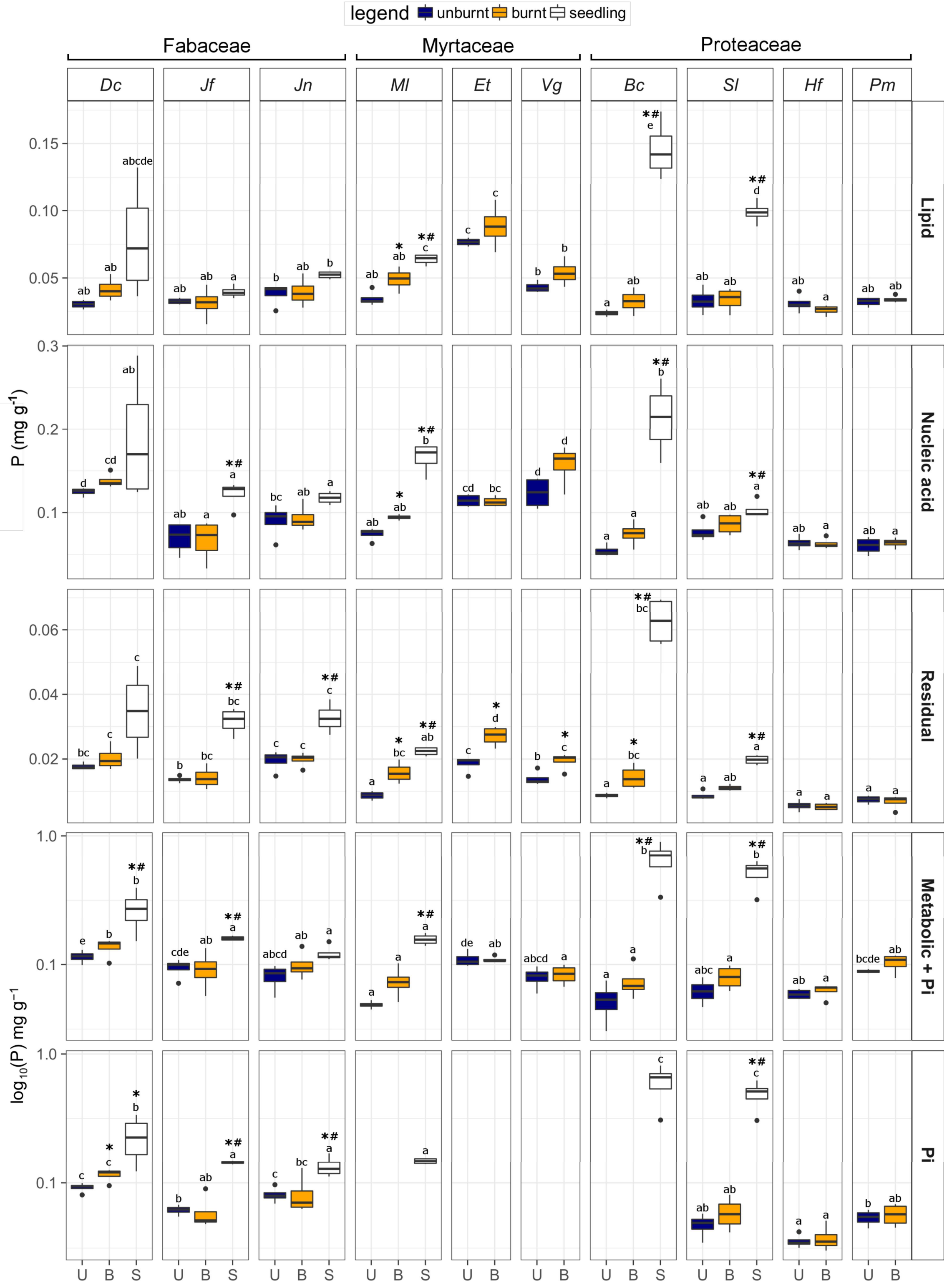


Figure 5. Leaf phosphorus (P) fractions (lipid P, nucleic acid P, residual P, metabolic P plus inorganic P (Pi), and Pi) concentrations (mg g^{-1} dry weight) of unburnt (U) and burnt (B) adult plants and seedlings (S) for 10 species collected in spring 2017 at Badgingarra National Park, Southwest Australia (Supplementary material Table S9-Table S13). Box plots show medians (horizontal lines) with quartiles (box margins); the lowest and the highest values within 1.5-

interquartile ranges (whiskers) and outliers (filled circles). Different letters indicate significant differences among species within each regeneration form, and symbols express significant differences among regeneration forms within each individual species (* relative to unburnt; # relative to burnt) based on generalised least squares models followed by the Tukey–Kramer honest test. *Daviesia chapmanii* (Dc), *Jacksonia floribunda* (Jf), *Jacksonia nutans* (Jn), *Melaleuca leuropoma* (Ml), *Eucalyptus todtiana* (Et), *Verticordia grandis* (Vg), *Banksia candolleana* (Bc), *Stirlingia latifolia* (Sl), *Hakea flabellifolia* (Hf), *Petrophile macrostachya* (Pm).

7. Photosynthesis

Among the Proteaceae species, only *B. candolleana* exhibited significantly faster net photosynthetic rates for burnt adults than for their respective unburnt counterparts (medians for the former and latter were 20.6 and 14.9 μmol CO_2 m^{-2} s^{-1}, respectively; Fig. 6; Supplementary material Table S14). Burnt adults of *S. latifolia* had 16% faster net photosynthetic rates than the unburnt ones, but the difference was not significant (*P*=0.068). However, stomatal conductance was significantly greater for burnt adults of all Proteaceae than for their unburnt conspecifics; 19% greater for *B. candolleana*, 43% for *S. latifolia*, 44% for *H. flabellifolia* and 100% for *P. macrostachya* (Table S15).

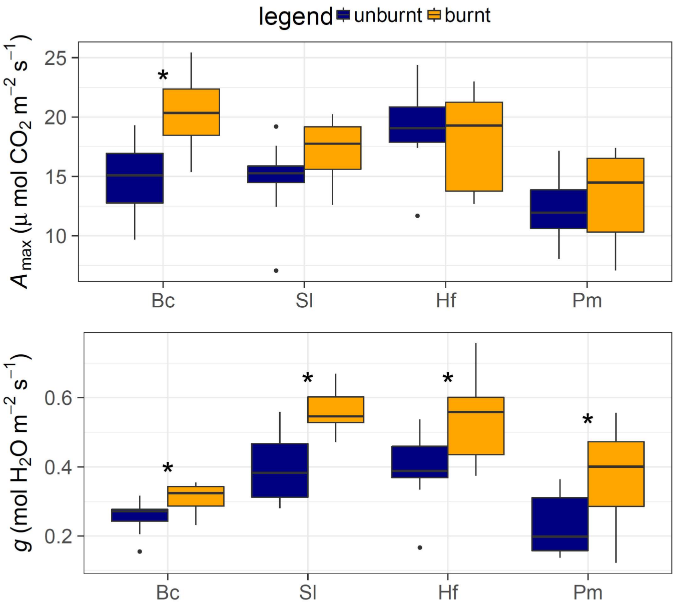


Figure 6. Net photosynthetic rates of the youngest fully-expanded leaves (A_{max}) and stomatal conductance (g) both at saturating light intensity plotted for unburnt and burnt adults of each of the Proteaceae species. Box plots show medians (horizontal lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile range (whiskers) and outliers (filled circles). (*) Significant differences among regeneration forms within each individual species based on generalised least squares models followed by the Tukey–Kramer honest test. *Banksia candolleana* (Bc), *Stirlingia latifolia* (Sl), *Hakea flabellifolia* (Hf), *Petrophile macrostachya* (Pm).

DISCUSSION

Leaf total phosphorus and LDMC

High leaf [P] in adult plants coincides with a capacity for rapid growth under productive conditions and a decline in productivity under limited supply of nutrients (Grime *et al.* 1997), whereas extremely low leaf [P] is related to resource retention and high P-use efficiency in severely P-impoverished environments (Wright, Reich, *et al.* 2004; Denton *et al.* 2007; Lambers *et al.* 2010). Here, we found that leaf [P] does not only vary with habitat productivity evidenced by previous studies, but also across regeneration forms. Our study showed major differences in leaf total [P] between seedlings and unburnt adults of species from three families, indicating a shift in P-use strategy during development. As we predicted by hypothesis 1, seedlings had greater leaf [P] than conspecific unburnt adults, consistent with developmental shifts observed in a P-limited rainforest (Liu *et al.* 2010). This pattern contrasts with that reported for plants on more fertile soils, where similar or higher leaf [P] is observed for adults (Schoettle 1994; Palow *et al.* 2012; Meisner *et al.* 2013; Liu *et al.* 2019). Therefore, our observations suggest a shift from a relatively acquisitive to a conservative life history strategy in plants from severely P-impoverished soils, which might be enabled by seed P and/or greater soil P in the soils from burnt areas. This would allow faster growth during early developmental stages and enhance establishment success, for instance, by maximising root growth to withstand drought during summer (Padilla & Pugnaire 2007).

The majority of burnt plants had higher leaf [P] than unburnt ones at the beginning of the rainy season, but this difference had disappeared for most species by the beginning of the dry season. Thus, our second hypothesis was not supported, as a time span for burnt plants needs to be taken into account, because the initial increase in leaf [P] of burnt adults was followed by a decrease within a year after fire. The decrease might be explained by the allocation of nutrients to reproductive structures and/or by a growth stimulation that dilutes the nutrient concentrations (Christensen 1977; Reich *et al.* 1990; Durán *et al.* 2010) during the rainy season.

The total leaf [P] results suggested that there are differences in P strategies among plant lineages. At the family level, our results show remarkable shifts in P fractions among developmental stages within Proteaceae: both unburnt and burnt adults in this family had the lowest [P] in all fractions, while seedlings had the highest. Differences among developmental stages were also observed in the other families, but were much less pronounced. This pronounced developmental shift in total leaf [P] of the Proteaceae may consist in another specialisation of the Southwest Australian members of this family to cope with severe P scarcity (Lambers *et al.* 2013; Lambers, Finnegan, *et al.* 2015).

High values of LDMC are associated with greater investment in leaf protection and slow growth, whereas the opposite holds for fast-growing species (Lambers & Poorter 1992). We found that LDMC was usually greater for unburnt adults than burnt adults, and lowest in seedlings. The lower LDMC in seedlings compared with unburnt adults agrees with previous observations of ontogenetic shifts in ecological strategies that favour faster rates of resource acquisition during early developmental stages to cope with their vulnerability (Dayrell *et al.* 2018). Interestingly, *B. candolleana* was the only exception, and did not exhibit changes in LDMC between developmental stages. Seedlings of this species appear to have a successful strategy allocating resources to durable leaves, rather than growth, despite their susceptibility to summer droughts (Enright & Lamont 1989). The lower LDMC of burnt adults relative to the unburnt ones also suggest a shift in strategy of the resprouting plants, similar to that of seedlings, to invest in faster growth.

As previously acknowledged (Lambers et al. 2010; Sulpice et al. 2014), the high dry matter content in leaves protects them and then inevitably leads to lower leaf [P] calculated per unit leaf dry weight. When we expressed the leaf [P] on a fresh weight basis, instead of dry weight basis, the differences between unburnt and burnt adults at the beginning of the wet season narrowed by 2 to 24%, but remained significant for six species. Notably, Proteaceae species exhibited large differences in leaf [P] between developmental stages and smaller differences for LDMC. These results suggest that leaf attributes other than lower dry matter content account for the differences found between unburnt adults and the two other regeneration forms. Further studies comparing regeneration forms in leaf anatomy and allocation of P to different cells within leaves are needed to clarify this matter.

Leaf Mn concentration, soil P fractions and seed P content

We investigated leaf [Mn] to screen for changes in root functioning related to P-mobilisation strategies among individuals at burnt and unburnt sites (Lambers, Hayes, *et al.* 2015). Interestingly, burnt adults of *P. macrostachya* exhibited lower leaf [Mn] in the beginning of the rainy season, suggesting that resprouting individuals released less carboxylates in the rhizosphere to mobilise soil P during this period (Lambers, Hayes, *et al.* 2015; Huang *et al.* 2017), because of a higher P availability at the burnt soil (Reich *et al.* 1990). However, no other species exhibited this pattern for leaf [Mn], which is consistent with observations that a higher leaf [P] in burnt plants results from an increase in the root:aboveground biomass ratio, rather than changes in soil nutrient availability (Christensen 1977). It is also possible that the ash resulting from the fire increased soil pH during the months prior to our measurement, thereby decreasing Mn availability and uptake (Lambers, Chapin, *et al.* 2008).

Seed P content was generally associated with leaf [P] in seedlings, corroborating our fourth hypothesis, and indicating that seed reserves are the major source of P for seedlings. Studies have often reported very high seed P content and concentrations for *kwongan* species (Kuo *et al.* 1982; Denton *et al.* 2007; Groom & Lamont 2010), suggesting that a significant nutrient reserve is key to successful seedling recruitment in nutrient poor habitats (Milberg & Lamont 1997; Milberg *et al.* 1998). The two Proteaceae had the highest seed P content which explains where the high leaf [P] comes from and also confirms that phylogenetic context is a major driver of P allocation strategies across regeneration forms. *Banksia candolleana* exhibited a remarkably high seed P content, which agrees with values previously reported for Proteaceae species from Southwest Australia (Kuo *et al.* 1982; Denton *et al.* 2007; Groom & Lamont 2010), and had a seed [P] much higher than that of all other species. On the other hand, seed [P] of *S. latifolia* did not differ from that of the other three species assessed in this study, and seed P content of this species resembled more those of more closely related Proteaceae from South Africa (e.g., *Protea* and *Leucadendron*) than those from Southwest Australia (Groom & Lamont 2010). This suggests that the remarkably high values reported for seed P content of Proteaceae from Southwest Australia might be restricted to a few closely related large genera, such as *Banksia*, *Grevillea* and *Hakea* (Groom & Lamont 2010).

Seed P content varied considerably and was highly associated with seed mass. This corroborates observations that there is no association between large seeds and low-nutrient soils (Leishman *et al.* 2000). Seed P content was lower for Fabaceae and Myrtaceae species, indicating that recruitment strategies other than high seed nutrient reserves are viable for plants in the *kwongan*. Although soil total [P] of the burnt site was still remarkably low when compared with values of the oldest and most severely P-impoverished coastal dune soils in south-western Australia (Turner & Laliberté 2015), variation in soil [Pi] and resin [P] was notably larger at the burnt site than that at the unburnt site. This agrees with observations that fire passage often creates microsites consisting of litter and sand with contrasting physical and chemical properties, which affects negatively seedling establishment of Proteaceae species, with relatively high seed mass (Lamont *et al.* 1993), but may be essential to recruit species with low seed P content, such as *M. leuropoma*.

Soil [P] and leaf [Mn] results suggest that the slightly higher P availability in the soil does not have a major and/or lasting effect on leaf [P] of burnt adults. One year after fire, burnt and unburnt adults of most species had similar leaf [P], and we found no support for the hypothesis that a greater P availability in the burnt soil is associated with more P allocation to leaves. Therefore, the increase in P availability experienced by some microsites may consist of an important source of nutrients for seedlings of some species, but does not seem to influence leaf function of the resprouting plants.

Leaf P fractions

We reject our fifth hypothesis of a preferential P allocation to the nucleic acid fraction over the other fractions in seedlings and in burnt adults. Although seedlings of most species exhibited an increase in nucleic acid [P], all of them allocated more P to the Pi fraction. A constant [Pi] is needed in the cytoplasm for metabolism, whereas excess Pi is stored in vacuoles as reserves that vary according to Pi availability (Veneklaas *et al.* 2012). Therefore, the higher leaf [Pi] in seedlings suggests that leaves serve as a site for P storage. As the cytosolic [Pi] is maintained within a narrow range, the remarkably high [Pi] for seedlings of both Proteaceae species indicates the storage function may be especially important for the Southwest Australian members of this family at this early developmental stage. This contrasts with the strategy of adults of *Banksia* species to store Pi in the stem, maintaining a low [P] in leaves (Jeschke & Pate 1995; Parks *et al.* 2000), and possibly indicates a developmental shift in organ function.

The nucleic acid pool in a plant mainly consists of rRNA (Bieleski 1968; Kanda *et al.* 1994), and rRNA content is positively correlated with protein synthesis, which supports demands of growth (Elser *et al.* 2010). We found that seedlings from four of the six species exhibited higher nucleic acid P concentrations than their conspecific unburnt adults, further suggesting that life history strategy shifts during development from faster growth rates at early stages to a more conservative slow growth later on. Proteaceae adults from Southwest Australia function at very low leaf rRNA levels, partly by separating leaf growth from photosynthetic maturation through delayed greening, which spreads out investment of P in rRNA over time (Sulpice *et al.* 2014). This process of delayed greening appears to be absent or less pronounced in seedlings (R. Dayrell, *pers. obs.*), possibly allowing for faster growth rates. Interestingly, seedlings of *D. chapmanii* and *J. nutans* (both Fabaceae) did not show the same functioning, and nucleic acid [P] was similar for both developmental stages. This might be related to the fact that some faster-growing Fabaceae may have a less plastic allocation of P to leaf P fractions and their adults tend to operate at relatively higher nucleic acid levels than species of the two other families (Yan *et al.* 2019).

Adults of *B. candolleana* and other Proteaceae species replace phospholipids with lipids that do not contain P during leaf development, resulting in a very pronounced decline in the fraction of lipid P from young to mature leaves (Lambers *et al.* 2012; Kuppusamy *et al.* 2014). Our results show that the lipid P fraction also varies during development, because lipid [P] was remarkably greater in seedlings than in unburnt adults of Proteaceae. This suggests that the greater P availability provided by seeds and the soil at the burnt site might enable plants to restrict replacement of lipid P fractions during early stages of development and/or continue expanding while photosynthesising and producing new leaves.

The residual P fraction, consisting mostly of phosphorylated proteins, was the smallest P pool, consistent with previous reports (Zhang *et al.* 2018; Yan *et al.* 2019). Residual [P] was greater in seedlings than in unburnt adults of five out of six species. Interestingly, this was the only fraction in which we also observed differences between burnt and unburnt adults in more than one species. The phosphorylation of proteins is a basic regulatory mechanism of biological processes (Ranjeva & Boudet 1987), and the higher concentration of phosphorylated proteins in seedlings is likely linked to greater metabolic and cellular activity (Lohrig *et al.* 2009).

Photosynthesis

We only found differences in net photosynthetic rates among unburnt and burnt adults of *B. candolleana* within the Proteaceae. Interestingly, the burnt plants of this species still had higher leaf [P] in October, and the residual [P] fraction was greater than that of the unburnt plants. This result contrasts with previous findings of net photosynthetic rates being more correlated to the lipid P and nucleic acid P fractions in a P-limited habitat (Zhang *et al.* 2018). The intrinsic high efficiency of the Proteaceae in using P in both nucleic acid and lipid fractions

(Lambers *et al.* 2012; Sulpice *et al.* 2014) might allow more P to be allocated to residual P and possibly to metabolic P fractions, thereby increasing productivity through higher metabolic activity (Yan *et al.* 2019).

Burnt adults of all Proteaceae species had greater stomatal conductance than unburnt plants. Since there was no difference in soil moisture between sites, this result can be explained by the increase in leaf area per unit of soil volume, and hence greater water availability for individual plants. The greater stomatal conductance can enhance P acquisition by transpiration-induced mass flow (solvent drag) in sand with a low P-buffering capacity (Huang *et al.* 2017), and may be a strategy of burnt adults to acquire more P from soil at the burnt site, without the costs of carboxylates exudation.

Conclusions

Plants from severely P-impoverished soils, such as those from the *kwongan* in Southwest Australia, typically exhibit a nutrient-conserving, slow-growing strategy (Specht & Rundel 1990; Lambers & Poorter 1992; Wright & Westoby 2003) with low leaf [P] and high photosynthetic P-use efficiency (Wright, Groom, *et al.* 2004; Lambers *et al.* 2012; Sulpice *et al.* 2014). In this study, we show that P-use and -allocation strategies of these plants shifted with plant development, and also after fire. Seedlings had greater leaf [P] than conspecific unburnt adults, compatible with a shift from a relatively acquisitive to a conservative life history strategy (Dayrell *et al.* 2018). Burnt adults had a short-term and less pronounced increase in leaf [P], possibly allocating the additional P to reproduction and/or growth in less than a year after fire (Christensen 1977; Reich *et al.* 1990; Durán *et al.* 2010). Seed P content and leaf [P] in seedlings varied considerably across species, suggesting that different recruitment strategies are viable in the *kwongan*. Some species, such as the Proteaceae in this study, seem to rely mostly on seed P content at their early stages, whereas seedling survival and growth of other species might depend on microsites with increased P availability in the soil after a fire (Lamont *et al.* 1993).

The greater leaf [P] in seedlings was mostly due to a greater leaf [Pi] than in unburnt adults, suggesting that leaves serve as a site for P storage (Veneklaas *et al.* 2012). Most seedlings exhibited higher levels in at least some of the organic P fractions, indicating that the greater leaf [P] is also used to support higher metabolic activity and growth rates (Elser *et al.* 2010; Veneklaas *et al.* 2012). The developmental shifts in concentrations of leaf P, Pi and organic P fractions (especially lipid P) were remarkably pronounced in the Proteaceae, and possibly indicate a unique regeneration strategy of the Southwest Australian members of this family to enhance establishment in severely P-impoverished soils.

Our study provides a novel comparative community-level perspective of P-allocation patterns of naturally coexisting unburnt and burnt adults and seedlings. We show potentially general relationships between P-use strategies and plant developmental stages, creating an opportunity to further understand community assembly on severely P-impoverished soils. The assessment of different species from three dominant families of the *kwongan*, revealed different patterns in P-use and -allocation strategies for seedlings of different species in the community, and further evidenced singular strategies of Proteaceae from Southwest Australia. Finally, we suggest that knowledge on regeneration forms, such as seedlings and resprouting individuals, is needed to fully appreciate the life history strategies of plants that cope with nutrient scarcity, underpinning conservation management.

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SUPPLEMENTARY MATERIAL

Table S1. List of species and information concerning the number of replicates (r) and number (n) of sampled individuals. Adult individuals for the nutrient analyses at the beginning of rainy season (BR) and at the beginning of dry season (BD). Seedlings were collected at BD for leaf dry matter content (LDMC) measurements and then pooled for P analysis.

Family/ Species	**Unburnt Adult** r (n)		**Burnt Adult** r (n)		**Seedling** r (n)	**Seedling** r (n)
	BR	**BD**	**BR**	**BD**	**LDMC**	**P analyses**
Fabaceae						
Daviesia chapmanii	6 (1)	4 (1)	6 (1)	4 (1)	10 (1)	4 (2-4)
Jacksonia floribunda	6 (1)	4 (1)	6 (1)	4 (1)	10 (1-2)	4 (2-4)
Jacksonia nutans	6 (1)	4 (1)	5 (1)	4 (1)	12 (1)	4 (3)
Myrtaceae						
Melaleuca leuropoma	5 (1)	4 (1)	6 (1)	4 (1)	6 (23-36)	4 (23-34)
Eucalyptus todtiana	6 (1)	4 (1)	6 (1)	4 (1)	-	-
Verticordia grandis	6 (1)	4 (1)	6 (1)	4 (1)	-	-
Proteaceae						
Banksia candolleana	6 (1)	4 (1)	6 (1)	4 (1)	4 (1)	4 (1)
Stirlingia latifolia	6 (1)	4 (1)	6 (1)	4 (1)	12 (1-2)	4 (3-6)
Hakea flabellifolia	6 (1)	4 (1)	6 (1)	4 (1)	-	-
Petrophile macrostachya	6 (1)	4 (1)	6 (1)	4 (1)	-	-

Table S2. Generalized Least Square models (GLSs) of leaf dry-matter content (LDMC; mg g^{-1}) for unburnt and burnt adult plants and seedlings within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AIC	ΔAIC	P
Banksia candolleana	1	LDMC ~ reg form	6	31.79	-51.58	-12.85	**0.0003**
	null	LDMC ~ 1	2	21.37	-38.73	-	
Daviesia chapmanii	1	LDMC ~ reg form	5	46.7	-83.4	-34	**<.0001**
	null	LDMC ~ 1	2	26.7	-49.41	-	
Eucalyptus todtiana	1	LDMC ~ reg form	3	32.63	-59.26	-9.63	**0.0006**
	null	LDMC ~ 1	2	26.82	-49.63	-	
Hakea flabellifolia	1	LDMC ~ reg form	4	28.56	-49.11	-8.78	**0.0017**
	null	LDMC ~ 1	2	22.17	-40.34	-	
Jacksonia floribunda	1	LDMC ~ reg form	4	62.86	-117.71	-68.77	**<0.0001**
	null	LDMC ~ 1	2	26.47	-48.95	-	
Jacksonia nutans	1	LDMC ~ reg form	5	59.81	-109.61	-47.18	**<0.0001**
	null	LDMC ~ 1	2	33.22	-62.43	-	
Melaleuca leuropoma	1	LDMC ~ reg form	5	50.05	-90.1	-58.63	**<0.0001**
	null	LDMC ~ 1	2	17.73	-31.47	-	
Petrophile macrostachya	1	LDMC ~ reg form	4	32.26	-56.52	-9.76	**0.001**
	null	LDMC ~ 1	2	25.38	-46.76	-	
Stirlingia latifolia	1	LDMC ~ reg form	6	48.61	-85.23	-32.27	**<.0001**
	null	LDMC ~ 1	2	28.48	-52.95	-	
Verticordia grandis	1	LDMC ~ reg form	3	27.95	-49.91	-13.75	**0.0001**
	null	LDMC ~ 1	2	20.08	-36.15	-	

Table S3. Generalized Least Square models (GLSs) of total leaf phosphorus (P; mg g^{-1} dry weight) and manganese (Mn; mg kg^{-1} dry weight) for unburnt and burnt adult plants within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold (*P* < 0.05). The regeneration forms (reg form) were unburnt and burnt adult plants.

Data	Model ID	Model	K	Log L	AIC	ΔAIC	P
Unburnt adult	MM	Total P ~ species	12	123.69	-223.38	-77.43	**<0.0001**
	null	Total P ~ 1	2	74.98	-145.95	-	
Burnt adult	MM	Total P ~ species	11	97.21	-172.42	-20.35	**<0.0001**
	null	Total P ~ 1	2	78.04	-152.07	-	
Banksia candolleana	1	Total P ~ reg form	3	23.77	-41.54	-9.27	**0.0008**
	null	Total P ~ 1	2	18.14	-32.27	-	
Daviesia chapmanii	1	Total P ~ reg form	3	19.26	-32.53	0.40	0.2063
	null	Total P ~ 1	2	18.47	-32.93	-	
Eucalyptus todtiana	1	Total P ~ reg form	3	22.05	-38.10	-2.94	**0.0262**
	null	Total P ~ 1	2	19.58	-35.16	-	
Hakea flabellifolia	1	Total P ~ reg form	4	25.34	-42.68	-12.90	**0.0002**
	null	Total P ~ 1	2	16.89	-29.78	-	
Jacksonia floribunda	1	Total P ~ reg form	3	19.96	-33.93	-2.89	**0.0271**
	null	Total P ~ 1	2	17.52	-31.04	-	
Jacksonia nutans	1	Total P ~ reg form	3	19.39	-32.78	-8.62	**0.0011**
	null	Total P ~ 1	2	14.08	-24.16	-	
Melaleuca leuropoma	1	Total P ~ reg form	3	17.06	-28.12	-7.81	**0.0017**
	null	Total P ~ 1	2	12.15	-20.31	-	
Petrophile macrostachya	1	Total P ~ reg form	3	20.98	-35.96	-5.95	**0.0048**
	null	Total P ~ 1	2	17.00	-30.01	-	
Stirlingia latifolia	1	Total P ~ reg form	3	30.89	-55.77	-33.59	**<0.0001**
	null	Total P ~ 1	2	13.09	-22.18	-	
Verticordia grandis	1	Total P ~ reg form	4	24.82	-41.64	-20.93	**<0.0001**
	null	Total P ~ 1	2	12.36	-20.72	-	
Unburnt adult	MM	Total Mn ~ species	12	-185.09	394.17	-118.36	**<0.0001**
	null	Total Mn ~ 1	2	-254.27	512.53	-	
Burnt adult	MM	Total Mn ~ species	12	-206.91	437.81	-92.72	**<0.0001**
	null	Total Mn ~ 1	2	-263.27	530.53	-	
Banksia candolleana	1	Total Mn ~ reg form	3	-53.00	112.01	0.26	0.1866
	null	Total Mn ~ 1	2	-53.87	111.75	-	
Daviesia chapmanii	1	Total Mn ~ reg form	3	-46.34	98.67	2.00	0.9654
	null	Total Mn ~ 1	2	-46.34	96.67	-	
Eucalyptus todtiana	1	Total Mn ~ reg form	3	-49.52	105.04	1.73	0.602
	null	Total Mn ~ 1	2	-49.66	103.31	-	
Hakea flabellifolia	1	Total Mn ~ reg form	4	-22.63	53.26	-6.30	**0.0058**
	null	Total Mn ~ 1	2	-27.78	59.56	-	
Jacksonia floribunda	1	Total Mn ~ reg form	3	-35.37	76.75	1.67	0.5633
	null	Total Mn ~ 1	2	-35.54	75.08	-	

Data	Model ID	Model	K	Log L	AIC	ΔAIC	P
Jacksonia nutans	1	Total Mn ~ reg form	3	-32.87	71.74	1.94	0.8024
	null	Total Mn ~ 1	2	-32.90	69.80	-	
Melaleuca leuropoma	1	Total Mn ~ reg form	3	-32.15	70.30	-8.77	**0.001**
	null	Total Mn ~ 1	2	-37.53	79.07	-	
Petrophile macrostachya	1	Total Mn ~ reg form	3	-24.03	54.06	-15.89	**<0.0001**
	null	Total Mn ~ 1	2	-32.97	69.95	-	
Stirlingia latifolia	1	Total Mn ~ reg form	3	-39.03	84.07	1.31	0.4064
	null	Total Mn ~ 1	2	-39.38	82.76	-	
Verticordia grandis	1	Total Mn ~ reg form	4	-51.07	110.14	-9.31	**0.0013**
	null	Total Mn ~ 1	2	-57.72	119.44	-	

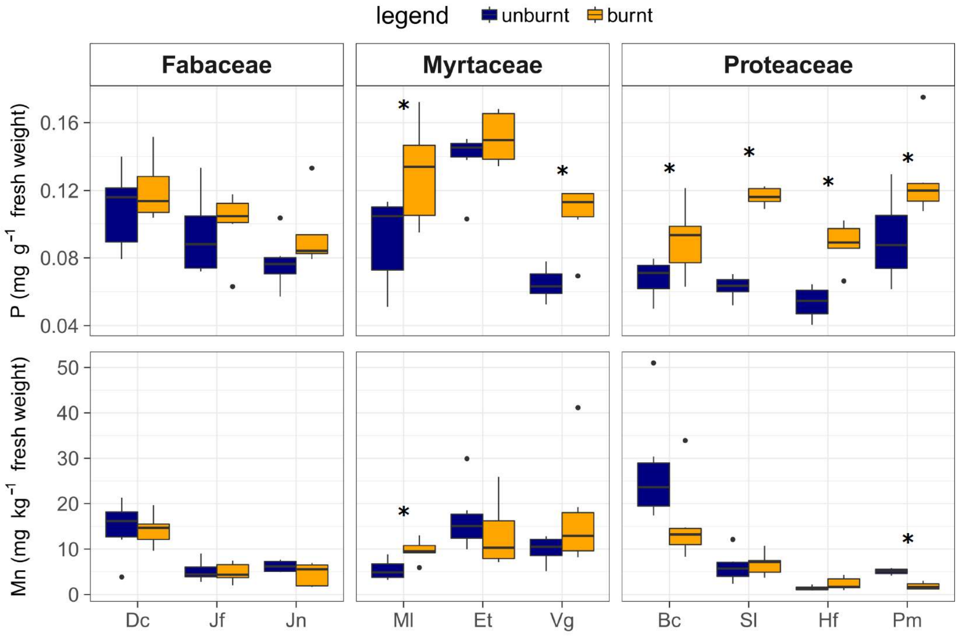

Figure S1. Leaf total phosphorus (P) and manganese (Mn) concentrations on a fresh weight basis of adults from an unburnt site (unburnt) and resprouting adults from a burnt site (burnt) for 10 species collected in June 2017 at Badgingarra National Park, Southwest Australia (Supplementary material Table S3). Box plots show medians (horizontal lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile ranges (whiskers) and outliers (filled circles). (*) Significant differences among regeneration forms within each individual species based on 95% confidence intervals from generalised least squares models (Supporting Information Table S2). *Daviesia chapmanii* (Dc), *Jacksonia floribunda* (Jf), *Jacksonia nutans* (Jn), *Melaleuca leuropoma* (Ml), *Eucalyptus todtiana* (Et), *Verticordia grandis* (Vg), *Banksia candolleana* (Bc), *Stirlingia latifolia* (Sl), *Hakea flabellifolia* (Hf), *Petrophile macrostachya* (Pm).

Table S4. Generalized Least Square models (GLSs) of total leaf phosphorus (P; mg g^{-1} fresh weight) and manganese (Mn; mg kg^{-1} fresh weight) for unburnt and burnt adult plants within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold (*P*<0.05). The regeneration forms (reg form) were unburnt and burnt adult plants.

Data	Model ID	Model	K	Log L	AIC	Δ AIC	P
Banksia candolleana	1	Total P ~ reg form	3	33.13	-60.26	-3.23	**0.0222**
	null	Total P ~ 1	2	30.52	-57.03	-	
Daviesia chapmanii	1	Total P ~ reg form	3	30.11	-54.22	1.12	0.3471
	null	Total P ~ 1	2	29.67	-55.33	-	
Eucalyptus todtiana	1	Total P ~ reg form	3	33.19	-60.38	0.06	0.1633
	null	Total P ~ 1	2	32.22	-60.44	-	
Hakea flabellifolia	1	Total P ~ reg form	3	37.94	-69.87	-14.32	**0.0001**
	null	Total P ~ 1	2	29.78	-55.56	-	
Jacksonia floribunda	1	Total P ~ reg form	3	29.73	-53.45	1.62	0.5379
	null	Total P ~ 1	2	29.54	-55.08	-	
Jacksonia nutans	1	Total P ~ reg form	3	29.24	-52.48	-0.53	0.1119
	null	Total P ~ 1	2	27.98	-51.95	-	
Melaleuca leuropoma	1	Total P ~ reg form	3	24.46	-42.92	-3	**0.0253**
	null	Total P ~ 1	2	21.96	-39.92	-	
Petrophile macrostachya	1	Total P ~ reg form	3	28.37	-50.75	-3.73	**0.0166**
	null	Total P ~ 1	2	25.51	-47.01	-	
Stirlingia latifolia	1	Total P ~ reg form	3	45.42	-84.84	-36.59	**<0.0001**
	null	Total P ~ 1	2	26.12	-48.24	-	
Verticordia grandis	1	Total P ~ reg form	3	34.48	-62.95	-12.27	**0.0002**
	null	Total P ~ 1	2	27.34	-50.69	-	
Banksia candolleana	1	Total Mn ~ reg form	3	-44.68	95.35	-1.55	0.0594
	null	Total Mn ~ 1	2	-46.45	96.91	-	
Daviesia chapmanii	1	Total Mn ~ reg form	3	-35.3	76.6	1.98	0.8943
	null	Total Mn ~ 1	2	-35.31	74.61	-	
Eucalyptus todtiana	1	Total Mn ~ reg form	3	-39.75	85.5	1.19	0.3668
	null	Total Mn ~ 1	2	-40.16	84.32	-	
Hakea flabellifolia	1	Total Mn ~ reg form	4	-13.69	35.38	-4.34	**0.0154**
	null	Total Mn ~ 1	2	-17.86	39.72	-	
Jacksonia floribunda	1	Total Mn ~ reg form	3	-25.4	56.8	1.92	0.7798
	null	Total Mn ~ 1	2	-25.44	54.88	-	
Jacksonia nutans	1	Total Mn ~ reg form	3	-21.83	49.67	-0.28	0.1309
	null	Total Mn ~ 1	2	-22.98	49.95	-	
Melaleuca leuropoma	1	Total Mn ~ reg form	3	-23.85	53.7	-5.53	**0.0061**
	null	Total Mn ~ 1	2	-27.61	59.22	-	
Petrophile macrostachya	1	Total Mn ~ reg form	3	-12.02	30.04	-21.21	**<0.0001**
	null	Total Mn ~ 1	2	-23.63	51.26	-	
Stirlingia latifolia	1	Total Mn ~ reg form	3	-29.2	64.39	1.86	0.7101
	null	Total Mn ~ 1	2	-29.27	62.53	-	
Verticordia grandis	1	Total Mn ~ reg form	4	-37.55	83.1	-7.67	**0.0029**
	null	Total Mn ~ 1	2	-43.38	90.77	-	

Table S5. Generalized Least Square models (GLSs) of total leaf phosphorus (P) (mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Total P ~ species	11	89.92	-148.42	-50.58	**<0.0001**
(10 species)	null	Total P ~ 1	2	51.08	-97.84	-	
Burnt adult	MM	Total P ~ species	11	75.83	-120.24	-32.96	**<0.0001**
(10 species)	null	Total P ~ 1	2	45.8	-87.28	-	
Seedling	MM	Total P ~ species	12	29.02	-34.05	-53.81	**<0.0001**
(6 species)	null	Total P ~ 1	2	-7.88	19.76	-	
Banksia candolleana	MM	Total P ~ reg form	5	16.67	-13.33	-36.65	**<0.0001**
(unburnt, burnt, seedling)	null	Total P ~ 1	2	-8.99	23.32	-	
Daviesia chapmanii	MM	Total P ~ reg form	5	18.42	-16.83	-14.75	**<0.0001**
(unburnt, burnt, seedling)	null	Total P ~ 1	2	3.71	-2.08	-	
Eucalyptus todtiana	MM	Total P ~ reg form	3	22.86	-33.73	3.75	0.1736
(unburnt, burnt)	null	Total P ~ 1	2	21.94	-37.48	-	
Hakea flabellifolia	MM	Total P ~ reg form	3	23.99	-35.98	5.49	0.74
(unburnt, burnt)	null	Total P ~ 1	2	23.94	-41.47	-	
Jacksonia floribunda	MM	Total P ~ reg form	4	21.05	-28.39	-7.19	**<0.0001**
(unburnt, burnt, seedling)	null	Total P ~ 1	2	13.27	-21.2	-	
Jacksonia nutans	MM	Total P ~ reg form	4	21.55	-29.38	-0.03	0.0149
(unburnt, burnt, seedling)	null	Total P ~ 1	2	17.34	-29.36	-	
Melaleuca leuropoma	MM	Total P ~ reg form	4	27.88	-42.05	-28.9	**<0.0001**
(unburnt, burnt, seedling)	null	Total P ~ 1	2	9.24	-13.15	-	
Petrophile macrostachya	MM	Total P ~ reg form	3	21.82	-31.65	3.11	0.1146
(unburnt, burnt)	null	Total P ~ 1	2	20.58	-34.76	-	
Stirlingia latifolia	MM	Total P ~ reg form	5	19.14	-18.29	-27.3	**<0.0001**
(unburnt, burnt, seedling)	null	Total P ~ 1	2	-1.84	9.01	-	
Verticordia grandis	MM	Total P ~ reg form	3	13.68	-15.35	1.88	0.0538
(unburnt, burnt)	null	Total P ~ 1	2	11.82	-17.23	-	

Table S6. Linear mixed-effect models (LMMs) of leaf total phosphorus (P) and P-containing leaf fractions (lipid P, nucleic acid P, residual P, metabolic P and inorganic P (Pi), and Pi; mg g^{-1} dry weight) for the three regeneration forms and the three families assessed in this study. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between minimal model (MM) and the null model (null). Random intercepts per species were specified in the model. The significant differences (ANOVA) between models are indicated in bold (*P* < 0.05). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings, and the families were Fabaceae, Myrtaceae and Proteaceae.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Total P	MM	P ~ reg form + reg form:family, random = species	14	154.37	-276.03	-274.64	**<0.0001**
	null	P ~ 1, random = species	3	3.82	1.39	-	
Lipid P	MM	P ~ reg form + reg form:family, random = species	14	323.15	-613.58	-173.51	**<0.0001**
	null	P ~ 1, random = species	3	223.16	-440.07	-	
Nucleic P	MM	P ~ reg form + reg form:family, random = species	14	260.64	-488.56	-127.2	**<0.0001**
	null	P ~ 1, random = species	3	183.8	-361.36	-	
Residual P	MM	P ~ reg form + reg form:family, random = species	14	440.26	-847.81	-222.51	**<0.0001**
	null	P ~ 1, random = species	3	315.77	-625.3	-	
Metabolic P	MM	P ~ reg form + reg form:family, random = species	14	232.84	-432.96	-332.97	**<0.0001**
and Pi	null	P ~ 1, random = species	3	53.11	-99.98	-	
Pi	MM	P ~ reg form * family, random = species	11	160.36	-294	-220.93	**<0.0001**
(Fabaceae and Proteaceae)	null	P ~ 1, random = species	3	39.72	-73.07	-	

Table S7. Generalised Least Square models (GLSs) of soil parameters, and soil phosphorus (P) concentration (mg g^{-1} dry weight) for burnt and unburnt sites at Badgingarra National Park, Southwest Australia. The sampled soil parameters were moisture, electrical conductivity (EC), pH in $CaCl_2$ and pH in H_20. The measured soil fractions were Total P, organic P (Po), inorganic P (Pi) and Resin-extractable P (resin P). Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	P
Total P	1	P ~ site	3	-27.72	61.45	-3.89	**0.0152**
	null	P ~ 1	2	-30.67	65.34	-	
Po	1	Po ~ site	3	-19.22	44.43	-0.74	0.0977
	null	Po ~ 1	2	-20.59	45.18	-	
Pi	1	Pi ~ site	4	-9.13	26.27	-14.42	**0.0001**
	null	Pi ~ 1	2	-18.34	40.69	-	
Resin P	1	Resin P ~ site	4	2.11	3.77	-8.77	**0.0017**
	null	Resin P ~ 1	2	-4.27	12.54	-	
Soil moisture	1	soil moisture ~ site	3	-20.83	47.66	-1.71	0.054
	null	soil moisture ~ 1	2	-22.69	49.37	-	
EC (μS cm^{-1})	1	EC ~ site	3	-50.40	106.80	-2.90	**0.0269**
	null	EC ~ 1	2	-52.85	109.70	-	
pH ($CaCl_2$)	1	pH ~ site	3	12.54	-19.07	1.29	0.4009
	null	pH ~ 1	2	12.18	-20.37	-	
pH (H_2O)	1	pH ~ site	3	26.85	-47.70	0.44	0.2117
	null	pH ~ 1	2	26.07	-48.14	-	

Table S8. Generalised Least Square models (GLSs) of seed phosphorus (P) content (μg $seed^{-1}$), seed P concentration ([P]; mg g^{-1}), and seed mass (mg) for five species from Badgingarra National Park, Southwest Australia. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. Δ AIC is the difference in AIC values between selected and null models. The significantly different value (ANOVA) is indicated in bold ($P < 0.05$).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	P
Seed P content	1	seed P ~ species	10	103.49	-186.97	-125.20	**<0.0001**
	null	seed P ~ 1	2	-28.89	61.77		
Seed P concentration	1	seed [P] ~ species	10	-10.45	40.89	-97.60	**<0.0001**
	null	seed [P] ~ 1	2	-67.25	138.50		
Seed mass	1	seed mass ~ species	7	-19.12	52.24	-197.88	**<0.0001**
	null	seed mass ~ 1	2	-121.97	250.12	-	

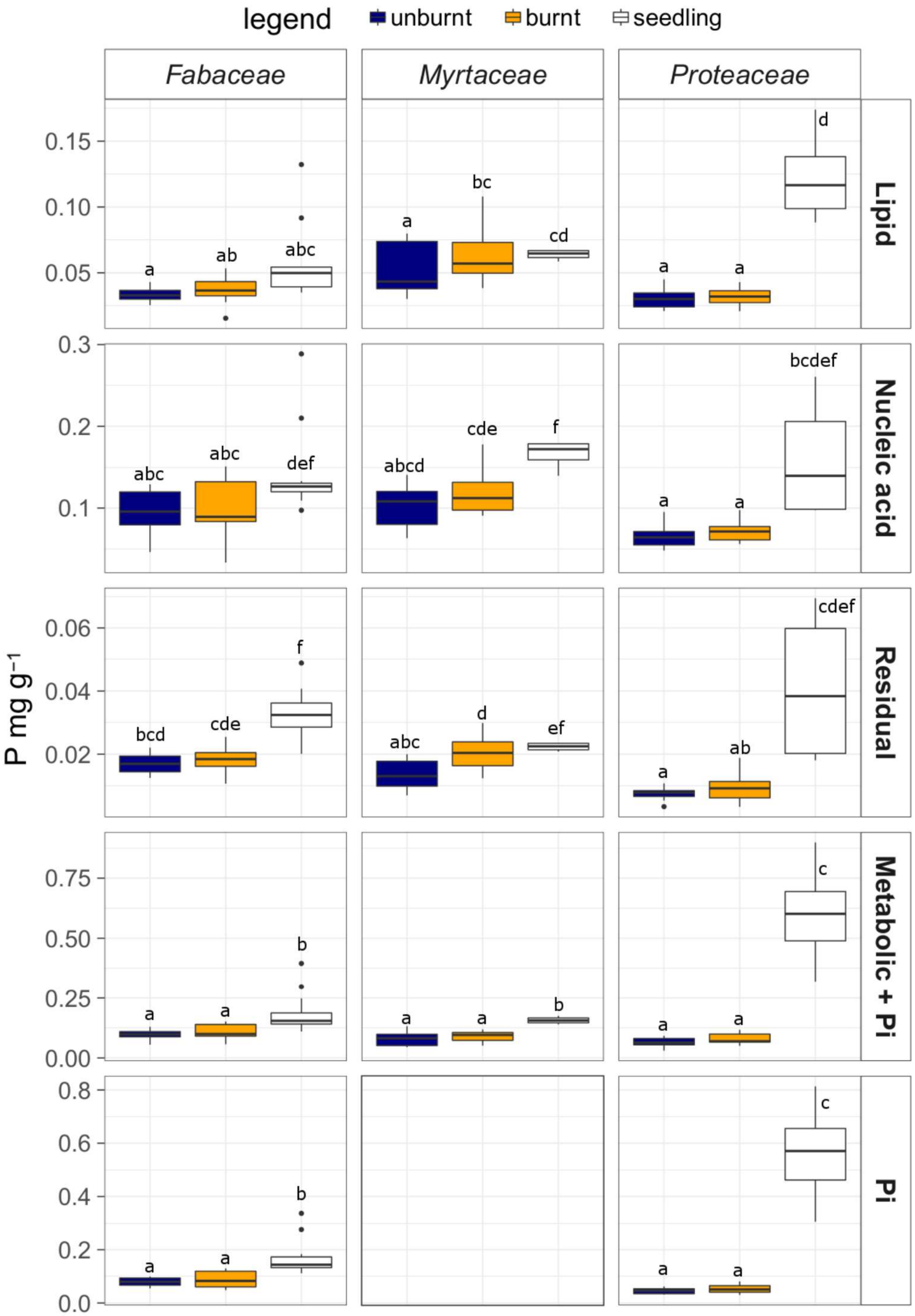

Figure S2. Leaf phosphorus (P) fractions (lipid P, nucleic acid P, residual P, metabolic P and inorganic P (Pi), and Pi) concentrations (mg g^{-1} dry weight) of unburnt and burnt adult plants and seedlings for Fabaceae, Myrtaceae and Proteaceae species collected at Badgingarra National Park, Southwest Australia. Box plots show medians (horizontal lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile range (whiskers) and outliers (filled circles). Different letters indicate significant differences between all regeneration forms based on 95% confidence intervals (CI) from linear mixed-effects model (Supporting Information Table S2).

Table S9. Generalized Least Square models (GLSs) of the lipid fraction of leaf phosphorus (P) (mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Lipid P ~ species	11	157.33	-283.22	-63.36	**<0.0001**
(10 species)	null	Lipid P ~ 1	2	112.09	-219.86	-	
Burnt adult	MM	Lipid P ~ species	11	134.48	-237.54	-38.54	**<0.0001**
(10 species)	null	Lipid P ~ 1	2	101.66	-199	-	
Seedling	MM	Lipid P ~ species	12	83.54	-114.72	-31.66	**<0.0001**
(6 species)	null	Lipid P ~ 1	2	43.81	-83.05	-	
Banksia candolleana	MM	Lipid P ~ reg form	5	41.05	-62.1	-32.61	**<0.0001**
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	17.42	-29.5	-	
Daviesia chapmanii	MM	Lipid P ~ reg form	5	39.28	-58.56	-13.76	**<0.0001**
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	25.07	-44.8	-	
Eucalyptus todtiana	MM	Lipid P ~ reg form	3	25.49	-38.98	3.25	0.1252
(unburnt, burnt)	null	Lipid P ~ 1	2	24.31	-42.23	-	
Hakea flabellifolia	MM	Lipid P ~ reg form	3	31.33	-50.66	3.7	0.1681
(unburnt, burnt)	null	Lipid P ~ 1	2	30.38	-54.36	-	
Jacksonia floribunda	MM	Lipid P ~ reg form	5	43.31	-72.9	5.05	0.1892
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	41.64	-77.95	-	
Jacksonia nutans	MM	Lipid P ~ reg form	5	42.53	-77.07	-3.28	**0.0262**
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	38.89	-73.79	-	
Melaleuca leuropoma	MM	Lipid P ~ reg form	5	45.43	-77.15	-12.48	**<0.0001**
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	35	-64.66	-	
Petrophile macrostachya	MM	Lipid P ~ reg form	3	35.61	-64.81	-4.57	0.3109
(unburnt, burnt)	null	Lipid P ~ 1	2	36.12	-60.24	-	
Stirlingia latifolia	MM	Lipid P ~ reg form	5	41.19	-68.67	-25.26	**<0.0001**
(unburnt, burnt, seedling)	null	Lipid P ~ 1	2	24.37	-43.41	-	
Verticordia grandis	MM	Lipid P ~ reg form	3	29.14	-52.28	-2.37	**0.0366**
(unburnt, burnt)	null	Lipid P ~ 1	2	26.95	-49.91	-	

Table S10. Generalized Least Square models (GLSs) of the nucleic acid fraction of leaf phosphorus (P) (mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Nucleic P ~ species	11	123.3	-215.17	-45.62	**<0.0001**
(10 species)	null	Nucleic P ~ 1	2	86.94	-169.55	-	
Burnt adult	MM	Nucleic P ~ species	11	118.79	-206.15	-51.12	**<0.0001**
(10 species)	null	Nucleic P ~ 1	2	79.68	-155.04	-	
Seedling	MM	Nucleic P ~ species	8	58.31	-91.01	-21.7	**<0.0001**
(6 species)	null	Nucleic P ~ 1	2	36.94	-69.31	-	
Banksia candolleana	MM	Nucleic P ~ reg form	5	33.66	-47.32	-24.24	**<0.0001**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	14.21	-23.09	-	
Daviesia chapmanii	MM	Nucleic P ~ reg form	5	35.22	-50.43	-16.77	**<0.0001**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	19.5	-33.66	-	
Eucalyptus todtiana	MM	Nucleic P ~ reg form	3	29.77	-47.53	5.52	0.7765
(unburnt, burnt)	null	Nucleic P ~ 1	2	29.73	-53.05	-	
Hakea flabellifolia	MM	Nucleic P ~ reg form	3	29.16	-46.33	5.53	0.7968
(unburnt, burnt)	null	Nucleic P ~ 1	2	29.13	-51.86	-	
Jacksonia floribunda	MM	Nucleic P ~ reg form	5	31.32	-48.93	-4.83	**0.0014**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	24.72	-44.11	-	
Jacksonia nutans	MM	Nucleic P ~ reg form	4	34.7	-61.4	-3.19	**0.0274**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	31.11	-58.21	-	
Melaleuca leuropoma	MM	Nucleic P ~ reg form	7	43.18	-44.35	-6.71	**<0.0001**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	21.49	-37.65	-	
Petrophile macrostachya	MM	Nucleic P ~ reg form	3	28.03	-44.07	5.16	0.5086
(unburnt, burnt)	null	Nucleic P ~ 1	2	27.81	-49.23	-	
Stirlingia latifolia	MM	Nucleic P ~ reg form	5	37.98	-62.25	-0.58	**0.0113**
(unburnt, burnt, seedling)	null	Nucleic P ~ 1	2	33.5	-61.66	-	
Verticordia grandis	MM	Nucleic P ~ reg form	3	20.37	-34.73	-2.64	**0.0313**
(unburnt, burnt)	null	Nucleic P ~ 1	2	18.05	-32.09	-	

Table S11. Generalized Least Square models (GLSs) of residual fraction of leaf phosphorus (P) (mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Residual P ~ species	11	201.36	-371.29	-66.86	**<0.0001**
(10 species)	null	Residual P ~ 1	2	154.38	-304.43	-	
Burnt adult	MM	Residual P ~ species	11	184.12	-336.81	-55.01	**<0.0001**
(10 species)	null	Residual P ~ 1	2	143.06	-281.8	-	
seedling	MM	Residual P ~ species	8	95.05	-164.5	-35.82	**<0.0001**
(six species)	null	Residual P ~ 1	2	66.63	-128.68	-	
Banksia candolleana	MM	Residual P ~ reg form	5	53.24	-86.48	-36.93	**<0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	27.45	-49.56	-	
Daviesia chapmanii	MM	Residual P ~ reg form	5	50.53	-81.05	-9.9	**<0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	38.25	-71.16	-	
Eucalyptus todtiana	MM	Residual P ~ reg form	3	36.75	-61.5	-5.68	**0.0008**
(unburnt, burnt)	null	Residual P ~ 1	2	31.11	-55.83	-	
Hakea flabellifolia	MM	Residual P ~ reg form	3	42.4	-72.81	5.45	0.7029
(unburnt, burnt)	null	Residual P ~ 1	2	42.33	-78.26	-	
Jacksonia floribunda	MM	Residual P ~ reg form	4	53.73	-93.75	-19.52	**<0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	39.79	-74.24	-	
Jacksonia nutans	MM	Residual P ~ reg form	4	52.42	-91.13	-11.04	**0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	42.72	-80.1	-	
Melaleuca leuropoma	MM	Residual P ~ reg form	4	58.26	-102.8	-19.08	**<0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	44.53	-83.72	-	
Petrophile macrostachya	MM	Residual P ~ reg form	3	40.43	-68.86	5.18	0.5161
(unburnt, burnt)	null	Residual P ~ 1	2	40.22	-74.04	-	
Stirlingia latifolia	MM	Residual P ~ reg form	4	64.55	-115.39	-27.07	**<0.0001**
(unburnt, burnt, seedling)	null	Residual P ~ 1	2	46.82	-88.32	-	
Verticordia grandis	MM	Residual P ~ reg form	3	37.78	-63.57	-2.04	**0.0057**
(unburnt, burnt)	null	Residual P ~ 1	2	33.96	-61.52	-	

Table S12. Generalized Least Square models (GLSs) of metabolic phosphorus (P) and inorganic P (Pi) fractions of leaf P (mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Metabolic P and Pi ~ species	11	121.35	-211.27	-33.83	**<0.0001**
(10 species)	null	Metabolic P and Pi ~ 1	2	90.89	-177.45	-	
Burnt adult	MM	Metabolic P and Pi ~ species	11	104.66	-177.89	-6.41	**0.0001**
(10 species)	null	Metabolic P and Pi ~ 1	2	87.9	-171.48	-	
seedling	MM	Metabolic P and Pi ~ species	8	39.02	-52.44	-54.11	**<0.0001**
(6 species)	null	Metabolic P and Pi ~ 1	2	1.45	1.67	-	
Banksia candolleana	MM	Metabolic P and Pi ~ reg form	5	21.3	-22.6	-33.5	**<0.0001**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	-2.78	10.9	-	
Daviesia chapmanii	MM	Metabolic P and Pi ~ reg form	5	26.33	-32.66	-13.56	**<0.0001**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	12.21	-19.1	-	
Eucalyptus todtiana	MM	Metabolic P and Pi ~ reg form	3	25.29	-38.57	5.58	0.8937
(unburnt, burnt)	null	Metabolic P and Pi ~ 1	2	25.28	-44.15	-	
Hakea flabellifolia	MM	Metabolic P and Pi ~ reg form	3	29.7	-47.41	4.89	0.3981
(unburnt, burnt)	null	Metabolic P and Pi ~ 1	2	29.35	-52.29	-	
Jacksonia floribunda	MM	Metabolic P and Pi ~ reg form	4	31.17	-48.63	-8.02	**0.0003**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	22.97	-40.6	-	
Jacksonia nutans	MM	Metabolic P and Pi ~ reg form	4	30.78	-47.85	1.16	**0.0271**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	27.17	-49.01	-	
Melaleuca leuropoma	MM	Metabolic P and Pi ~ reg form	4	34.9	-56.08	-22.55	**<0.0001**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	19.43	-33.53	-	
Petrophile macrostachya	MM	Metabolic P and Pi ~ reg form	3	24.81	-37.62	2.57	0.0816
(unburnt, burnt)	null	Metabolic P and Pi ~ 1	2	23.29	-40.19	-	
Stirlingia latifolia	MM	Metabolic P and Pi ~ reg form	5	26.09	-32.18	-35.3	**<0.0001**
(unburnt, burnt, seedling)	null	Metabolic P and Pi ~ 1	2	1.1	3.12	-	
Verticordia grandis	MM	Metabolic P and Pi ~ reg form	3	23.41	-34.82	5.41	0.6588
(unburnt, burnt)	null	Metabolic P and Pi ~ 1	2	23.31	-40.22	-	

Table S13. Generalized Least Square models (GLSs) of leaf inorganic phosphorus (Pi; mg g^{-1} dry weight) for different species within the three regeneration forms, and for the different regeneration forms within each species individually. Model identification and rationale, number of parameters (*K*), log likelihood (log L). The Akaike's information criterion with a correction for finite sample sizes (AICc) represents the uncertainty of the model, whereby lower AICc values represent the more parsimonious models. ΔAICc is the difference in AICc values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$). The regeneration forms (reg form) were unburnt and burnt adult plants and seedlings.

Data	Model ID	Model	K	Log L	AICc	Δ AICc	P
Unburnt adult	MM	Pi ~ species	7	85.0664	-149.133	-35.0782	**<0.0001**
(six species)	null	Pi ~ 1	2	59.313	-114.055		
Burnt adult	MM	Pi ~ species	7	65.1293	-109.259	-12.3873	**<0.0001**
(six species)	null	Pi ~ 1	2	50.7213	-96.8712		
seedling	MM	Pi ~ species	8	38.2039	-50.8078	-48.883	**<0.0001**
(six species)	null	Pi ~ 1	2	3.2482	-1.9249		
Daviesia chapmanii	MM	Pi ~ reg form	5	30.6721	-41.3443	-19.241	**<0.0001**
(unburnt, burnt, seedling)	null	Pi ~ 1	2	13.7183	-22.1033		
Hakea flabellifolia	MM	Pi ~ reg form	3	29.3088	-46.6177	5.343	0.6122
(unburnt, burnt)	null	Pi ~ 1	2	29.1803	-51.9607		
Jacksonia floribunda	MM	Pi ~ reg form	5	40.5588	-61.1175	-23.415	**<0.0001**
(unburnt, burnt, seedling)	null	Pi ~ 1	2	21.5179	-37.7025		
Jacksonia nutans	MM	Pi ~ reg form	4	29.345	-44.9757	-1.9417	**0.0057**
(unburnt, burnt, seedling)	null	Pi ~ 1	2	24.1837	-43.034		
Petrophile macrostachya	MM	Pi ~ reg form	3	26.9833	-41.9665	-5.295	0.5808
(unburnt, burnt)	null	Pi ~ 1	2	26.8308	-47.2615		
Stirlingia latifolia	MM	Pi ~ reg form	5	27.3136	-34.6271	-37.2985	**<0.0001**
(unburnt, burnt, seedling)	null	Pi ~ 1	2	1.331	2.6714		

Table S14. Generalized Least Square models (GLSs) of net photosynthetic rates of youngest fully expanded leaves at saturating light intensity (A_{max}) for two regeneration forms, adults from unburnt site and burnt adults from burnt site, within each of the Proteaceae species. Model identification and rationale, number of parameters (K), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	*P*
Banksia candolleana	1	$A_{\mathrm{max}} \sim$ reg form	3	-49.5837	105.1674	11.59327	**0.0002**
	null	$A_{\mathrm{max}} \sim 1$	2	-56.3803	116.7607		
Hakea flabellifolia	1	$A_{\mathrm{max}} \sim$ reg form	3	-53.8392	113.6785	1.486905	0.4738
	null	$A_{\mathrm{max}} \sim 1$	2	-54.0958	112.1916		
Petrophile macrostachya	1	$A_{\mathrm{max}} \sim$ reg form	3	-51.2842	108.5685	1.460084	0.4625
	null	$A_{\mathrm{max}} \sim 1$	2	-51.5542	107.1084		
Stirlingia latifolia	1	$A_{\mathrm{max}} \sim$ reg form	3	-48.9444	103.8888	1.333028	0.0679
	null	$A_{\mathrm{max}} \sim 1$	2	-50.6109	105.2218		

Table S15. Generalized Least Square models (GLSs) of stomatal conductance (g) of youngest fully expanded leaves at saturating light intensity for two regeneration forms, adults from unburnt site and burnt adults from burnt site, within each of the Proteaceae species. Model identification and rationale, number of parameters (K), log likelihood (log L). The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. ΔAIC is the difference in AIC values between selected and null models. The significantly different values (ANOVA) are indicated in bold ($P < 0.05$).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	*P*
Banksia candolleana	1	$g \sim$ site	3	35.45518	-64.9104	5.866671	**0.005**
	null	$g \sim 1$	2	31.52185	-59.0437		
Hakea flabellifolia	1	$g \sim$ site	3	16.57902	-27.158	6.283387	**0.004**
	null	$g \sim 1$	2	12.43733	-20.8747		
Petrophile macrostachya	1	$g \sim$ site	3	14.50568	-23.0114	3.871614	**0.0154**
	null	$g \sim 1$	2	11.56987	-19.1398		
Stirlingia latifolia	1	$g \sim$ site	3	23.69344	-41.3869	14.58336	**<0.0001**
	null	$g \sim 1$	2	15.40176	-26.8035		

Chapter 4

Does spatial heterogeneity influence seedling establishment in ironstone outcrops?

ABSTRACT

Environmental filters select species with viable ecological strategies from a larger species pool, thereby limiting the set of potentially coexisting species in plant communities. Paradoxically, some of the world's most biodiverse communities are subjected to strong abiotic filters, especially at the vulnerable seedling stage. Spatial heterogeneity within communities broadens the range of viable strategies, by providing a variety of microhabitats. Here, we investigated fine-scale abiotic and biotic drivers of seedling establishment to unveil the role of microhabitat heterogeneity in promoting species coexistence and diversity in a harsh megadiverse ironstone outcrop ecosystem. We characterised environmental heterogeneity associated with the presence of two dominant shrubs, generally taller than the other species, *Mimosa calodendron* (Fabaceae) and *Lychnophora pinaster* (Asteraceae), and tested whether seedling abundance and adult richness of all species are greater under presumably milder conditions in microhabitats associated with these shrubs. We also assessed whether seedling growth and survival differed between specific microhabitats. Spatial heterogeneity in the community was mostly driven by differences in substrate properties, and plots associated with *M. calodendron* and *L. pinaster* exhibited different biotic and abiotic conditions than those lacking these shrubs. Microhabitat under these two species had greater seedling abundance and adult richness, suggesting an apparent beneficial effect of the environmental conditions associated with these plants. However, we identified two potential negative plant-plant interactions: larger crown area of the two dominant shrubs was negatively associated with species richness, and greater understorey plant cover with seedling abundance. None of the measured attributes were good predictors of seedling survival, and relative growth rates of seedlings were lower in plots with the dominant shrubs and in fragmented rock substrates, and could reflect differences in resource availability among microhabitats. Our study shows that regeneration from seed in ferruginous *campo rupestre* is marked by complex spatial heterogeneity driven by abiotic and biotic factors that affect seedling growth and abundance, thereby allowing species coexistence and promoting species diversity.

INTRODUCTION

Plants are at highest risk of mortality during early stages of development (Stearns 1976; Moles & Westoby 2004; Kitajima & Fenner 2009); hence, regeneration of plant communities is fine-tuned with environmental conditions that allow seed germination, successful seedling establishment and growth to ensure species persistence (Grubb 1977; Leck *et al.* 2008). Many environmental factors related to substrate and plant cover affect seedling survival, including microtopography (Hughes *et al.* 2016), soil properties (Ailstock *et al.* 2008), litter layer (Loydi *et al.* 2013), canopy cover (Nicotra *et al.* 2010; Ibáñez *et al.* 2015), plant-plant interactions (Rodríguez-García *et al.* 2011; Boeken 2018), and plant-microbe interactions (Reynolds *et al.* 2003; Mangan *et al.* 2010). Seedling survival is also influenced by complex interactions between environmental conditions and species traits (Milberg & Lamont 1997; Lloret *et al.* 1999; Larson & Funk 2016b), hindering our understanding of demographic patterns, and our ability to predict the impact of environmental change on regeneration processes (Larson & Funk 2016a).

Environmental conditions vary in space, and drive community assembly processes across different scales, from biomes to microhabitats (Conti *et al.* 2017; Stark *et al.* 2017; Scherrer *et al.* 2019). Seed germination and seedling establishment are mostly driven by biotic and abiotic conditions at the microenvironmental level, that is, not larger than the largest individual plants in the plant community (Kollmann 2000; Mangan *et al.* 2010), and variation in seedling traits within communities can be as great as that between them (Wright & Westoby 1999). Thus, to understand the spatial structure and dynamics of plant communities, we need to investigate seedling abundance, growth and survival in different microhabitats.

By providing a variety of different microhabitats, spatial and temporal heterogeneity within communities is often considered one of the main drivers of species coexistence, and consequently of diversity in plant communities (Wright 2002; Adler *et al.* 2013; Kraft *et al.* 2015; Falster *et al.* 2017). For instance, *campo rupestre* is an old and nutrient-impoverished montane grassland (Hopper *et al.* 2016; Silveira *et al.* 2016), where spatial heterogeneity is associated with remarkable species diversity (Silveira *et al.* 2016; Carmo & Jacobi 2016). This ecosystem consists of a mosaic of grasslands associated with rocky outcrops, where soil depth varies with local topography and faulting/fracturing, providing a diversity of soil environments that have led to the assembly of distinct plant communities within the same landscape (Alves & Kolbek 2010; Le Stradic *et al.* 2015; Carmo & Jacobi 2016; Abrahão *et al.* 2019). At a very fine scale, variation in rock microrelief within rocky outcrops also affects species composition, and is associated with different strategies to cope with water shortage and mechanical resistance to root growth (Carmo *et al.* 2016).

At the community scale, stressful abiotic conditions in *campo rupestre* represent a strong biological filter for both seedlings and mature plants, selecting species with similar strategies (Negreiros *et al.* 2014; Dayrell *et al.* 2018). Despite these harsh conditions, ferruginous *campo rupestre* (i.e. established in ironstone outcrops), locally known as *canga*, comprises more than 1,436 plant species in a small area of 1,580 km^2 in Southeast Brazil (Carmo *et al.* 2018). The large number of species that coexist in this harsh environment pose a question of whether a variety of microhabitats for seedlings plays an essential role in species coexistence and species diversity. Studies on seedlings of ironstone outcrops are limited in number and mostly focused on specific groups (e.g., Poot & Lambers 2003; Yates *et al.* 2011), and we still do not understand patterns of regeneration processes from a community perspective. Since this is a habitat extremely threatened by mining-related activities (Salles *et al.* 2019), this information is relevant to better inform conservation and restoration of the remaining areas.

Two dominant shrubs, *Mimosa calodendron* (Fabaceae) and *Lychnophora pinaster* (Asteraceae) occur in soil-filled depressions, steps and crevices, where soil and water accumulate, thus occupying microhabitats with milder environmental conditions within ferruginous *campo rupestre* (Jacobi *et al.* 2007; Carmo & Jacobi 2016). Here, we studied seedling establishment in microhabitats directly influenced by one of these two dominant species, as compared with microhabitats lacking both species. We aimed to unveil fine-scale abiotic and biotic drivers of seedling establishment, and to understand how regeneration from seed occurs in this type of environment. Specifically, we tested whether 1) microhabitats beneath *M. calodendron* and *L. pinaster* are different to those lacking both species, 2) seedling abundance, growth and survival, and adult richness of understorey species are greater under the crown of these two shrubs, and are positively correlated with shrub cover and soil depth.

METHODS

Study site

The Iron Quadrangle is a mountainous region in Southeast Brazil and constitutes an ecotonal area between two global biodiversity hotspots, the *Cerrado* and the Atlantic Rainforest ecoregions (Myers *et al.* 2000). For tens of millions of years, chemical and physical weathering of the iron-rich rocks in the higher plateaus and in the mountain crests of the Iron Quadrangle formed crusts of iron oxy-hydroxides, which are generally called ironstone outcrops, locally known as *canga* (Schaefer *et al.* 2016). The soils in these habitats are shallow, acidic and remarkably phosphorus (P)-impoverished (Schaefer *et al.* 2016; Carmo & Jacobi 2016), constituting a strong edaphic filter that resulted in islands of ferruginous *campo rupestre* embedded in an Atlantic Forest and *Cerrado* matrix (Silveira *et al.* 2016; Carmo & Jacobi 2016; Salles *et al.* 2019). This ecosystem is also heavily impacted by mining-related activities, as it is strongly associated with iron ore deposits, having lost more than 50% of its original area (Salles *et al.* 2019).

This study was conducted in a landscape of ferruginous *campo rupestre* at *Serra da Calçada* (located in the Iron Quadrangle region; 20°06'S, 43°58'W, altitude of approximately 1,500 m a.s.l.). The study site had no history of topsoil removal activities and no signs of invasion by exotic plant species. Temperature is 10–26°C in winter and 18–30°C in summer, and mean annual rainfall is 1,485 mm, of which nearly 90% falls between October and March (Instituto Nacional de Meteorologia, average between 1981 and 2010 from Estação Ibirité; http://www.inmet.gov.br/portal/index.php?r=clima/normaisclima tologicas).

The study site encompasses an area dominated by two sclerophyllous and perennial shrubs, *M. calodendron* and *L. pinaster*, which are generally prevalent in ironstone outcrops at the Iron Quadrangle (Viana & Lombardi 2007; Carmo *et al.* 2016; Carmo & Jacobi 2016).

Lychnophora pinaster is restricted to *campo rupestre* of Minas Gerais state (Loeuille 2015), whereas *M. calodendron* is restricted only to ironstone outcrops at the Iron Quadrangle (Carmo *et al.* 2018). In general, individuals of *M. calodendron* were taller and exhibited a larger crown area than those of *L. pinaster*. Adult individuals of *L. pinaster* sampled in this study had an average height of 56 ± 10 mm (mean ± SD, range 37–66 mm) and a crown area of 0.65 ±0.5 m^2 (range 0.2–1.9), while those of *M. calodendron* had an average height of 86 ± 14 mm (range 67–109 mm) and a crown area of 1.1 ±0.6 m^2 (range 0.3–2.8).

Sampling design

We established three parallel 50 m long transects, each 100 m apart from each other. Five blocks were established in each transect (at each 10 m). Each plot consisted of three 50 x 50 cm plots differing according to the presence of: 1) *M. calodendron*, 2) *L. pinaster*, 3) neither of these species, i.e. 1 m away from the crown of these two species (Figure 1; n = 15 plots associated with each species, n = 45 plots in total). We first selected the adult individual of *M. calodendron* closest to each 10 m mark. We then randomly drew a direction and picked an adult individual of *L. pinaster* distancing 1 m from any adult individual of *M. calodendron*, moving the plot clockwise whenever necessary. Finally, the same process was used to select a plot distancing 1 m from any adult individual of *M. calodendron* and *L. pinaster.* The size of the plot was chosen to maximise the number of seedlings sampled, while allowing for the search of very small seedlings and preventing the plot from exceeding the average area under the shrub crown.

Figure 1. Plots in ferruginous *campo rupestre* with the presence of a) *Mimosa calodendron*, b) *Lychnophora. pinaster*, c) neither, i.e. 1 m away from the crown of these two species. Quadrats measuring 25 x 25 cm (a quarter of the plot) with 16 subdivisions (shown in the three photos) were used to aid visual estimation of ground cover and map the location of seedlings.

Plot survey

Our survey was carried out from April to early June 2015, at the rainy-to-dry season transition. We recorded species richness (i.e. species number) and seedling abundance and composition for each plot. The percentage of understorey plant cover (including all plants under the crown of *M. calodendron* and *L. pinaster*), ferricrete cover (i.e. bare rock) and fragmented rock (gravel) cover was estimated visually, adding up to the 100% ground cover within each plot. We also estimated visually the litter cover percentage of total ground cover. Quadrats measuring 25 x 25 cm with 16 subdivisions were used to aid visual estimation (Figure 1), following the method described by Wikum & Shanholtzer (1978), but adapted to a finer scale. Substrate depth (cm; i.e., soil plus litter layer) was measured with a calliper until it reached the rock at 16 points within each plot (eight equally-spaced points in each diagonal of the plot). The substrate depth of the 16 points was then averaged for each plot. Maximum (A) and minimum (B) crown diameters of shrubs were measured to calculate crown area, considering crown shape as an ellipse:

$$\text{Crown area} = \pi * A/2 * B/2 \qquad \text{(eqn. 1)}$$

Seedlings

We defined seedlings as structurally simple, small individuals, unbranched shoot, with no signs of resprouts or clonal origin and absence of reproductive organs. The presence of embryonic structures such as persistent cotyledons was not a requirement, and, thus, our definition comprises both seedlings and juveniles (*sensu* Gatsuk *et al.* 1980). In each plot, we identified all seedlings in the plots, and measured their initial heights (H0), either with a digital calliper or a measuring tape. Due to absence of reproductive structures, many seedlings could not be identified past the genus-level. We also recorded the type of substrate (fragmented rocks; or associated with at least some soil, ferricrete, or on other plants and biocrusts) seedlings were established on at the rooting point (stem base) and the presence of a litter layer.

To assess seedling survival and growth, we mapped the position of all seedlings in each plot during the survey in 2015 with the aid of the quadrats (Figure 1) and reassessed them in the subsequent onset of the dry season in May 2016. At the time of reassessment in 2016, two plots had been vandalised and six seedlings were lost and excluded from survival and growth analysis. Missing individuals were scored as dead. We measured final height (Hf) of the surviving individuals and calculated the relative growth rate (RGR) over a time period (tp) of one year according to the following equation:

$$RGR = \frac{\ln Hf - \ln H0}{tp} \qquad \text{(eqn. 2)}$$

Statistical analyses

Statistical analyses were performed with the R software platform (R Development Core Team 2017) and graphs created with ‘ggplot2’ package (Wickham 2016). We explored the measured attributes within the plots using a principal component analysis (PCA) to assess whether the multivariate structure of the dataset could effectively represent both substrate- and plant-driven spatial heterogeneity and to investigate the relative importance of the attributes and the relationships among them. First, attribute values of each plot were transformed to provide distributions as close as possible to normality. Then, a PCA of transformed attribute values was performed using the ‘prcomp’ function computed with the correlation method. We used LMMs as described below to assess differences in principal components (PC1, PC2 and PC3) between associated species.

To investigate the environmental variables associated with plant richness and seedling establishment, we used generalised linear mixed-effects models (GLMMs) and linear mixed-effects models (LMMs) with block nested within transect as random effects to avoid pseudo-replication (Pinheiro & Bates 2000). Separate analyses were conducted for each of the two dependent variables associated with the plots, species richness and seedling abundance (Table 1). The potential explanatory variables initially considered as fixed effects in both analyses were associated species (*Lychnophora pinaster, Mimosa calodendron* or neither), crown area (m^2), understorey plant cover (%), fragmented rock cover (%), litter cover (%) and substrate depth (mm). Initial analysis showed that fragmented rock cover and ferricrete cover (%) were confounded, and thus the later was excluded from the model. We then tested whether associated species could explain differences in seedling abundance of the most prevalent species individually (seedling number > 5; Table 1, Table 2).

Finally, analyses were conducted separately for each of the two dependent variables associated with each individual seedling: survival and growth (Table 1). The potential explanatory variables initially considered as fixed effects in both analyses were: associated species (*M. calodendron* or *L. pinaster* or neither); establishment in fragmented rocks (yes or no); establishment on other plants and biocrusts (yes or no); presence of litter layer (yes or no); seedling species; seedling initial height and the interaction of these two last factors. To ensure a minimum number of species per group, all species with less than five seedlings recorded for all 45 plots were grouped as dicots (n = 17) and monocots (n = 10; Table 2).

Species richness and seedling growth were analysed using LMMs, performed with the 'nlme' package (Pinheiro *et al.* 2017). Due to heteroscedasticity, variance structure of the data was modelled first using the 'weights' option in the 'lme' function with best variance structure determined according to AIC comparisons (Zuur et al. 2009). For all other analyses, we used GLMMs with different error distribution models that better fitted each dependent variable. The lme4 package (Bates *et al.* 2015) was used to fit GLMMs with negative binomial distribution for seedling abundance, and a binomial distribution for seedling survival. The glmmTMB package (Brooks *et al.* 2017) was used to fit GLMMs with Poisson distribution for seedling abundance of *M. calodendron* and zero-inflated Poisson distribution for seedling abundance of all other prevalent species. Significance of models was estimated by comparing a minimal model with the null model. In GLMMs and LMMs, the minimal models were obtained by a stepwise deletion of non-significant parameters and their interaction (P>0.05), starting from a full factorial model. In the minimal model, only significant factors remained. Comparisons between significant parameters within each model were determined by the post-hoc Tukey–Kramer honest test (McCulloch & Neuhaus 2005) by using 'lsmeans' package (Lenth 2016).

Table 1. List of dependent and independent (potential explanatory) variables analysed, with respective sampling units. Each dot (●) indicates one variable and indented text indicate levels within variables when present. Units of measurement are shown in parenthesis. One model was constructed for each dependent variable. Block was included as random effect in all models.

Sampling unit	**Dependent variables**	**Independent variables**
plot	● species richness ● seedling abundance	● associated species *Lychnophora pinaster* *Mimosa calodendron* neither ● crown area (m^2) ● understorey plant cover (%) ● fragmented rock cover (%) ● litter cover (%) ● substrate depth (mm)
plot	seedling abundance of most prevalent species: ● *Lychnophora pinaster* ● *Microstachys corniculata* ● *Mimosa calodendron* ● *Symphyopappus brasiliensis* ● *Vellozia* spp.	● associated species *Lychnophora pinaster* *Mimosa calodendron* neither
seedling (each individual)	● seedling survival ● seedling growth	● associated species *Lychnophora pinaster* *Mimosa calodendron* neither ● establishment in fragmented rocks yes or no ● establishment on other plants, and biocrusts yes or no ● presence of litter layer yes or no ● seedling species *Lychnophora pinaster* *Microstachys corniculata* *Mimosa calodendron* *Symphyopappus brasiliensis* *Vellozia* spp. dicots monocots ● seedling initial height (mm)

Table 2. List of species with seedlings in ferruginous *campo rupestre* at *Serra da Calçada,* Southeast Brazil. The column "seedlings (*n*)" indicates the total number of seedlings found for each species in the 45 plots; the column "plots (*n*)" indicates the number of plots, out of 45, in which the species was found. Survival refers to rate of seedling survival: individuals that lived (L) divided by the total initial number (T), and survival percentage (in parentheses). Height is the initial height and values are shown as mean (±SE). Non-identified (NI).

Family / species	seedlings (*n*)	plots (*n*)	survival L/T	(%)	height (mm)		
Apocynaceae							
Ditassa sp. ^{D}	1	1	1/1	(100)	124		
Asteraceae							
Lychnophora pinaster *	13	8	6/12	(50)	45	±	5
Symphyopappus brasiliensis *	13	7	8/13	(62)	48	±	12
Bromeliaceae							
Dyckia consimilis ^{M}	4	3	2/4	(50)	15	±	5
Euphorbiaceae							
Microstachys corniculata *	6	4	4/6	(67)	53	±	13
Fabaceae							
Mimosa calodendron *	28	7	17/28	(61)	36	±	5
Orchidaceae							
Hoffmannseggella caulescens ^{M}	1	1	0/1	(0)	26		
Rubiaceae							
NI ^{D}	4	2	4/4	(100)	50	±	19
Velloziaceae							
Vellozia spp. *	100	17	66/95	(69)	9	±	1
Verbenaceae							
Stachytarpheta sp. ^{D}	3	3	2/3	(67)	32	±	25
NI	2	1	0/2	(0)	24		
NI (dicots) ^{D}	7	4	3/7	(43)	24	±	4
NI (monocots) ^{M}	5	5	1/5	(20)	40	±	13

* most common species. ^{D} species grouped as dicots. ^{M} species grouped as monocots (see methods).

RESULTS

Substrate- and plant-driven spatial heterogeneity

Principal component 1 explained 40.6% of the variance of measured attributes amongst all sampled plots in the ferruginous *campo rupestre*, and correlated positively with ferricrete cover (Pearson's r = 0.80) and negatively with litter cover, fragmented rock cover, substrate depth and crown area (Pearson's r > 0.70, Figure 2). This axis separated plots where the ironstone was fragmented and litter cover was greater from those where ferricrete cover prevailed. Principal component 2 explained 20.6% of the variance, and correlated negatively with understorey plant cover (Pearson's r = 0.86) and positively with seedling abundance (Pearson's r = 0.52) and fragmented rock cover (Pearson's r = 0.47). Thus, PC2 separated plots with more understorey plant cover from those where seedlings were more abundant. Principal component 3 accounted for an additional 12.8% of the total variance in attributes and was mainly correlated with species richness (Pearson's r = 0.78), which did not correlate well with the first two axes. Principal component 1 and 2, but not PC3, separated plots where *M. calodendron* and *L. pinaster* were present from those where neither of these two species were present (Table S1).

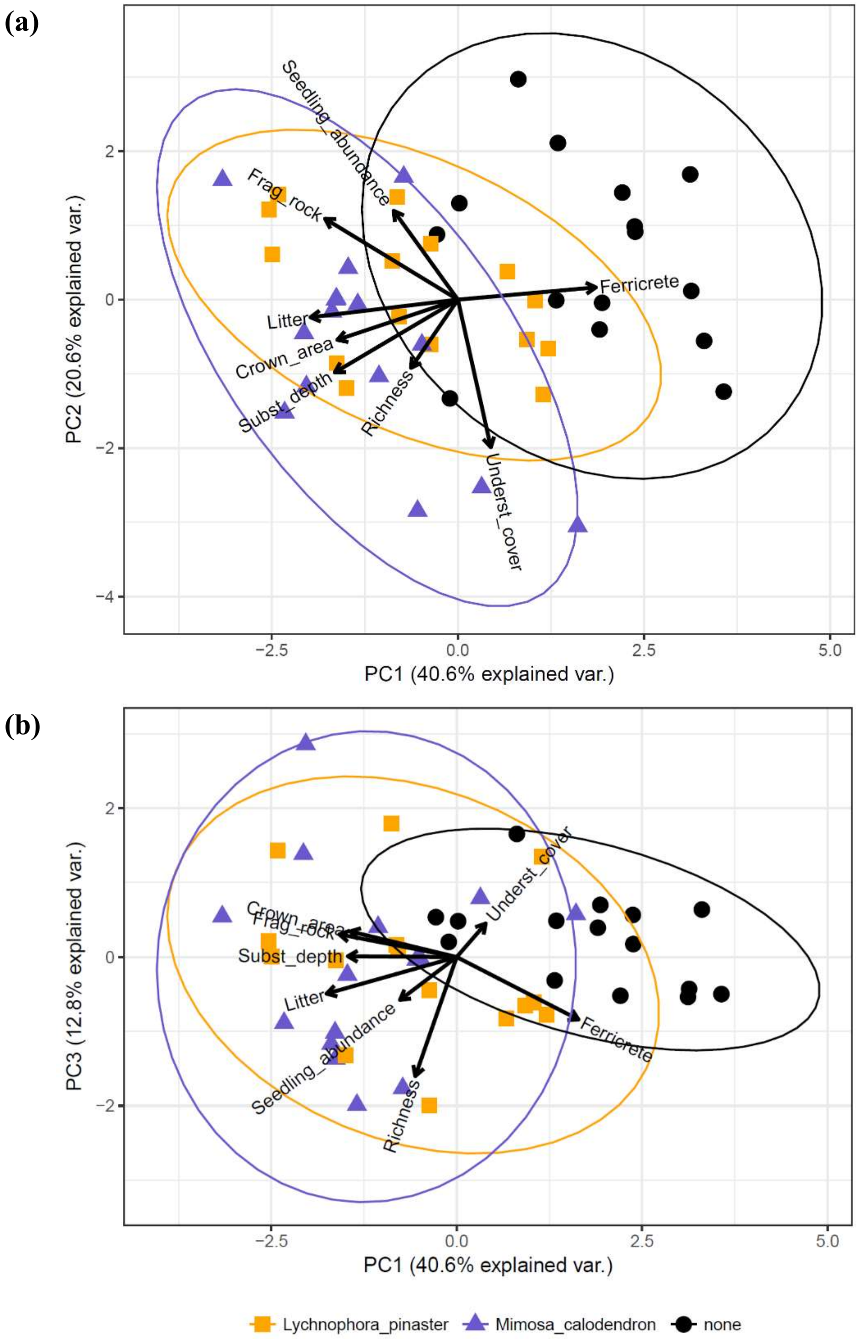

Figure 2. A principal components analysis (PCA) showing the main axes of variability in measured attributes amongst 45 plots in a ferruginous *campo rupestre*, Southeast Brazil. Axis 1 versus axis 2 (a) and axis 1 versus axis 3 (b) are shown. Plot scores are clustered into three groups according to the presence of adults of *Mimosa calodendron* (purple triangle) or *Lychnophora pinaster* (orange square) or the absence of both species (black dots). Confidence ellipses represent 95% intervals of confidence around the groups. Solid arrows indicate direction and weighing of loadings (vectors) representing the eight variables assessed in each plot. Measured variables are understorey plant cover (underst_cover), ferricrete cover (ferricrete), fragmented rock cover (frag_rock), litter cover (litter), crown area, substrate depth (subst_depth), seedling abundance and species richness (richness).

Among all environmental variables assessed within the plots (i.e. associated species, crown area, understorey plant cover, fragmented rock cover, litter cover and substrate depth), only associated species and crown area had a significant effect on species richness ($P<0.0001$; Table S2). Species richness was greater in plots were *M. calodendron* was present, followed by plots associated with *L. pinaster*, and it was lowest in plots with neither of these species (Figure 3a). Despite greater species richness in plots associated with the two shrubs, species richness decreased with increasing crown area (Figure 3c). Both associated species and understorey plant cover had an effect on seedling abundance ($P=0.0082$; Table S2): seedling abundance was greater in plots where *M. calodendron* was present compared with plots with neither of the two associated species (Figure 3c), and negatively associated with understorey plant cover (Figure 3d).

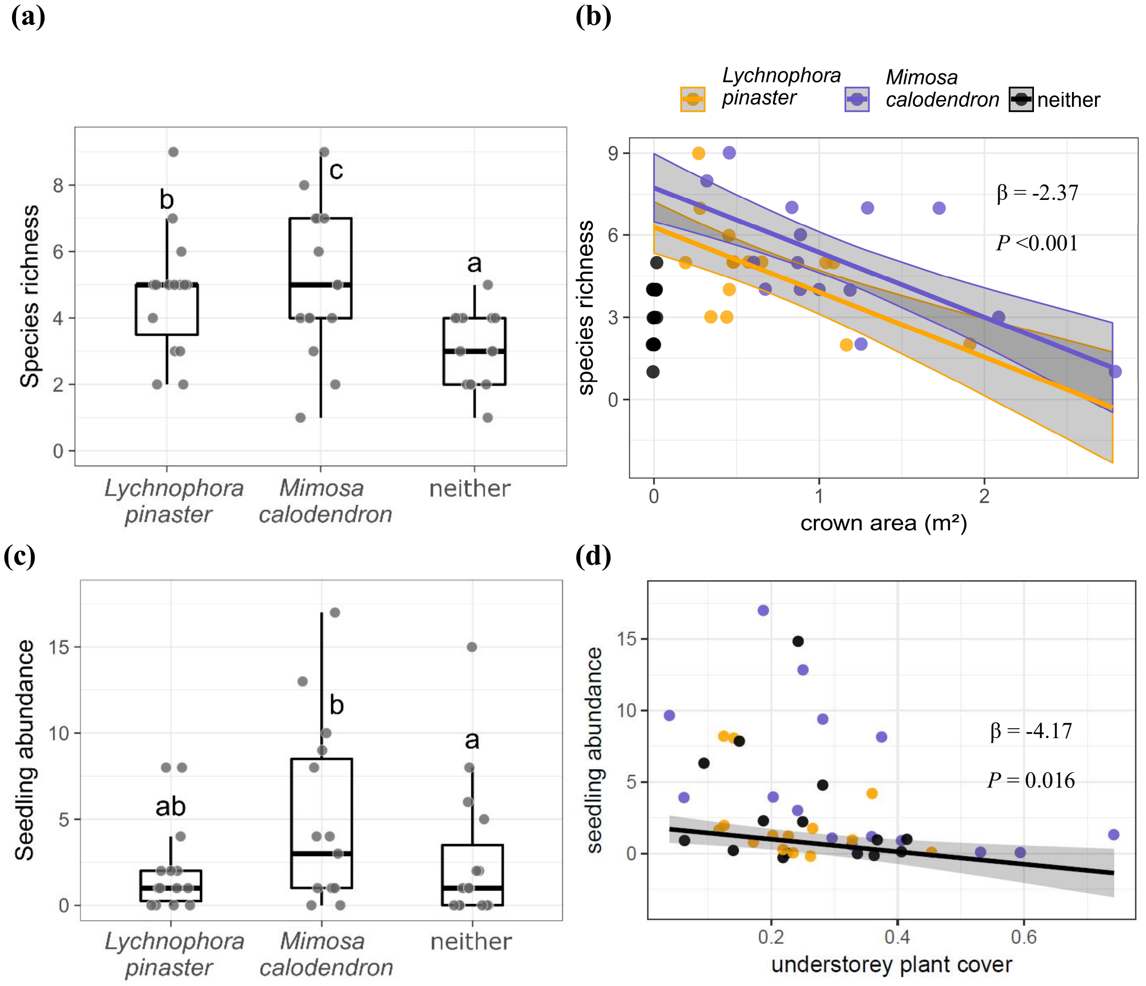

Figure 3. Environmental variables associated with species richness and seedling abundance for 45 plots in ferruginous *campo rupestre*, Southeast Brazil. Environmental variables were associated species (a, c), crown area (m^2) of the associated species (b) and fraction of understorey plant cover (d). Each line represents the line of best fit and grey areas indicate the 95% confidence interval derived from generalised linear mixed-effect models (GLMMs; Table S2). Dots are values of individual plots coloured according to the presence of *Mimosa calodendron* (purple) or *Lychnophora pinaster* (orange) adult shrubs or absence of either of the two species (black). Model coefficients (β; slope of the line) and P-values (*P*). Black and larger dots (in b and d) represent means, grey and smaller dots are individual samples, error bars are SE, and different lower-case letters represent significant differences between groups based on GLMMs (Table S2) followed by the Tukey–Kramer honest test. Seedling abundance of one plot with *L. pinaster* as associated species (value = 43) was omitted in (c and d) to improve visualisation.

Understorey adult plants

Adult plants of some species and plant groups, apart from *L. pinaster* and *M. calodendron*, were commonly found on the study site (Table S3). The most dominant families were Velloziaceae and Poaceae, which were found in 82% and 76% of the plots, respectively. The most abundant species of other families were *Hoffmannseggella caulescens* (Orchidaceae; 53% of the plots), *Trilepis lhotzkiana* (Cyperaceae; 33%), *Symphyopappus brasiliensis* (Asteraceae; 27%) and *Microstachys corniculata* (Euphorbiaceae; 11%).

Seedling spatial patterns

A total of 187 seedlings were recorded in the 45 plots (Table 2), equivalent to a density of 16.6 seedlings per m^2. More than half of the seedlings were *Vellozia* spp. (n=100, Figure 4a), present in over a third of the plots. *Mimosa calodendron* was the second most abundant species (n=28, Figure 4b), followed by *L. pinaster* and *S. brasiliensis* (n=13 each, Figure 4c), and *M. corniculata* (n=6). Some rare species were *Ditassa* sp., *Stachytarpheta* sp., *Dyckia consimilis* and *H. caulescens*. Twelve of the 45 plots (~27%) had no seedlings.

Figure 4. Seedlings of a) *Vellozia* spp., b) *Mimosa calodendron*, and c) *Lychnophora pinaster* in ferruginous *campo rupestre*. a) Yellow arrow heads show seedlings amongst the fragmented rock substrate, and the white arrow shows a pencil.

Seedling survival and growth

Overall, 63% of the seedlings survived over the measured year. None of the measured attributes (i.e. associated species, establishment in fragmented rocks, establishment on other plants and biocrusts, presence of litter, initial height, and species) was a good predictor of seedling mortality. Establishment on fragmented rocks was the best predictor amongst all attributes, but had no significant effect on seedling survival compared with the null model (*P*=0.104; Table 3; Figure S1, Figure S2). On the other hand, associated species, establishment in fragmented rocks, seedling species, initial height and the interaction between the last two had a significant effect on seedling growth (*P*<0.0001; Table 3). This reflected the differential growth rates among species (faster relative growth rates when the two shrub species were absent, slower growth with increase in seedling size) and the greater growth of seedlings established on substrates other than fragmented rocks, that is, organic substrate (either litter or other plants and biocrusts), since no seedlings were found on ferricrete (Figure 5; Figure S3). In plots where a shrub of *M. calodendron* was present, seedlings of *M. calodendron* were more abundant, while seedlings of *Vellozia* spp. were less abundant compared with the plots with the presence of *L. pinaster* or neither of the two associated species (Figure S4; Table S4).

Table 3. Generalised linear mixed-effect models (GLMMs) of seedling survival and growth for plots in a ferruginous *campo rupestre*. The potential explanatory variables initially considered as fixed effects in the full model were associated species (associated sp; *Lychnophora pinaster, Mimosa calodendron*, neither), establishment on fragmented rocks (frag), establishment on other plants and biocrusts, presence of litter layer, seedling species, seedling initial height, and the interaction of the last two (sp * h0). Random intercepts per blocks nested within transects were specified in the model. The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. Δ AIC is the difference in AIC values between minimal model (MM) and the null model (null). Model identification is (Model ID), rationale (Model), number of parameters (K), and log likelihood (Log L). The significant differences (ANOVA) between minimal and null models are indicated in bold (*P*<0.05). Analysed seedlings species shown in Table 2.

Data	Model ID	Model	*K*	Log L	AIC	Δ AIC	*P*
Total seedlings	MM[1]	survival ~ frag random = transect/block	4	-97.6	203.2	-0.8	0.0976
	null[1]	survival ~ 1 random = transect/block	3	-99.0	204.0		
Surviving seedlings	MM[2]	RGR ~ associated sp + sp * h0 + frag random = transect/block	21	5.0	32.0	-80.6	**<0.0001**
	null	RGR ~ 1 random = transect/block	4	-52.3	112.7		

[1] binomial distribution specified; [2] normal distribution with specified variance structure.

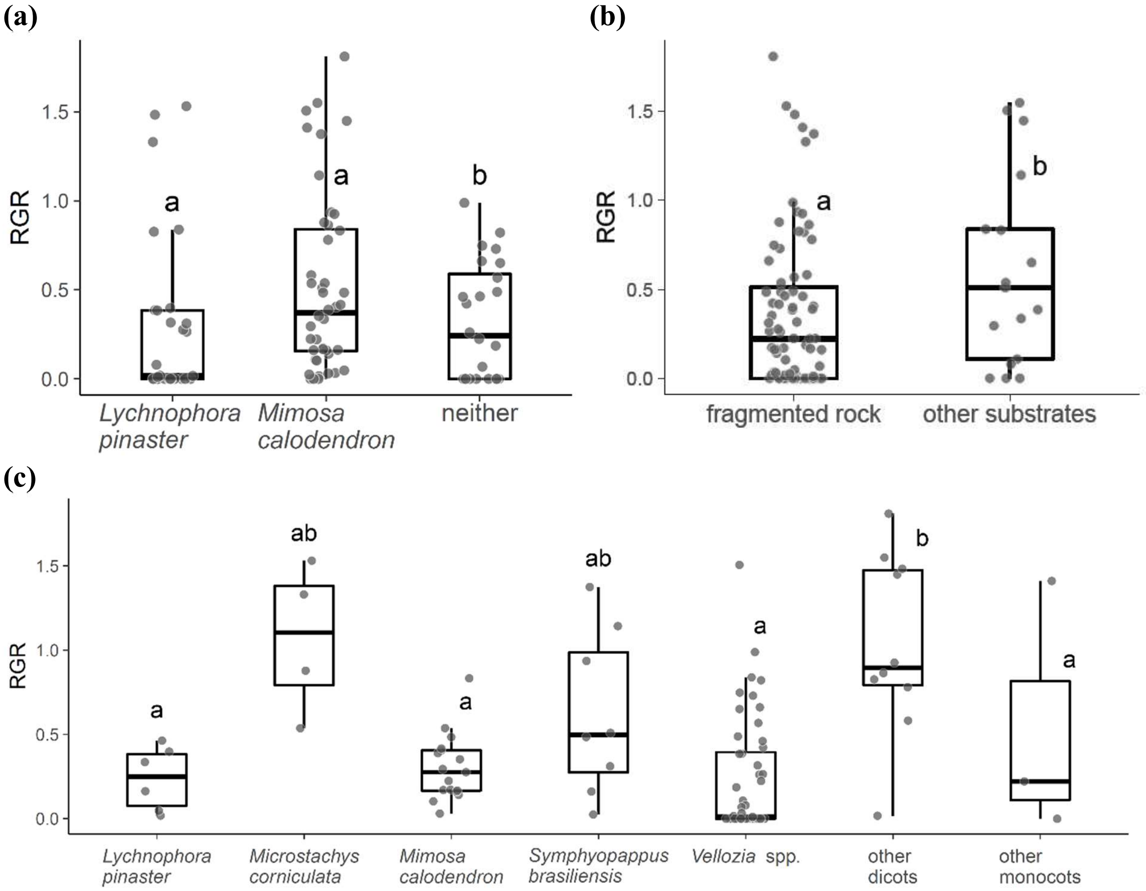

Figure 5. One-year relative growth rates (mm mm^{-1} year^{-1}) of surviving seedlings in a ferruginous *campo rupestre* in Southeast Brazil for: a) seedlings established under different associated species (*Lychnophora pinaster*, *Mimosa calodendron*, neither), b) seedlings established in fragmented rocks or on other substrates, c) most prevalent species. Black and bigger dots represent means, lighter and smaller dots are individual samples, error bars are SE (n=96), and different lower-case letters represent significant differences between groups based on generalised linear mixed-effect models (Table 3) followed by the Tukey–Kramer honest test. Other dicots and other monocots are specified in Table 2.

DISCUSSION

Patterns of spatial heterogeneity

Habitat heterogeneity is a well-known driver of species coexistence in many biodiversity hotspots (Thuiller *et al.* 2006; Cramer & Verboom 2017; Morellato & Silveira 2018), and an important driver of successful establishment for specific plant species in these environments (Lamont *et al.* 1993; Poot & Lambers 2003; Yates *et al.* 2011). However, the main drivers of seedling survival and growth in many biodiverse communities remain poorly understood, limiting our knowledge of the assembly of these plant communities (Grubb, 1977; Paine et al., 2015). Here, we identified important drivers of spatial heterogeneity in a plant community of ferruginous *campo rupestre*. Principal component analysis identified that there were substantial differences in environmental characteristics between plots containing either of the two shrub species and plots where the shrubs did not occur, which can be interpreted as spatial heterogeneity across the site. Variation among plots was mostly driven by substrate-related attributes (i.e. ferricrete cover, fragmented rock cover, litter cover, and substrate depth), which was associated with crown area. Understorey plant cover was also associated with spatial variability in the ferruginous *campo rupestre*, and was mostly unrelated to soil development and crown area. As predicted, plots where *M. calodendron* and *L. pinaster* were present had more developed substrate and greater understorey plant cover, and were clearly separated from plots lacking both species. The microenvironment in plots associated with these shrub species can be considered as more benign for plant growth, as their crowns provide shade, the more developed substrate offers less mechanical resistance to root growth, and litter provides organic matter and some moisture retention. This agrees with previous reports on different microhabitats within ironstone outcrops (Jacobi *et al.* 2007) which should allow co-occurrence of species with distinct requirements, including seed germination and seedling establishment (Dalling & Hubbell 2002; Pearson *et al.* 2003), contributing to the great species diversity of ferruginous *campo rupestre*.

Seedlings were more abundant in plots with the presence of *M. calodendron* than in plots without the two associated species, and the potentially milder microenvironment in the ironstone outcrop might provide a safer site for seed germination and/or seedling emergence. Plots with *L. pinaster* had intermediate values of seedling abundance and did not differ from the other two types of plots. Although *M. calodendron* had greater average crown size than *L.*

pinaster, crown area of shrubs did not explain seedling abundance in plots. Thus, the intermediate values of seedling abundance in plots with *L. pinaster* may be due to specific interactions between shrub species and seedlings. For example, *M. calodendron* may ameliorate its vicinity playing a role as a N_2 fixer (dos Reis Jr *et al.* 2010), and *L. pinaster* could exert some allelopathic effect on seedling establishment due to its secondary metabolic compounds (Haber *et al.* 2009).

Plots with the presence of *M. calodendron* do not seem to be the most suitable site for the establishment of all species, as seedling abundance exhibited a trend of species-specific spatial distribution. Seedlings of *M. calodendron* were preferentially found under the crown of conspecific shrubs which is at least partly due to dispersal limitation. As for many species in old and nutrient-poor landscapes, *M. calodendron* lacks specialised means of seed dispersal, which can be interpreted as an adaptive trait when microsites close to the parent plants are more likely to exhibit attributes that enable seed germination and seedling establishment for this species (Hopper 2009; Silveira *et al.* 2016). On the other hand, seedlings of *Vellozia* spp. were found preferentially in plots where *M. calodendron* shrubs were absent. Seed germination of *Vellozia* species typically requires light and temperatures that are higher than the average for *campo rupestre* species (Nunes *et al.* 2016), and therefore milder microsites associated with *M. calodendron* shrubs might be less suitable for them. Some *Vellozia* species also have root specialisations to obtain nutrients from bare rocks, thus allowing them to occupy less-developed substrates (Teodoro *et al.* 2019), where they are less likely excluded by faster-growing species with less-efficient mechanisms of nutrient acquisition (Lambers & Poorter 1992). It is possible that some of the other surveyed species also have a habitat preference, but the low number of seedlings precluded identifying significant associations. Taken together, these results add to evidence that spatial heterogeneity contributes to the species richness, probably by promoting species coexistence in *campo rupestre* (Brum *et al.* 2017; Morellato & Silveira 2018).

As biotic interactions tend to shift from negative (competition) to positive (facilitation) with increase in environmental stress (Bertness & Callaway 1994; Brooker *et al.* 2008), we expected to find evidence of positive plant interactions in ferruginous *campo rupestre*, as previously found for other old and nutrient-poor landscapes (Muler *et al.* 2014; Teste *et al.* 2014; Lambers *et al.* 2018). Indeed, adult species richness was greater in plots with *M. calodendron*, intermediate in plots with *L. pinaster* and lowest in plots lacking both species. However, unexpectedly, adult richness decreased with increasing crown area. Thus, any benefit resulting from milder conditions in plots with the two associated shrub species seemed to be offset by adverse factors as the shrubs grow bigger. Shrub roots often access deep and/or intermediate soil layers (Brum *et al.* 2017), and thus larger shrubs with higher capacity for space preemption may exclude other shrubs since the availability of gaps in the bedrock is limited (Schwinning 2010). Larger canopies will also cast shade on the species that are essentially adapted to abundant light (Lüttge *et al.* 2007; Pereira *et al.* 2018), possibly compromising their growth and survival. Moreover, microhabitats under shrubs with larger crowns possibly accumulate more allelopathic chemicals or pathogens, which may limit the growth of some plant species.

Our study identified a second potential negative plant-plant interaction, as understorey plant cover had a negative effect on seedling abundance. This agrees with previous observations that competitive interactions exist to some extent in less-stressed microsites within these harsh environments (Lamont *et al.* 1993; Loydi *et al.* 2013). Although 10% of the seedlings (mostly *Vellozia* spp.) were found on top of other plants and biocrusts (no roots in substrate), these seedlings did not exhibit enhanced growth or survival compared with that on the other substrates. Therefore, our study provides no evidence of a positive interaction between seedlings and understorey plants in ferruginous sites, but a tendency to benefit from microenvironmental conditions on plots with *M. calodendron* and *L. pinaster*. Overall, our results indicate that habitat heterogeneity in harsh environments may determine the outcome of plant-plant interactions, shifting from positive effects on patches with low plant density, to negative effects on better-quality microsites (Bertness *et al.* 1999; Boeken 2018).

Our study design does not allow us to pinpoint the causes of greater species richness and seedling abundance in plots with the presence of the shrubs. One of the explanations is that *M. calodendron* and *L. pinaster* ameliorate the microhabitat around them, and consequently facilitate seedling establishment, as previously suggested (Jacobi *et al.* 2008). However, it is also possible that the two species established and grew in microhabitats that were already favourable before any of these shrubs were present. Indeed, we found no seedlings of *M. calodendron* and only one of *L. pinaster* (out of 28 and 13, respectively) in plots without the two shrubs which suggests that recruitment of these species may be limited to the more favourable microhabitats. Experimental approaches are needed to disentangle causes and effects of habitat heterogeneity, and confirm facilitation in ferruginous *campo rupestre* (Castanho *et al.* 2015).

Seedling spatial patterns

High seedling mortality in old nutrient-poor landscapes is often attributed to water deficit during the dry season (Coelho *et al.* 2008; Yates *et al.* 2011; Hallett *et al.* 2014), while survival could be related to the ability of a plant to access water before the onset of the dry season (Lamont *et al.* 1993; Poot & Lambers 2003; Shi *et al.* 2019). Here we found that more than 60% of the seedlings survived over the year, agreeing with previous findings that juveniles from *campo rupestre* are stress-tolerant (Dayrell *et al.* 2018). Mortality rates in old and nutrient-poor landscapes are predicted by growth rates (i.e., fast growing species have higher mortality risk; Negreiros *et al.* 2016), and establishment in unfavourable microsites (Lamont *et al.* 1993). Interestingly, none of the attributes we measured had an effect on seedling mortality. It is possible that the low number of recorded seedlings, most belonging to few species, and the merging of seedlings and juveniles concealed existing patterns. Another explanation is that, the interplay between species traits not addressed here – such as nutrient-acquisition strategies (Shi *et al.* 2019), belowground interactions (Lambers *et al.* 2018), and water-use strategies (Brum *et al.* 2017) –, and stochastic factors, e.g., success in locating cracks in the underlying rock (Poot & Lambers 2003) might drive seedling survival in ferruginous *campo rupestre*.

Overall, growth of seedlings over the year was remarkably slow, and some seedlings did not grow at all which is the expected pattern for stress-tolerant seedlings (Dayrell *et al.* 2018). Interestingly, RGR of seedlings in plots without the two associated shrub species was higher than that of seedlings in the plots with the shrubs, which could be related to differences in availability of nutrients (Milberg *et al.* 1998), light (Rice & Bazzaz 1989) and water (Lamont *et al.* 1993). The higher RGR of seedlings in substrates other than fragmented rock could also be explained by more resource availability in these substrates, which included thin soil layer, cracks in ferricrete cover, plants (e.g. mosses) and biocrusts.

Despite the striking dominance of monocot adults in the plots, very few seedlings of monocots other than *Vellozia* spp. were recorded. Clonal growth is observed in 70% of the monocots in ferruginous *campo rupestre* (Jacobi *et al.* 2007), and probably is their main strategy for species persistence. *Vellozia* spp. were the most abundant group of adults and seedlings in number of plots. This agrees with the typically high seed germination percentages of *Vellozia* species compared with those of other *campo rupestre* species (Mota & Garcia 2013; Dayrell *et al.* 2017). However, an earlier demographic study with populations of *Vellozia* spp. failed to find seedlings and juveniles, and reported the youngest specimens as being several decades old (Alves 1994). The small size and remarkably-slow seedling growth might hinder their detection in the field, and it is possible that individuals of *Vellozia* rarely grow past early developmental stages. Altogether, our study points to a need to design specific studies addressing the demography and seedling ecology of species from old and nutrient-impoverished landscapes.

Conclusions

Our study supported the view that substrate is an important driver of spatial heterogeneity in ferruginous *campo rupestre* (Carmo *et al.* 2016) and showed that microhabitats associated with *M. calodendron* and *L. pinaster* shrubs exhibited different biotic and abiotic conditions from those lacking these shrubs. Plots with these species had greater adult richness and seedling abundance. It is unclear, however, whether *M. calodendron* and *L. pinaster* shrubs modified microhabitats around them, thereby facilitating seedling establishment as previously suggested (Jacobi *et al.* 2008), or only thrived in favourable microhabitats. Such insights can provide valuable guidance for restoration programmes that could either use these species to increase species richness and seedling abundance, or use certain species for specific habitats according to their requirements. Also, similar studies should be conducted in other sites to allow for broader generalisations of the results. Interestingly, we identified two potential negative plant-plant interactions, with larger crown area being associated with a decrease in species richness, and greater understorey plant cover with lower seedling abundance. This suggests that habitat heterogeneity may allow not only coexistence of species, but also modulate the interplay of positive and negative interactions, as previously reported for other harsh environments (Bertness *et al.* 1999; Boeken 2018).

Seedlings of ferruginous *campo rupestre* are stress-tolerant (Dayrell *et al.* 2018), withstand harsh environmental conditions, and exhibit very slow growth. None of the measured attributes was a good predictor of seedling survival. Experimental studies controlling for the number and age of seedlings, while manipulating environmental variables associated with the plots, as well as investigation of belowground traits involving water and nutrient acquisition are recommended to unveil drivers of seedling mortality. Relative growth rate of seedlings was higher in plots without *M. calodendron* or *L. pinaster* shrubs and also higher in substrates other than fragmented rock, which suggests different resource availability among microhabitats within the community. Despite similar rates of seedling survival among species, some species had an overall growth greater than others, indicating the existence of different ecological strategies within the community. Altogether, our study shows that regeneration from seeds in ferruginous *campo rupestre* is marked by complex spatial heterogeneity, driven by abiotic and biotic factors that affect seedling growth and abundance, thereby allowing species coexistence and promoting species diversity.

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SUPPLEMENTARY MATERIAL

Table S1.Linear mixed-effect models (LMMs) of principal components 1, 2 and 3 for plots according to the presence of associated species (associated sp; *Lychnophora pinaster, Mimosa calodendron* or neither) in a ferruginous *campo rupestre*. Random intercepts per blocks nested within transects were specified in the model. The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. Δ AIC is the difference in AIC values between minimal model (MM) and the null model (null). Model identification is (Model ID), rationale (Model), number of parameters (*K*), and log likelihood (log L). The significant differences (ANOVA) between minimal and null models are indicated in bold (*P*<0.05).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	*P*
plot	MM[1]	PC1 ~ associated sp random = transect/block	6	-66.0	144.0	-43.6	**<0.0001**
	null	PC1 ~ 1 random = transect/block	4	-89.8	187.7		
plot	MM[2]	PC2 ~ associated sp random = transect/block	8	-59.9	135.8	-14.0	**0.0002**
	null	PC2 ~ 1 random = transect/block	4	-70.9	149.8		
plot	MM[2]	PC3 ~ associated sp random = transect/block	8	-58.9	133.9	-0.1	0.0887
	null	PC3 ~ 1 random = transect/block	4	-63.0	134.0		

[1] normal distribution; [2] normal distribution with specified variance structure.

Table S2. Generalised linear mixed-effect models (GLMMs) of species richness (richness) and seedling abundance (seedling abund) for plots in a ferruginous *campo rupestre*. The potential explanatory variables initially considered as fixed effects were associated species (associated sp; *Lychnophora pinaster, Mimosa calodendron* or neither), crown area (m^2), understorey plant cover (underst cover; %), fragmented rock cover (%), litter cover (%) and substrate depth (mm). Random intercepts per blocks nested within transects were specified in the model. The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. Δ AIC is the difference in AIC values between minimal model (MM) and the null model (null). Model identification is (Model ID), rationale (Model), number of parameters (*K*), and log likelihood (log L). The significant differences (ANOVA) between minimal and null models are indicated in bold (*P*<0.05).

Data	Model ID	Model	K	Log L	AIC	Δ AIC	*P*
plot	MM[1]	richness ~ associated sp + crown area random = transect/block	7	-76.7	167.5	-28.2	**<0.0001**
	null	sp richness ~ 1 random = transect/block	4	-93.9	195.7		
plot	MM[2]	seedling abund ~ associated sp + underst cover random = transect/block	7	-105.1	218.1	-4.0	**0.01876**
	null[2]	seedling abund. ~ 1 random = transect/block	4	-100.1	214.1		

[1] normal distribution; [2] negative binomial distribution specified.

Table S3. List of species of adults in a ferruginous *campo rupestre* at *Serra da Calçada,* Southeast Brazil. The column "plots (*n*)" indicates the number of plots, out of 45, in which the species was found. The presence of *Lychnophora pinaster* and *Mimosa calodendron* was used as a criterion for plot selection, and therefore these two species were not included in this list. Non-identified (NI).

Family / species	plots (*n*)
Apocynaceae	
Ditassa sp.	4
Asteraceae	
Symphyopappus brasiliensis	12
Baccharis sp.	1
Bromeliaceae	
Dyckia consimilis	4
NI	1
Cactaceae	
Arthrocereus glaziovii	3
NI	1
Cyperaceae	
Trilepis lhotzkiana	15
Euphorbiaceae	
Microstachys corniculata	5
Loranthaceae	
Struthanthus flexicaulis	1
Melastomataceae	
Pleroma heteromallum	3
Orchidaceae	
Acianthera teres	2
Epidendrum secundum	1
Hoffmannseggella caulescens	24
Poaceae	
NI	34
Rubiaceae	
NI	5
Velloziaceae	**37***
Vellozia albiflora	35
Vellozia graminea	9
Vellozia caruncularis	4
Vellozia compacta	1
Verbenaceae	
Stachytarpheta sp.	2

* number of plots on which *Vellozia* spp. were found.

Table S4. Generalised linear mixed-effect models (GLMMs) seedling abundance (seedling abund) of most prevalent species for plots with different associated species (associated sp; *Lychnophora pinaster, Mimosa calodendron*, neither). Random intercepts per blocks nested within transects were specified in the model. The Akaike's information criterion (AIC) represents the uncertainty of the model, whereby lower AIC values represent the more parsimonious models. Δ AIC is the difference in AIC values between minimal model (MM) and the null model (null). Model identification is (Model ID), rationale (Model), number of parameters (K), and log likelihood (log L). The significant differences (ANOVA) between minimal and null models are indicated in bold (*P*<0.05). Analysed seedlings species: *Lychnophora pinaster*, *Microstachys corniculata*, *Mimosa calodendron*, *Symphyopappus brasiliensis*, *Vellozia* spp.

Data	Model ID	Model	*K*	Log L	AIC	Δ AIC	*P*
Lychnophora pinaster	1[1]	seedling abund ~ associated sp random = transect/block	6	-29.3	70.5	3.0	0.6004
	null[1]	seedling abund ~ 1 random = transect/block	4	-29.8	67.5		
Microstachys corniculata	1[1]	seedling abund ~ associated sp random = transect/block	6	-15.2	42.5	0.3	0.1124
	null[1]	seedling abund ~ 1 random = transect/block	4	-17.4	42.8		
Mimosa calodendron	1[1]	seedling abund ~ associated sp random = transect/block	6	-27.6	67.2	-13.7	**0.0001**
	null[1]	seedling abund ~ 1 random = transect/block	4	-36.5	80.9		
Symphyopappus brasiliensis	1[1]	seedling abund ~ associated sp random = transect/block	6	-24.5	61.0	-4.2	**0.0171**
	null[1]	seedling abund ~ 1 random = transect/block	4	-28.6	65.2		
Vellozia spp.	1[1]	seedling abund ~ associated sp random = transect/block	6	-62.4	136.8	-30.9	**<0.0001**
	null[1]	seedling abund ~ 1 random = transect/block	4	-79.9	167.7		

[1] Zero-inflated Poisson distribution specified.

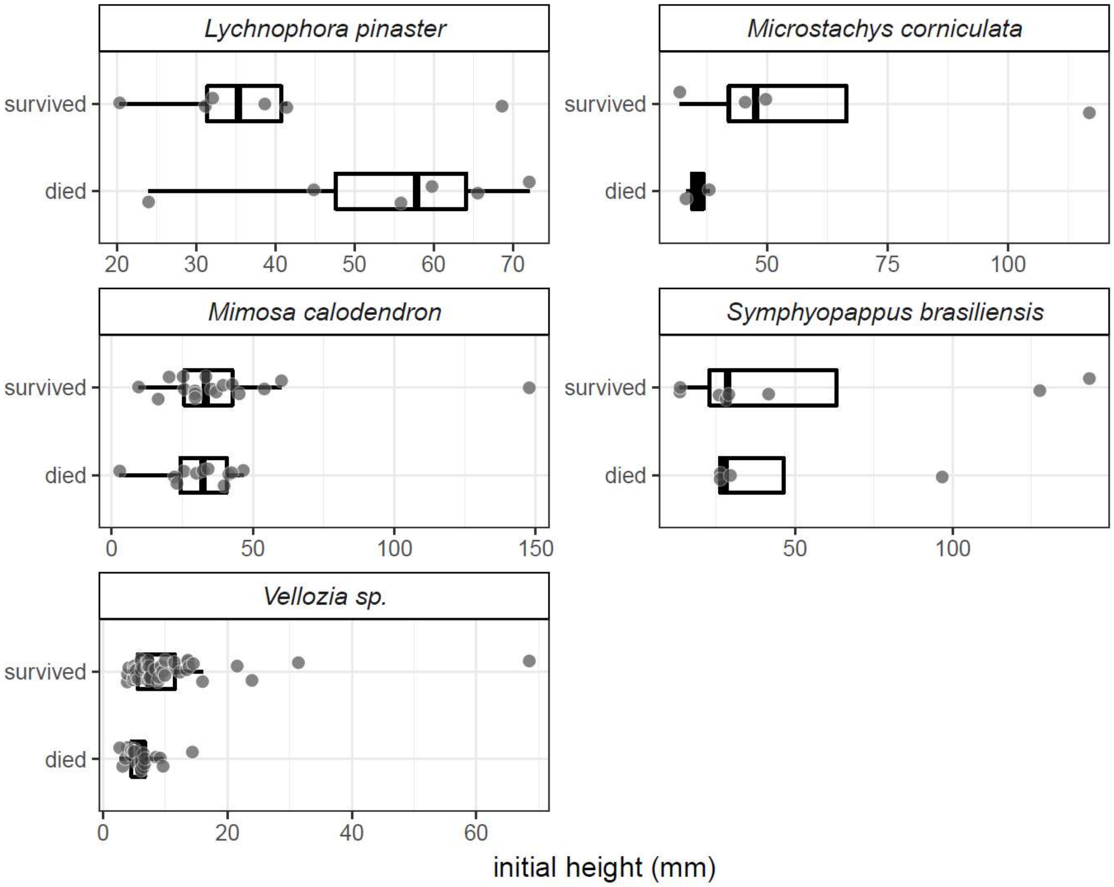

Figure S1. Survival of seedlings over a one-year period against their initial height (mm) in a ferruginous *campo rupestre*, Southeast Brazil. Data shown for most prevalent species of seedlings. Box plots show medians (vertical lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile ranges (whiskers). Grey dots are individual samples. Most prevalent species of seedlings: *Lychnophora pinaster*, *Microstachys corniculata*, *Mimosa calodendron*, *Symphyopappus brasiliensis*, *Vellozia* spp.

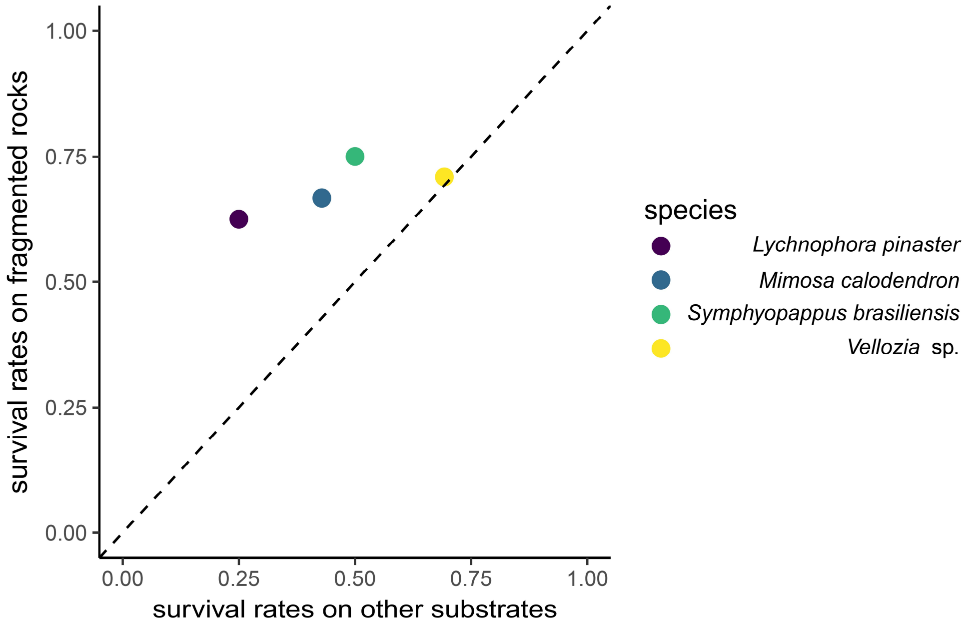

Figure S2. Seedling survival rate on fragmented rocks against survival rate on other substrates over a one-year period in a ferruginous *campo rupestre* in Southeast Brazil. Different colours show rates for most-prevalent species of seedlings individually and least common species were grouped as monocots and dicots. Dashed line is where survival rate on both substrates is numerically the same. Most-prevalent species of seedlings: *Lychnophora pinaster*, *Mimosa calodendron*, *Symphyopappus brasiliensis*, *Vellozia* spp. Groups with less than three replicates per substrate (i.e. *Microstachys corniculata*, other dicots and other monocots) were omitted from the plot.

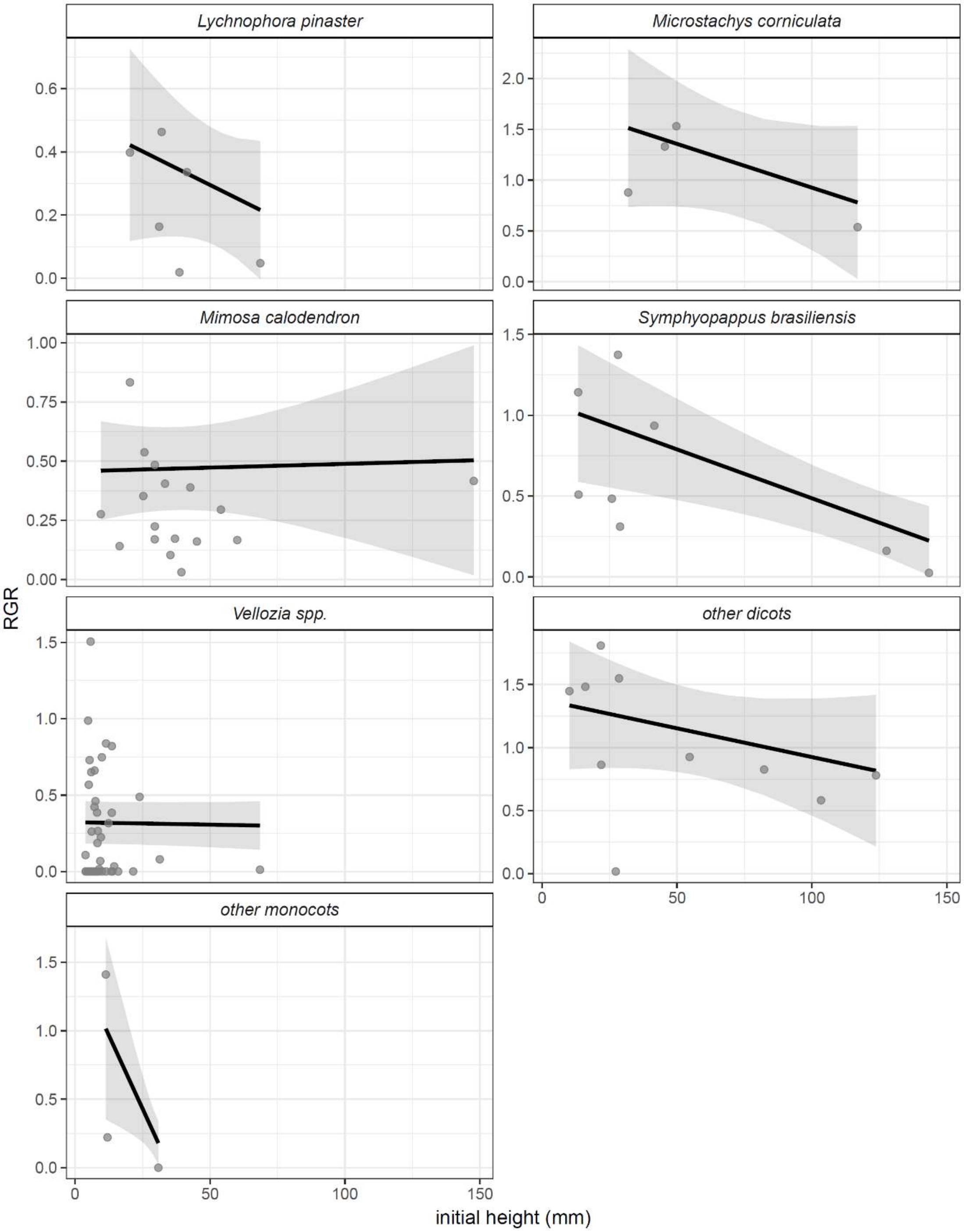

Figure S3. Relative growth rates (RGR) of seedlings over a one-year period against their initial height (mm) in a ferruginous *campo rupestre*, Southeast Brazil. Box plots show medians (vertical lines) with quartiles (box margins), the lowest and the highest values within 1.5-interquartile ranges (whiskers). Grey dots are individual samples. Other dicots and other monocots are specified in Table 2.

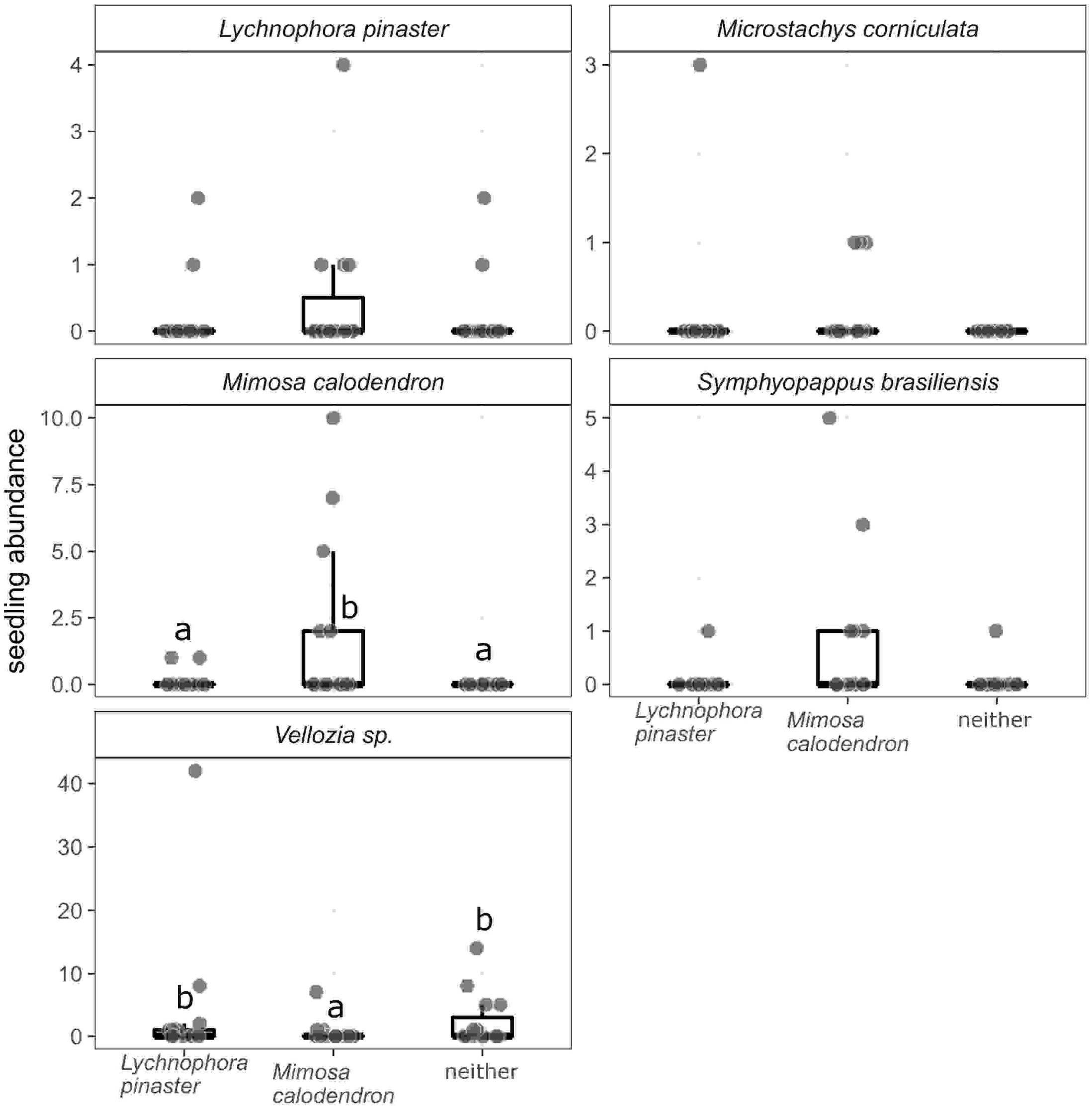

Figure S4. Seedling abundance of most-prevalent species and other monocots and dicots for plots with different associated species (*Lychnophora pinaster, Mimosa calodendron*, neither) in a ferruginous *campo rupestre*. Black and bigger dots represent means, lighter and smaller dots are individual samples, error bars are SE, and different lower-case letters represent significant differences between groups based on generalised linear mixed-effect models (Table 2) followed by the Tukey–Kramer honest test. Analysed seedlings species: *Lychnophora pinaster*, *Microstachys corniculata*, *Mimosa calodendron*, *Symphyopappus brasiliensis*, *Vellozia* spp.

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RESPONSE ARTICLE

Overcoming challenges on using native seeds for restoration of megadiverse resource-poor environments: a reply to Madsen et al.

Roberta L. C. Dayrell[1,2], André J. Arruda[1], Elise Buisson[3], Fernando A. O. Silveira[1]

Madsen et al. (2016) reviewed several major limiting factors to establishment of seedlings in nonforest ecosystems (NFE), and proposed seed enhancement technologies to overcome these restoration barriers. However, biodiverse nutrient-poor NFE present additional hurdles that preclude landscape-scale seed-based restoration and were not mentioned in their review. Here, we discuss issues related to native seed availability and provenance, and shortfalls in knowledge on seed quality testing and dormancy release that severely hamper restoration of degraded nutrient-impoverished NFE. We present alternatives for overcoming these challenges and highlight the need for investments to find more practical and cost-effective options for broad-scale restoration.

Key words: campo rupestre, OCBIL, P limitation, plant rarity, seed availability, seed sourcing

Implications for Practice

- Restoration of nutrient-poor nonforest ecosystems is severely hampered because of native seed-related barriers.
- Issues related to seed sourcing, seed quality/availability, and dormancy-breaking comprise relevant hurdles that limit our capacity to use native seeds for restoration in resource-poor environments.
- Knowledge to understand the causes of low seed quality and strategies for producing seed of higher quality should benefit restoration in these landscapes.

Despite increasing rates of anthropogenic impacts on nonforest ecosystems (NFE), knowledge of NFE restoration is still scarce compared to that of forest ecosystems (Veldman et al. 2015). Seed-based restoration techniques are fundamentally relevant in community reassembly after degradation. Hence, it was with great interest that we read Madsen et al.'s (2016) paper, which deals with seed enhancement technologies for overcoming barriers to NFE restoration.

We acknowledge and concur with the issues raised by Madsen et al. (2016) regarding seed-based restoration of NFE, and here address additional topics that are key to restoration of nutrient-poor NFE. Old, climatically buffered, infertile landscapes (OCBILs; Hopper 2009) constitute emblematic examples of NFEs that harbor extremely high levels of plant endemism and are damaged by anthropogenic activities that demand active restoration. OCBILs occur in at least 12 biodiversity hotspots that include some of the world's richest biological flora (e.g. the *kwongkan* in southwestern Australia, the *fynbos* in the Cape Floristic region, and the *campo rupestre* in eastern Brazil; Hopper et al. 2016).

OCBIL restoration studies are rare, but evidence suggests that effective strategies are extremely challenging and costly (Standish & Hobbs 2010; Le Stradic et al. 2014). Restoration techniques developed in temperate grasslands (e.g. hay transfer and trait-based approach to avoid weed invasion) are often not applicable for OCBILs, mostly because of seed-related issues (Standish & Hobbs 2010; Le Stradic et al. 2014). Here, we aim to discuss challenges constraining the success of seed-based landscape-scale restoration in OCBILs that were not included in Madsen et al.'s review, and to present options for overcoming these restoration hurdles (Fig. 1).

Genetic Issues

An appropriate selection of local provenances increases the probability of restoration success and avoids potentially negative consequences of introducing nonlocal genotypes (McKay et al. 2005). Short-distance differentiation is commonplace in OCBIL species, reflecting high levels of genetic divergence among populations (Krauss & He 2006). Hence, the seed supply for highly localized provenances may be low or nonexistent in OCBILs, which constitutes one of the major critiques against

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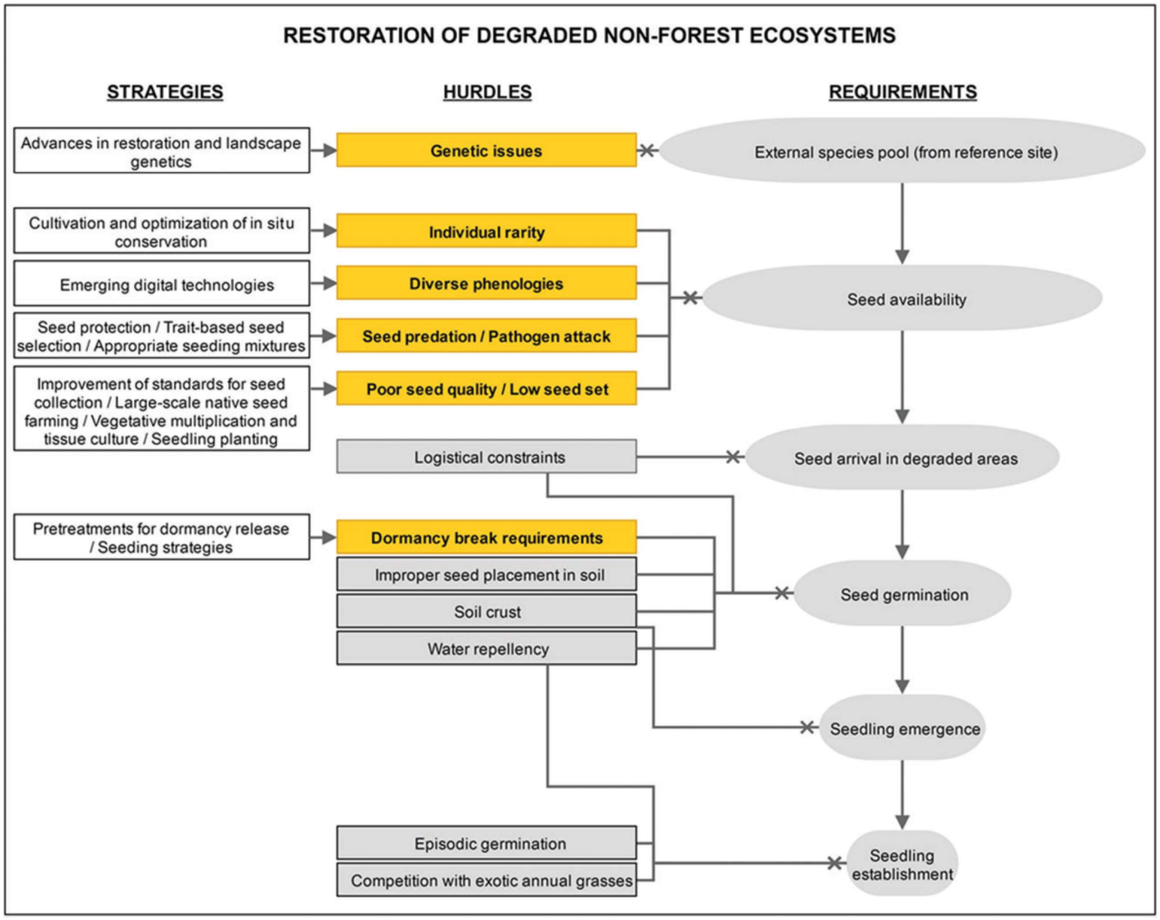

Figure 1. Key requirements and hurdles for seed-based restoration of nutrient-impoverished nonforest ecosystems, and strategies to overcome hurdles. Gray boxes indicate barriers addressed by Madsen et al. (2016) and yellow boxes show additional challenges discussed here. Links between hurdles and requirements are represented by lines with “x mark” at the tip close to requirements boxes. Vertical arrows represent the movement of species through the filters.

the “local is best” paradigm (Broadhurst et al. 2008). The field of restoration and landscape genetics, especially in next generation sequencing methods, may bring fresh perspectives to solve provenance issues in the future (Williams et al. 2014).

Seed Availability

Sourcing sufficient quantities of germinable seeds to restore biodiverse communities presents a major hurdle for landscape-scale restoration (Merritt & Dixon 2011), especially in OCBILs (Standish & Hobbs 2010). Below we address four commonly encountered issues that, when combined or even in isolation, hamper collection of sufficient amounts of native seeds for restoration of these ecosystems.

Individual Rarity

Species with low frequency and abundance are common in many plant communities (Zobel et al. 1998). This implies the presence of a very large number of individually rare species in biodiverse ecosystems (Hopper 2009). Considering the demands of landscape-scale restoration, these rare species constitute a very limited source of seeds. Ex situ seed production in glasshouses is a potential solution, if caution is taken to avoid cumulative inbreeding depression (Kephart et al. 1999). The reintroduction of rare species into restored habitats can simultaneously increase population size and diversify their gene pool (Lofflin & Kephart 2005), optimizing in situ conservation and restoration goals. However, preserving rare NFE species in restored ecosystems can be a difficult task.

Diverse Phenology

Due to their great diversity and heterogeneity, species in OCBIL communities display a wide variety of phenological patterns, which can require increased efforts/costs for seed collection. Additionally, many OCBIL species have supra-annual phenological patterns or rarely reproduce (Le Stradic 2012). Emerging digital technology advances such as satellite images, field cameras, aerial photography, and remote sensing (Morellato et al. 2016) are making data acquisition easier and thus may provide phenological data useful for restoration in the near future.

Poor Seed Quality and Low Seed Set

Compared with plants from other communities, plants in phosphorus (P)-limited landscapes invest little in sexual reproduction (Fujita et al. 2014). For instance, the association of severely P-impoverished soils and high concentrations of seed P often result in a low seed set per individual (Stock et al. 1989). Moreover, unusually high levels of seeds that lack embryos or are otherwise nonviable are common among OCBIL species (Dayrell et al. 2016). Besides P-limitation, the resilience strategy of many OCBIL plants of resprouting after fire may also explain this low fecundity, due to resource or breeding system limitations, or genetic load (Lamont & Wiens 2003).When germination cannot be achieved, methods for providing plant materials include vegetative multiplication and tissue culture. Research to develop techniques may be required for some species (Koch 2007). The hurdles of native seed supply has led some authors to encourage the improvement of standards to guide seed collection and utilization (Broadhurst et al. 2015) and large-scale native seed farming enterprises to generate seeds required to meet demands for landscape-scale restoration (Merritt & Dixon 2011).

Seed Predation/Pathogen Attack

Predation pressure can further reduce seed availability in seed-limited communities. Thus, the exclusion or control of seed predators may be required in some restoration programs (Orrock et al. 2009). The use of mulch (Nyamai et al. 2011) and chemical repellants (Brakes & Smith 2005) has been reported to protect the seeds, and late-season seeding and drill seeding may effectively reduce the impacts of granivory on seedling establishment (Gurney et al. 2015). Additionally, selection of species based on least preferred seed traits (Suazo et al. 2013) and appropriate abundance of seeds in seeding mixtures can also increase on-site retention of heavily predated seeds (Beard et al. 2013; Dudenhoffer et al. 2016).

Dormancy Break Requirements

Plants from natural communities disperse seeds with varying types and degrees of dormancy. Therefore, seeds may be wasted if sown without proper pretreatments or timing for dormancy release (Merritt & Dixon 2011). Although a wide variety of dormancy release and germination stimulation methods have been described (Merritt et al. 2007), germination requirements for most species remain to be investigated. Nevertheless, many techniques have been shown to improve germination outcomes in OCBILs, particularly those related to fire-related cues, such as smoke water and karrikinolide (Commander et al. 2009). These techniques, however, may not be useful for all OCBIL taxa and/or plant communities (Le Stradic et al. 2015).

Conclusion

OCBIL restoration is extremely difficult, and the new technologies reviewed by Madsen et al. (2016) will be key in restoring those exceptionally biodiverse, resource-poor NFE. However, issues related to seed sourcing, dormancy-breaking, and seed quality/availability comprise relevant hurdles that limit our capacity to employ seed-based techniques in OCBIL restoration. Despite moderate progress, a much greater investment in research addressing these issues is urgently needed to reduce costs, and improve the chances for successful restoration in these landscapes.

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Chapter 6

General Discussion

Introduction

Combinations of viable plant traits are restricted by underlying universal trade-offs in function and determine the success of individuals in different habitats (Lambers & Poorter 1992; Grime *et al.* 1997; Wright *et al.* 2004; Chave *et al.* 2009; Reich 2014; Díaz *et al.* 2016). Each suite of traits can be interpreted as a plant "strategy" that allows a plant to thrive under a specific set of environmental conditions (Grime & Pierce 2012). Although most plant life stages are sessile, different stages of plant development may experience different environmental filters, e.g., some forest seedlings live in deep shade, while adults may access abundant light (Valladares & Niinemets 2008). The inherent capacities of the plant also change over time, as the plant accrues biomass and augments its abilities to access resources, e.g., enabling some plants to prioritise resource use to photosynthetic performance as saplings, and to defence against herbivory and mechanical damage in later stages (Ishida *et al.* 2005). Accordingly, different stages may exhibit different combinations of functional traits (Gatsuk *et al.* 1980; Farnsworth 2004; Mason *et al.* 2013) which suggests that a shift in strategy throughout ontogeny maximises plant fitness.

While the associations between traits of adult individuals and environmental conditions are well understood (Garnier *et al.* 2016), there has been less progress in characterising suites of regeneration traits, despite their notorious importance in driving community assembly and species distribution (Grubb 1977; Paine *et al.* 2015; Larson & Funk 2016a). Seedlings and young juveniles inherently exhibit a higher mortality risk compared with adults (Stearns 1976;

Harms & Dalling 1997; Moles & Westoby 2004), suggesting strong selection pressure during these developmental stages. Knowledge on strategies of seedlings and juveniles for coping with biotic and abiotic conditions have been mostly limited to tolerance to drought and/or shade in a few ecosystems, such as tropical rainforests and temperate grasslands and shrublands (e.g., Kahmen & Poschlod 2004; Poorter & Bongers 2006; Poorter & Markesteijn 2007; Kitajima *et al.* 2013; Larson & Funk 2016b), limiting our understanding of regeneration in ecosystems with contrasting environmental conditions.

In this thesis, I aimed to investigate regeneration in old, climatically-buffered, infertile landscapes – OCBILs (Hopper 2009; Hopper *et al.* 2016). I specifically carried out my research in two OCBILs: the *kwongan* in Southwest Australia and the *campo rupestre* in Brazil (Hopper 2009; Hopper *et al.* 2016; Silveira *et al.* 2016). The evolution of plants in localised places subjected to minor soil disturbance and climatic buffering for millions of years provided prolonged opportunities for evolution to run its course, resulting in specific strategies for OCBIL lineages (Hopper 2009). Soil infertility, especially severe phosphorus (P) deficiency, is a strong environmental filter that leads to a clear predominance of nutrient-conserving, slow-growing strategies among OCBIL plant species (Specht & Rundel 1990; Lambers & Poorter 1992; Wright *et al.* 2002; Wright & Westoby 2003). While adults of OCBIL species have a very low P concentration ([P]) in leaves, seed [P] is often very high (Kuo *et al.* 1982; Denton *et al.* 2007; Groom & Lamont 2010), enabling seedlings to survive, without the need to obtain nutrients from the soil (Milberg & Lamont 1997). In the following sections, I highlight the major finding of this research on additional traits and strategies pertaining to early developmental stages of OCBIL plants. I also discuss the broader implications of my results and the areas on which I believe future studies should focus.

Regeneration in OCBILs

In the first study (Chapter 2), I focused on regeneration patterns of the plant community in an OCBIL (*campo rupestre*), comparing them with those of a more productive nearby habitat (forest). Ontogenetic shifts in plant ecological strategies amongst species seem to be ubiquitous, regardless of habitat productivity. Plants from the *campo rupestre* and the forest exhibited relatively acquisitive strategies that favoured less costly leaves and faster growth rates in the juvenile stage compared with conspecific adults, possibly due to the greater vulnerability of early developmental stages to damage (Harms & Dalling, 1997; Moles & Westoby, 2004). In absolute terms, however, the ecological strategies of juveniles were mainly stress-tolerant (marked by nutrient conservation and slow growth) in *campo rupestre* juveniles. In forest, juveniles invested in both competitive (larger leaf area to capture light) and stress-tolerant strategies. These results indicate that habitat filtering of juveniles resemble those of adults in these environments, despite the ontogenetic shifts in strategy. The OCBIL juveniles, similar to adults, invest mainly in traits to withstand abiotic stresses, at the expense of growth rates.

In the second study (Chapter 3), I investigated shifts in the use and allocation of leaf P in the plant community of an OCBIL (*kwongan*). I aimed to identify the difference between physiological strategies of unburnt adults and two regeneration forms after a fire: resprouting burnt adults and seedlings. The use and allocation of leaf P of the studied plant species changed with plant development. Seedlings had greater leaf [P] than conspecific unburnt adults which was compatible with a shift from a relatively acquisitive to a conservative life history strategy (Chapter 2, i.e. Dayrell *et al.* 2018). The greater leaf [P] was mostly due to greater leaf inorganic phosphorus concentration ([Pi]), suggesting that leaves at early developmental stages may serve as a site for P storage (Veneklaas *et al.* 2012). Higher levels in at least some of the organic P fractions (i.e. phospholipids, nucleic acid P, metabolic P, and a residual P fraction that contains phosphorylated proteins and unidentified molecules) also accounted for greater leaf [P] in seedlings, suggesting that greater [P] is also used to support a higher metabolic activity and growth rate at early life stages (Elser *et al.* 2010; Veneklaas *et al.* 2012). Seed P content markedly varied across species, being positively associated with leaf [P] in seedlings. Although burnt adults had a higher specific leaf area (SLA), and thus a more acquisitive strategy compared with unburnt adults, they exhibited only a short-term and less pronounced increase in leaf [P] compared with seedlings. This suggests that their pattern of leaf P use and -allocation does not resemble that of early developmental stages.

Species from the three most species-rich families of the *kwongan,* i.e., Fabaceae, Myrtaceae and Proteaceae (Mucina *et al.* 2014), exhibited different patterns of developmental shifts, showing that different strategies are viable for seedlings, and that these are strongly associated with plant lineages. Developmental shifts were remarkably pronounced in the Proteaceae, especially in *Banksia candolleana*, and particularities of the seedling stage may consist in another strategy of the Southwest Australian members of this family to cope with severe P scarcity (Lambers *et al.* 2013, 2015). Among all species, the Proteaceae typically exhibited the lowest leaf [P] in the adult stage, and the highest leaf [P] in the seedling stage. Among the organic P fractions, Proteaceae seedlings allocated considerably more P to phospholipids than adults did. Adults of *B. candolleana* and other Proteaceae species replace phospholipids with lipids that do not contain P during leaf development, and thus the fraction of lipid P remarkably decreases from young to mature leaves (Lambers *et al.* 2012; Kuppusamy *et al.* 2014). Therefore, the greater lipid [P] in the leaves of seedlings suggests that plants restrict replacement of lipid P fractions during early stages of development and/or continue expanding while photosynthesising and producing new leaves. Seed P content varied considerably across species. Only *B. candolleana* had higher seed [P] compared to all other species, and thus, seed mass was the major driver of differences between seed P content. This adds to the evidence that large-seededness could be a strategy of some plants occurring in high-light, nutrient- impoverished habitats as a means of maximising initial root growth (Milberg *et al.* 1998). The variation of seed P content suggests that seedling survival and growth of these species might depend on microsites with different P availability in the soil after a fire (Lamont *et al.* 1993).

The substrate in OCBILs typically varies on a very fine scale, e.g., due to rock micro relief and fracturing, ash deposition after fire, and local topography, providing a diversity of microhabitats within the same landscape which influence seedling survival (Lamont *et al.* 1993) and species composition (Alves & Kolbek 2010; Le Stradic *et al.* 2015; Carmo *et al.* 2016; Carmo & Jacobi 2016; Abrahão *et al.* 2019). In Chapter 4, I aimed to screen for differences in regeneration between microhabitats in an OCBIL: the ferruginous *campo rupestre,* a highly-threatened ironstone outcrop ecosystem. Microhabitats with the presence of two native shrubs that are dominant in the area (*Lychnophora pinaster* – Asteraceae; and *Mimosa calodendron* - Fabaceae) exhibited more developed substrates associated with greater adult species richness and seedling abundance than those lacking these two shrubs. However, larger crown area of the two shrubs and greater plant cover under the shrubs (understorey plant cover) were negatively associated with species richness and seedling abundance, respectively. This suggests that habitat heterogeneity may allow for both positive and negative plant-plant interactions, as previously reported for other harsh environments (Bertness *et al.* 1999; Boeken 2018). Seedlings of ferruginous *campo rupestre* were able to withstand harsh environmental conditions and exhibited very little growth over the course of one year, confirming my previous findings that a stress-tolerant strategy prevails in *campo rupestre* species since early stages of development (Chapter 2, i.e. Dayrell *et al.* 2018). None of the measured attributes were good predictors of seedling survival, but some species were found mainly in association with specific microhabitats. For instance, seedlings of *Vellozia* sp. were more abundant in microsites where

M. calodendron shrubs did not occur and grew very little over one year. On the other hand, *M. calodendron* seedlings were more abundant in microsites where conspecific shrubs were present, and grew considerably more than *Vellozia* sp. seedlings. Relative growth rates of seedlings were lower in plots with the dominant shrubs and in fragmented rock substrate, which could reflect different resource availability among microhabitats. Overall, my results suggest differences in seedling recruitment and species richness and composition associated with different microhabitats within the *campo rupestre*, and partially explain species coexistence in stressful ecosystems (Chapter 3).

Theoretical model of plant recruitment in OCBILs

The study of seedlings and juveniles in OCBIL illustrates how evolutionary effects add to direct biotic and abiotic factors to influence the dynamics of plant recruitment. Biotic and abiotic environments (including the level of soil fertility), in conjunction with the disturbance regime of an ecosystem select for viable strategies of seeds, seedlings and juveniles, thereby driving plant recruitment through evolutionary processes (Fig. 1). The combination of soil infertility (especially P deficiency) and historical stability (minor soil disturbance and climatic buffering) have led to specific species functional traits and vegetation patterns in OCBILs (Lambers *et al.* 2008; Hopper 2009; Hopper *et al.* 2016). A conservative (slow-growing nutrient-conserving) strategy prevails in plants living in P-impoverished soils (Specht & Rundel 1990; Lambers & Poorter 1992; Wright *et al.* 2002; Wright & Westoby 2003) since early stages of development (Chapters 2, 3, and 4). Although seedlings and juveniles are mostly stress-tolerant (Chapter 2, Dayrell *et al.* 2018), their mortality rates range between 40 and 100% after seasonal droughts (Chapter 4; Enright & Lamont 1989; Coelho *et al.* 2008; Yates *et al.* 2011), and emergence and/or survival will mostly depend on the species establishment in a suitable microhabitat (Chapter 4; Lamont *et al.* 1993). The slow growth during early developmental stages, which can be as drastic as the negligible growth of *Vellozia* seedlings over one year's time (Chapter 4), also implies remaining in a small, and thus more vulnerable stage for a long period which further decreases the chances of reaching maturity. Moreover, the great investment in persistent structures in the conservative strategy has been linked to limited investment in sexual reproduction (Bazzaz *et al.* 1987; Fujita *et al.* 2014; Gomes *et al.* 2018). For instance, some OCBIL plants allocate a disproportionally large amount of P to seeds which results in lower seed output (Stock *et al.* 1989; Henery & Westoby 2001). There have also been some reports of species in OCBILs dispersing very few viable seeds (Dayrell *et al.* 2017) and of low P availability in soils being associated with reduction in seed set (Vaughton & Ramsey 1998). The conservative strategy, therefore, seems to be associated with limited seed supply in OCBILs, which probably further constrains the number of seedlings produced each year.

The slow growth imposed by the conservative strategy in P-impoverished ecosystems can only be viable in the absence of major disturbances that kill adult individuals before each of them can successfully produce at least one other adult individual to maintain the population. Long-lived perennial organisms dominate OCBIL floras, probably as a result of the slow growth rate of plants (Hopper 2009; Negreiros *et al.* 2014), and have many opportunities to leave descendants in their lifetime. Therefore, regeneration in OCBILs happens on a very different time scale than that in productive, frequently-disturbed habitats that exhibit annual cycles of plant recruitment events, or to gap dynamics and ecological succession in forests (Bazzaz 1979). The old habitats seem to regenerate at a much slower pace, and it may take decades until one mature plant's descendant reaches the reproductive stage, and yet this sustains the population's persistence under natural conditions. Historical absence of large-scale disturbances in OCBILs led to the evolution of plants that are very successful in persisting in their stable natural habitat, but generally lack effective strategies for habitat colonisation (Hopper 2009). Time is, therefore, a crucial variable in order to understand regeneration in OCBILs. Measurements of reproductive output of species need to be considered relative to their growth rates, and total seed output over the plant's lifetime should be considered relative to their average longevity.

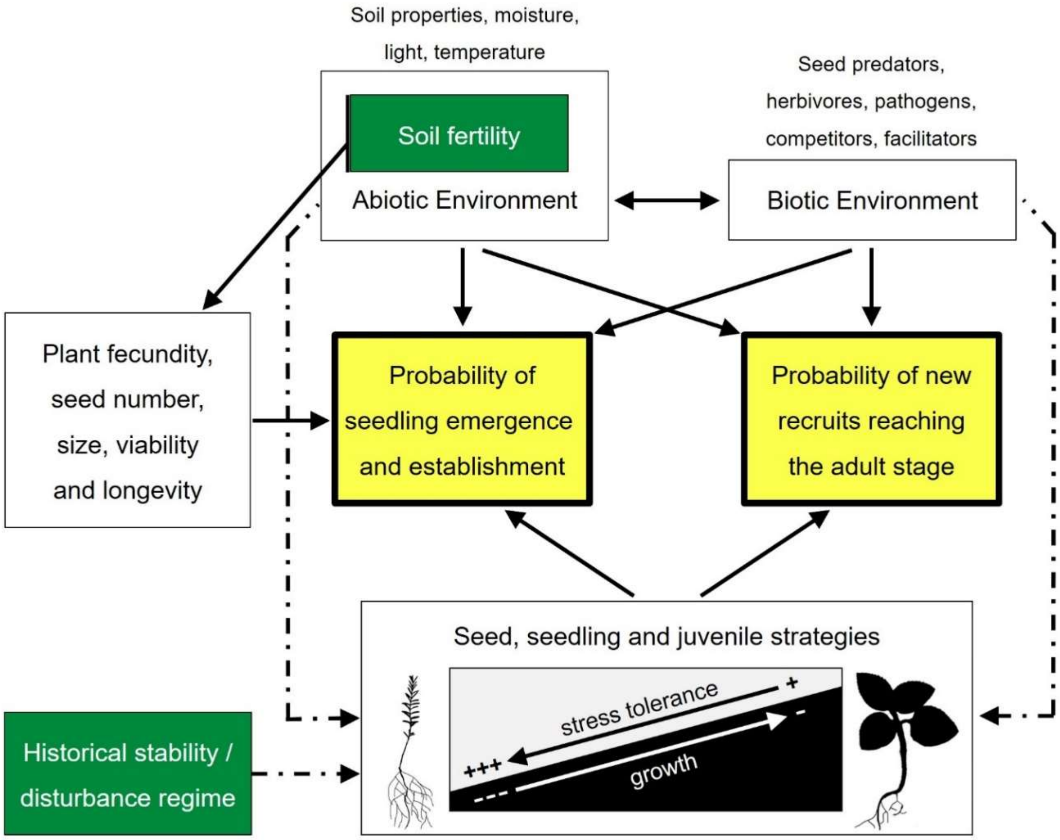

Figure 1. Flow diagram illustrating the interactions determining plant recruitment in landscapes taking into account evolutionary processes. This theoretical model explicitly shows the influence of historical stability and soil fertility (green boxes), as illustrated by studies carried out in old climatically-buffered infertile landscapes – OCBILs. The chances of a seed to become an adult depend on the combined probability of two processes: 1) seedling emergence and establishment, and 2) new recruits reaching the adult stage (yellow boxes). The microhabitat of seed fall in the landscape determines the abiotic and biotic environments influencing each other and the probability of the seed reaching the adult stage. Intrinsic seed, seedling, and juvenile strategies affect how the potential recruit responds to the abiotic and

biotic environments, determining the probability of species and individuals' propagules to reach the adult stage. The trade-off between stress-tolerance and growth of seedlings and juveniles (represented in 'seed, seedling and juvenile strategies' box) exemplifies: at one extreme a stress-tolerance strategy, in which plants exhibit slow growth rates, small, sclerophyllous and relatively costly leaves, and high biomass allocation to root system; on the other extreme a fast-growth strategy, in which plants are more susceptible to stress, and exhibit relatively larger and cheaper leaves, and more biomass allocation to the aerial part of the plant (Reich 2014). Soil fertility is part of the abiotic environment and directly influences plant fecundity, seed number, size, viability and longevity. Arrows with solid lines represent direct effects; arrows with dashed lines represent evolutionary effects.

How to reconcile slow regeneration with increasing need for restoration?

OCBILs, such as the *kwongan* in Southwest Australia and the *campo rupestre* in Brazil, have been subjected to major anthropogenic impacts that resulted in extremely large losses of their original areas (Coates *et al.* 2014; Silveira *et al.* 2016; Fernandes *et al.* 2018; Souza-Filho *et al.* 2019; Salles *et al.* 2019). Natural regeneration is not an effective management tool to restore these landscapes, since regeneration in pristine OCBIL areas occurs on a very slow time scale (as described in the previous section). Landscape-scale seed-based restoration of degraded areas is extremely challenging and costly (Standish & Hobbs 2010; Hallett *et al.* 2014; Le Stradic *et al.* 2018), and has not yet been successful in many OCBILs (Le Stradic *et al.* 2014; Buisson *et al.* 2019).

Given that the strategy of seedlings and juveniles is mainly conservative, the majority of OCBIL species will exhibit inherently slow growth. However, two main tactics that might speed up the recovery of the landscape emerge from the theoretical model proposed here: 1) increase seed availability, and 2) recreate biotic and abiotic environments that favour seedling survival (Fig. 1). I discussed causes of limited seed availability in OCBILs that hampers landscape-scale restoration, and the alternatives and directions for overcoming these challenges (Chapter 5; Dayrell *et al.* 2016). Sourcing sufficient quantities of germinable seeds is the most relevant hurdle to restore OCBILs (Standish & Hobbs 2010), due to factors such as embryoless and/or non-viable seeds, low seed set, individual rarity, and diverse phenologies. Some alternatives to these issues include large-scale native seed farming enterprises to generate seeds (Merritt & Dixon 2011), seed processing technologies that have recently emerged with promising results (Erickson *et al.* 2017; Pedrini *et al.* 2019), the use of digital image capture and processing technology to obtain phenological data (Morellato *et al.* 2016), vegetative multiplication (Hitchcock & Rebelo 2017) and tissue culture (Koch 2007). OCBIL species also commonly exhibit high levels of genetic divergence among populations (Krauss & He 2006), and new advances in landscape genetics can help guide the appropriate selection of seed provenance (Williams *et al.* 2014). For instance, species/populations adapted to conditions under climate change scenarios may result in better restoration outcomes than seeds locally sourced (Harris *et al.* 2006; Breed *et al.* 2013). Advances in control of invasive species can improve restoration outcomes (Richardson & Kluge 2008; Krupek *et al.* 2016). The progress in enhancing seedling survival is still in its infancy, and more studies are needed to identify important biotic and abiotic factors that maximise plant recruitment (Standish *et al.* 2007; Daws *et al.* 2013; Hallett *et al.* 2014; Waller *et al.* 2016). Overall, a much greater investment in research is urgently needed to reduce costs, and improve techniques that are appropriate for landscape-scale restoration of OCBILs.

Conclusion

My thesis unveiled general regeneration patterns for OCBILs. Species in these landscapes typically exhibit low seedling recruitment, likely due to low seed quality and quantity and/or lack of suitable sites for germination and seedling establishment. Seedlings are stress-tolerant and able to endure harsh conditions, such as seasonal droughts, but their inherently slow growth associated with the intrinsic vulnerability of this life stage severely hampers their chances to make it to adulthood. The community approach used in this thesis has expanded our knowledge to other plant families beyond the better-known Proteaceae and Fabaceae, and has shed light on the range of strategies that exist within OCBIL systems. Regeneration patterns were highly determined by a complex interaction between spatial heterogeneity and variability in species strategies, allowing their coexistence and thus promoting local and regional species diversity.

Future research to better understand the strategy of early developmental stages would need to include investigations of the root systems of seedlings and juveniles and their interactions with the soil, disentangling morphological, structural and physiological responses to two confounding stress factors in OCBILs: drought and nutrient deficiency (see Lamont *et al.* 1993). It would be crucial to continue studies on different regenerative strategies by choosing species with various seed sizes, contrasting seed P content, different phylogenetic backgrounds and abilities to resprout. Field experiments controlling for strategies and microhabitats at the same time would unveil how these two components interact, and provide a better understanding of recruitment patterns in OCBILs.

Regeneration traits examined here are suitable to maintain populations of long-lived individuals in old and stable habitats, however, these communities are highly vulnerable to anthropogenic impacts, such as more frequent disturbance or nutrient addition. Therefore, *in situ* and *ex situ* conservation strategies are imperative to avoid an irreversible loss of biodiversity. Investments in research to increase seed supply and recreate suitable microhabitats for seed germination and seedling establishment are needed to improve the chances of successful restoration of OCBILs.

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